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Total Synthesis of the Antimitotic Bicyclic Peptide Celogentin C

Bing Ma, Biplab Banerjee, Dmitry N. Litvinov, Liwen He, and Steven L. Castle*

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602

Received November 21, 2009; E-mail: scastle@chem.byu.edu

Abstract: An account of the total synthesis of celogentin C is presented. A right-to-left synthetic approach to this bicyclic octapeptide was unsuccessful due to an inability to elaborate derivatives of the right-hand ring. In the course of these efforts, it was discovered that the mild Braslau modification of the McFadyen−Stevens reaction offers a useful method of reducing recalcitrant esters to aldehydes. A left-to-right synthetic strategy was then examined. The unusual Leu−Trp side-chain cross-link present in the left-hand macrocycle was fashioned via a three-step sequence comprised of an intermolecular Knoevenagel condensation, a radical conjugate addition, and a SmI_2-mediated nitro reduction. A subsequent macrolactamization provided the desired ring system. The high yield and concise nature of the left-hand ring synthesis offset the modest diastereoselectivity of the radical conjugate addition. Formation of the Trp−His side-chain linkage characteristic of the right-hand ring was then accomplished by means of an indole−imidazole oxidative coupling. Notably, Pro−OBn was required as an additive in this reaction. Detailed mechanistic investigations indicated that Pro−OBn moderates the concentration of NCS in the reaction mixture, thereby minimizing the production of an undesired dichlorinated byproduct. The natural product was obtained after macrolactamization and deprotection. The chemical shifts of the imidazole hydrogen atoms exhibited significant dependence on temperature, concentration, and pH. Antitumor screening indicated that celogentin C inhibits the growth of some cancer cell lines.

Introduction

In 2001, Kobayashi and co-workers isolated the bicyclic octapeptide celogentin C (**1**, Figure 1) from the seeds of the flowering plant *Celosia argentea*.[1] In addition to **1**, the related natural products celogentin A (**2**), celogentin B (**3**), and moroidin (**5**) were also discovered during this investigation. Peptide **5** had been previously isolated by Williams and co-workers in 1986 from the leaves of the bush *Laportea moroides*, which is native to the Australian rainforest.[2] A re-examination of the seeds of *Celosia argentea* by Kobayashi and co-workers yielded six additional bicyclic members of the celogentin family: celogentin D (**4**), celogentins E−H (**6**−**9**), and celogentin J (**10**).[3] The celogentin family can be organized into three structural classes according to variations in the right-hand macrocycle structure. Thus, the right-hand ring of **1** is a tetrapeptide that contains a proline, whereas the right-hand rings of **2**−**4** are tripeptides that lack this residue. Peptides **5**−**10** incorporate glycine into their right-hand macrocycles, resulting in tetrapeptides that presumably adopt a different conformation than the proline-containing right-hand ring of **1**. Two unusual cross-links, one connecting the leucine β-carbon with the indole C-6 of tryptophan and the other joining indole C-2 with the imidazole N-1 of histidine, are common to the entire celogentin family. The structural assignment of moroidin (**5**) has been confirmed by X-ray crystallography.[4]

The seeds of *Celosia argentea* have been employed as a traditional Chinese and Japanese herbal medicine for the treatment of liver and eye diseases.[5] An investigation of the antimitotic activity of the celogentins by the Kobayashi group revealed that the ability of peptides **1**−**10** to inhibit tubulin polymerization was strongly dependent on the structure of the right-hand ring.[1,3,6] Celogentin C (**1**), the only family member to possess a proline residue in its right-hand ring, exhibits the greatest potency in this assay (IC_{50} 0.8 μM), which exceeds that of the anticancer agent vinblastine (IC_{50} 3.0 μM). In contrast, the moroidin-type compounds (**5**−**10**) are roughly equipotent with vinblastine (IC_{50} 2.0−4.0 μM), while celogentins A, B, and D (**2**−**4**) are significantly less active (IC_{50} 20−30 μM). Despite the potential of **1** and **5**−**10** as antitumor agents, to the best of our knowledge, their toxicity to cancer cells has not yet been described.

The combination of striking molecular architecture and significant biological activity has prompted several groups to address the synthesis of the celogentins. The laboratories of

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(2) (a) Leung, T.-W. C.; Williams, D. H.; Barna, J. C. J.; Foti, S.; Oelrichs, P. B. *Tetrahedron* **1986**, *42*, 3333. (b) Kahn, S. D.; Booth, P. M.; Waltho, J. P.; Williams, D. H. *J. Org. Chem.* **1989**, *54*, 1901.
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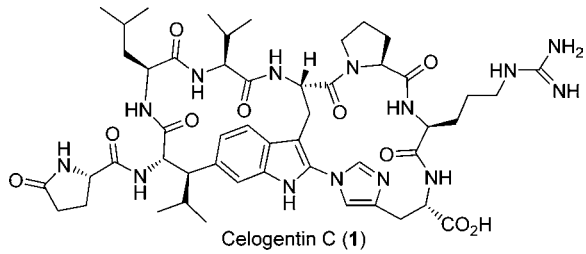

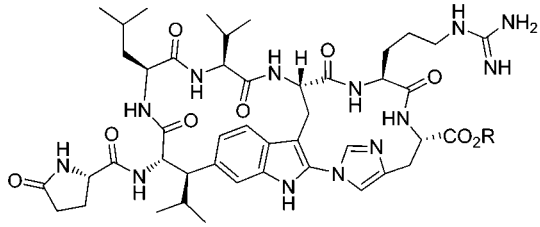

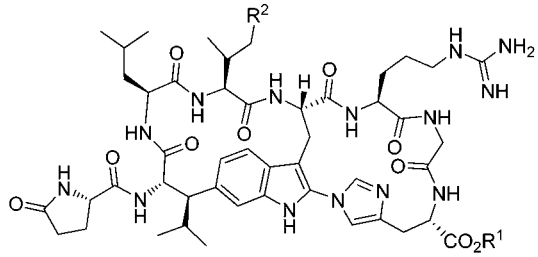

Figure 1. Celogentins A−H, celogentin J, and moroidin.

Hutton,[7] Wandless,[8] and Campagne[9] have each reported synthetic studies in this area. Notably, the efforts of Moody and co-workers[10] culminated in the construction of stephanotic acid methyl ester, a derivative of a monocyclic tetrapeptide that is very similar to the left-hand ring of the celogentins[11] (vide infra). Our group targeted celogentin C for total synthesis, and in 2003 we disclosed the construction of a functionalized tryptophan derivative suitable for elaboration into **1**.[12] Later, we prepared the right-hand ring of **1**,[13] and recently we achieved the total synthesis of celogentin C.[14] This accomplishment provides the first synthetic entry into the celogentin family of bicyclic peptides. Herein, we provide a detailed account of this venture, including the evolution of our strategy and the discovery of methodology which should have further applications. An indole−imidazole oxidative coupling reaction played a central role in the construction of the right-hand ring, and an unusual additive (Pro−OBn) was critical to the success of this process. We have conducted further experiments that led us to modify our initially proposed mechanistic rationale[14] for this intriguing transformation.

Results and Discussion

At the outset of the project, we envisioned developing an intramolecular Knoevenagel condensation−radical conjugate addition sequence for installing the key Leu−Trp linkage present in the left-hand ring of **1** (Scheme 1). Although intramolecular Knoevenagel condensations are rare, isolated examples exist.[15] Accordingly, it was our hope that α-nitroacetamido aldehyde **11** would cyclize to afford α,β-unsaturated α-nitroamide **12**. Examination of molecular models of **12** indicated that a twist in the indole ring relative to the plane defined by the double bond and its substituents would result in shielding of the bottom face of the alkene by the indole C-7 hydrogen. Therefore, the conjugate addition of an isopropyl radical[16] to **12** should occur from the top face of the olefin as required. We then planned to take advantage of the acidic α-nitroamide stereocenter in adduct **13**. Simple molecular mechanics minimization of **13** and its epimer at the nitro-bearing carbon demonstrated that **13** was ca. 3 kcal/mol lower in energy. Consequently, if *epi*-**13** were generated in the hydrogen atom abstraction step of the radical reaction, then treatment of the adduct with a weak base should form **13** via selective epimerization of the acidic α-nitro stereocenter.[17] Finally, nitro reduction and subsequent coupling to pyroglutamic acid would deliver **14**, the left-hand ring of celogentin C. Model studies with simple β-aryl-substituted α,β-unsaturated α-nitroamide substrates demonstrated the feasibility of the radical conjugate addition-nitro reduction sequence.[18] Unfortunately, despite numerous attempts, we were unable to construct an intramolecular Knoevenagel condensation substrate corresponding to **11**. Challenges inherent to installing the nitro and aldehyde functional groups in the same molecule ultimately thwarted our efforts.[19]

Our failure to prepare the left-hand ring of **1** via an intramolecular Knoevenagel condensation necessitated formulation of a second-generation plan, which is presented in Scheme 2. Prior studies by our group established the viability of an indole−imidazole oxidative coupling/macrolactamization sequence as a means of constructing the right-hand ring of **1**.[13] These results prompted us to pursue a "right-to-left" synthetic strategy for the total synthesis. Thus, oxidative coupling of modified Trp−Pro dipeptide **15** with Arg−His dipeptide **16** would afford heterobiaryl-linked tetrapeptide **17**. Deprotection and macrolactamization would then provide **18**, a suitably functionalized version of the celogentin C right-hand ring. Next, reduction of the methyl ester would deliver aldehyde **19**, substrate for an intermolecular Knoevenagel condensation. Adduct **20** would be converted into **1** via a sequence of transformations including radical conjugate addition, nitro

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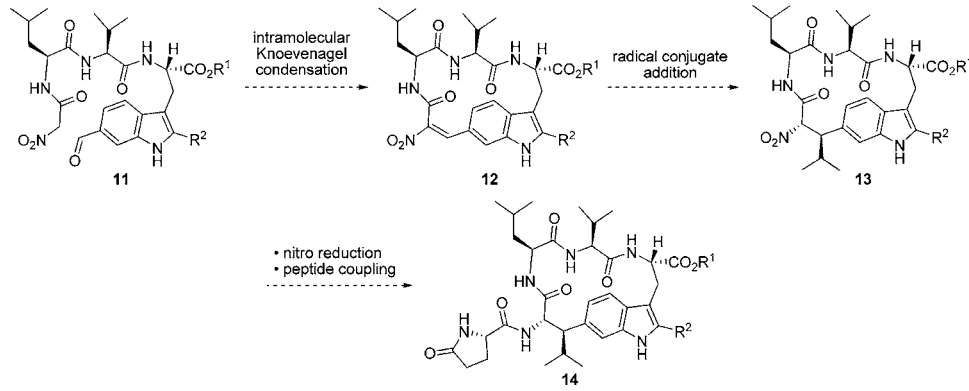
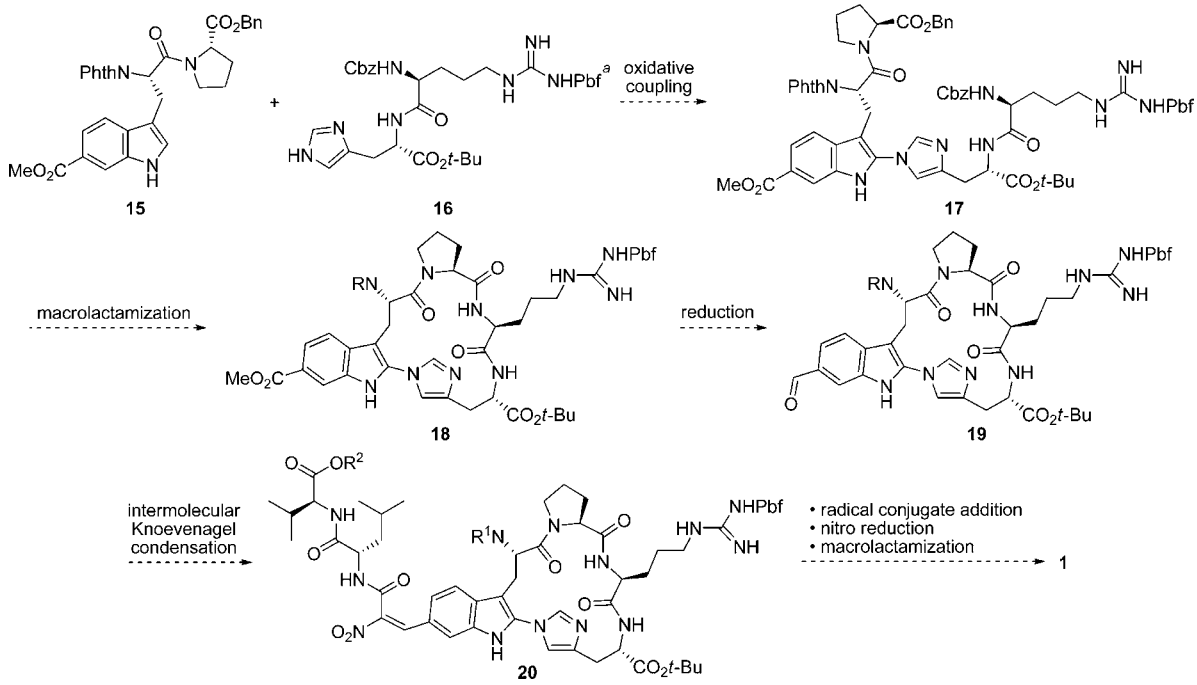
Scheme 1. Intramolecular Knoevenagel−Radical Conjugate Addition Approach to Left-Hand Ring



Scheme 2. Right-to-Left Synthetic Strategy for the Synthesis of **1**[a]



[a] Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl.

reduction, and macrolactamization. The most critical reaction in the endgame was likely to be the radical conjugate addition. In the second-generation route, the electron-deficient radical acceptor would be located in an acyclic portion of the substrate (cf., **20**), in contrast to our prior approach that imbedded the radical acceptor within a macrocycle (cf., **12**, Scheme 1). This change would presumably reduce the prospects of achieving satisfactory levels of substrate-directed stereocontrol in the reaction. Accordingly, we planned to employ a chiral Lewis acid in the radical conjugate addition.[20]

Another concern with the new plan involved the reduction of methyl ester **18** to aldehyde **19**. The electron-rich nature of the indole ring attached to the methyl ester could attenuate its reactivity toward reducing agents. In fact, the paucity of similar reductions in the literature[21] suggested that conversion of **18** into **19** may not be straightforward. Accordingly, we resolved to test the indolyl methyl ester reduction on a simpler substrate than macrocycle **18**.

The synthesis commenced with the preparation of C-6 carbomethoxy-substituted tryptophan **25** as outlined in Scheme 3. Previously, screening of various chiral phase-transfer catalysts in the alkylation of glycinate Schiff base **21** with triethylsilyl propargyl bromide demonstrated that the trifluorobenzyl-substituted hydrocinchonidine derivative **22** of Park and Jew[22] gave the best results for production of propargylglycine **23**.[12] Further optimization of this reaction in conjunction with scale-up efforts resulted in the excellent yield (91%) and ee (94%) shown in Scheme 3. The alkylation conditions, including the toluene−chloroform mixed solvent system, are based on those reported by Park and Jew.[22] The relatively labile benzophenone imine moiety of **23** was then exchanged for a more robust Cbz group, affording carbamate **24** in 96% yield. Earlier, we had performed the Larock-type heteroannulation[23] of **24** with a

(20) For a review of Lewis acid promoted radical reactions, see: Renaud, P.; Gerster, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2562.
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Scheme 3. Synthesis of C-6 Carbomethoxy Tryptophan **25**

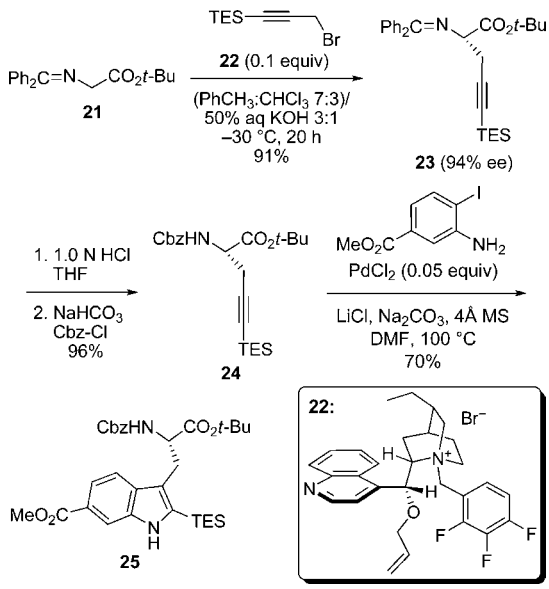
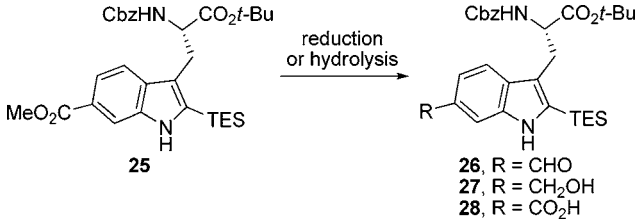


Table 1. Attempted Reduction/Hydrolysis of **25**



reagent	result
DIBAL-H	*tert*-butyl ester reduction
$LiAlH_4$	*tert*-butyl ester reduction
LiOH	complex mixture
Me_3SnOH	Cbz cleavage

hydroxymethyl-substituted *o*-iodoaniline coupling partner by utilizing a slight modification of the conditions developed by Cook and co-workers ($Pd(OAc)_2$, LiCl, Na_2CO_3).[12,24] The synthesis of C-6 carbomethoxy-substituted tryptophan **25** required the more electron-deficient 2-iodo-5-(methoxycarbonyl)-aniline[25] as coupling partner. We found that substitution of $PdCl_2$ for $Pd(OAc)_2$ and inclusion of 4 Å MS in the reaction mixture provided the best yields of **25**, which was obtained as a single detectable regioisomer.

Tryptophan **25** was employed as a model system for investigating the reduction of methyl ester **18** to aldehyde **19**, and the results of this study are summarized in Table 1. Surprisingly, attempts to reduce **25** with DIBAL-H or $LiAlH_4$ resulted in exclusive *tert*-butyl ester reduction, leaving the methyl ester intact. We then examined selective hydrolysis of the methyl ester, in hopes of either reducing the carboxylic acid or converting the acid into a reducible functional group (i.e., acid chloride, thioester, or selenoester). Unfortunately, exposure of **25** to LiOH generated a complex mixture from which the desired acid **28** could not be identified. Moreover, the use of

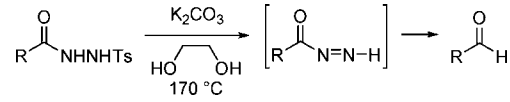
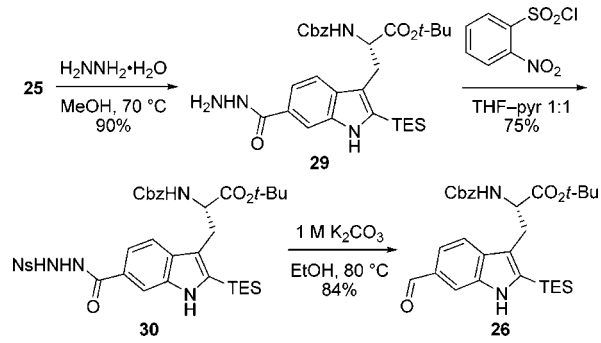


Figure 2. McFadyen–Stevens reduction.

Scheme 4. Modified McFadyen–Stevens Reduction of **25**



Me_3SnOH[26] unexpectedly caused cleavage of the Cbz group. Clearly, our fears regarding the recalcitrant nature of the C-6 methyl ester were realized.

As we searched for alternative methods of transforming methyl ester **25** into aldehyde **26**, we were drawn to a report by Braslau and co-workers of a mild variant of the McFadyen–Stevens reaction.[27] In the classical process, a tosyl-substituted acyl hydrazine is converted to an aldehyde by treatment with base at high temperature. Presumably, elimination of the tosyl group to generate an acyl diazene is followed by loss of N_2, providing the aldehyde (Figure 2).[28] In the Braslau modification, the tosyl group on the acyl hydrazine is replaced with an *o*-nitrobenzenesulfonyl (nosyl) moiety. This substitution allows the elimination to proceed at a lower temperature (ca. 80 °C). Because acyl hydrazines can be readily prepared from methyl esters,[29] we tested this protocol with methyl ester **25**. We were pleased to discover that exposure of **25** to a 1:2 mixture of hydrazine hydrate and MeOH at 70 °C provided acyl hydrazine **29** in 90% yield (Scheme 4). If the reaction was conducted in neat hydrazine hydrate, then the corresponding diacyl hydrazine derived from *tert*-butyl ester cleavage was obtained. Nosylation of **29** was best accomplished in a 1:1 THF–pyridine mixture, and the elimination–fragmentation of nosyl-substituted acyl hydrazine **30** proceeded readily at 80 °C in EtOH, delivering aldehyde **26** in 84% yield. Importantly, chiral HPLC analysis of **26** demonstrated that no epimerization of the amino acid α-stereocenter occurred during the three-step sequence.

With a means of accessing the indolyl C-6 aldehyde, we proceeded to construct the fully functionalized celogentin C right-hand ring as detailed in Scheme 5. Our previously developed method for synthesizing the model right-hand ring[13] was generally applicable, although some modification was necessary. First, C-6 carbomethoxy-substituted tryptophan **25** was converted into dipeptide **15** via a three-step process

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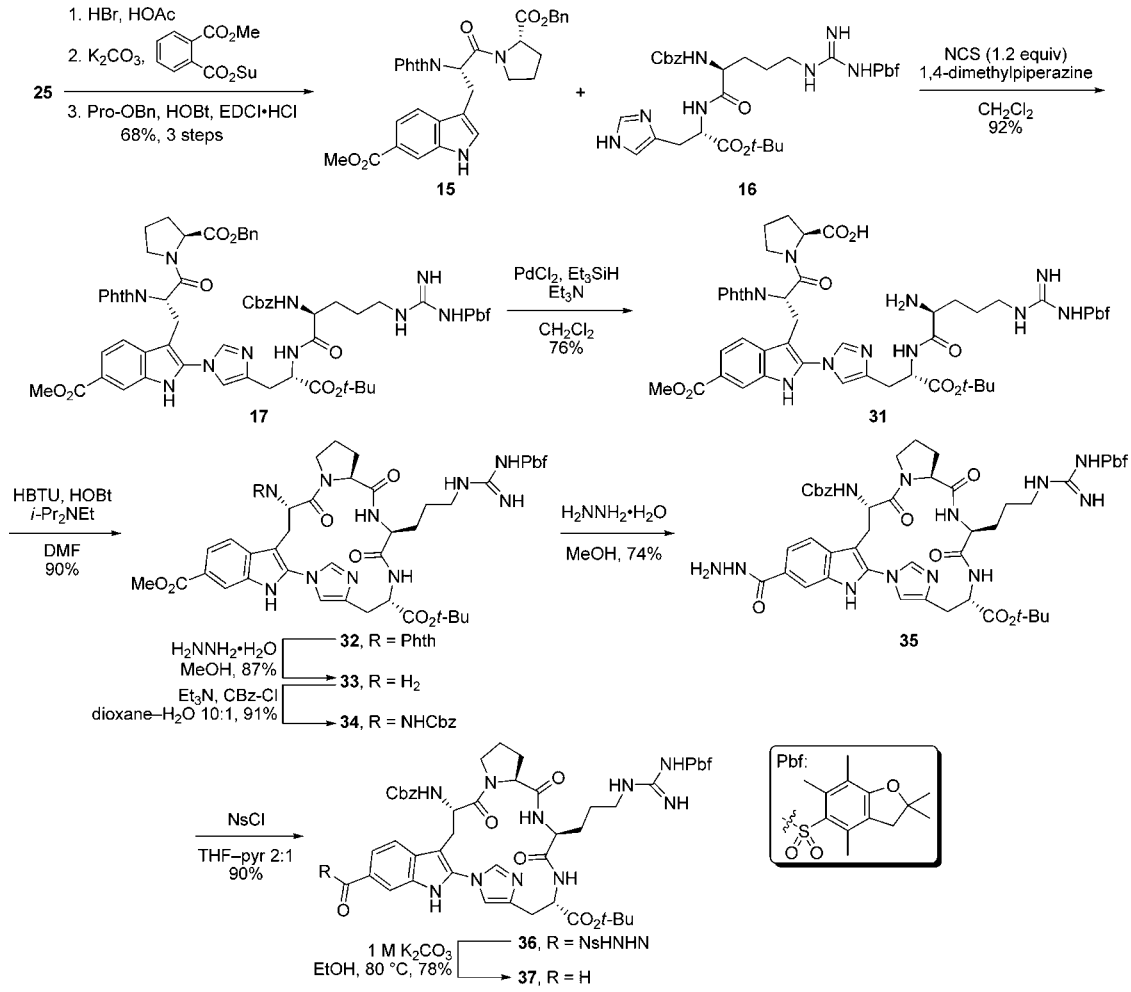
Scheme 5. Synthesis of Right-Hand Ring Aldehyde **37**



consisting of simultaneous *N*-Cbz, *tert*-butyl ester, and triethylsilyl deprotection (HBr in HOAc), phthalimide installation (methyl 2-((succinimidooxy)carbonyl)benzoate),[30] and peptide coupling (Pro−OBn, HOBt, EDCI·HCl). Oxidative coupling[31] of **15** with Arg−His dipeptide **16**[13] then proceeded readily, affording indole−imidazole-linked tetrapeptide **17** in 92% yield. This represents a significant improvement over the analogous oxidative coupling used to construct the model right-hand ring (58% yield, 25−30% recovery of each SM).[13] In the present case, optimization of the reaction conditions established that a slight excess (1.2 equiv) of NCS allowed for complete consumption of the reactants without degrading the product. Our prior studies established the clear superiority of NCS over NBS, NIS, and *t*-BuOCl,[13] so alternative oxidants were not examined.

Surprisingly, attempts to cleave the *N*-Cbz and benzyl ester moieties from **17** enlisting transfer hydrogenation conditions (10% Pd/C, HCO_2NH_4) used with the model system[13] failed to remove the carbamate group. Switching the H_2 source to 1,4-cyclohexadiene yielded the same result, as did employing H_2 at both atmospheric and elevated (70 atm) pressures. Fortunately, $PdCl_2$ in conjunction with triethylsilane[32] effected simultaneous *N*-Cbz and benzyl ester deprotection, delivering macrolactamization substrate **31** in 76% yield. Cyclization of **31** to afford **32**, the C-6 carbomethoxy-substituted celogentin C right-hand ring, then proceeded smoothly in the presence of HBTU and HOBt. In preparation for acyl hydrazine installation, the phthalimide moiety, which prevented the tryptophan *N*-terminus from engaging the chloroindolenine intermediate of the oxidative coupling reaction, was exchanged for a Cbz group. Transformation of the resulting carbamate **34** into aldehyde **37** was accomplished via the McFadyen−Stevens-based strategy; however, some modifications were necessary. Specifically, the hydrazinolysis step required a 1:1 mixture of hydrazine hydrate and MeOH instead of the 1:2 mixture used previously with ester **25**. Moreover, the reaction mixture was stirred at room temperature and concentrated under high vacuum at 0 °C to prevent diacyl hydrazine formation. Nosylation of the hydrazine was best accomplished in a 2:1 THF−pyridine solvent mixture, and the Braslau-modified McFadyen−Stevens reaction of nosylate **36** provided aldehyde **37** in good (78%) yield.

With the right-hand ring aldehyde in hand, we now required a suitable nitroacetamide derivative for the key intermolecular Knoevenagel condensation (see **19** → **20**, Scheme 2). Coupling

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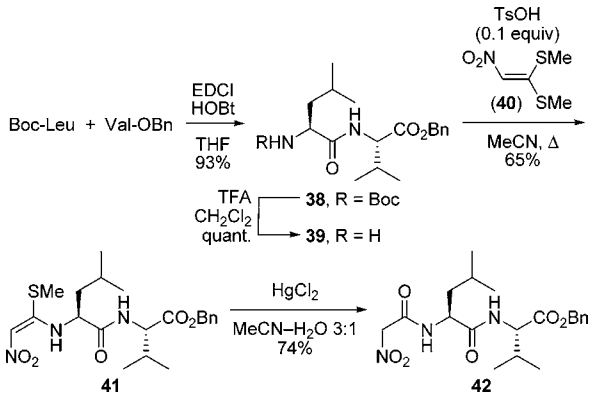
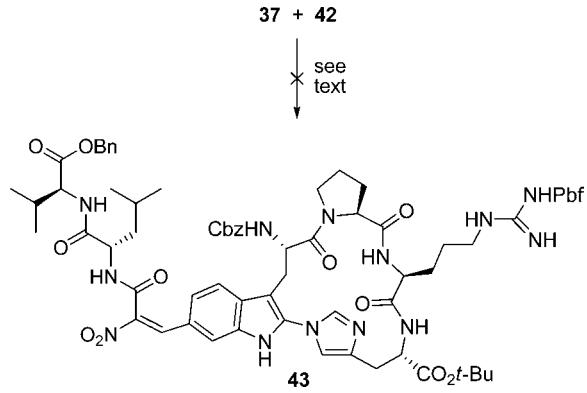
Scheme 6. Synthesis of Nitroacetamide **42**



Scheme 7. Failed Knoevenagel Condensation



of Boc−Leu and Val−OBn followed by Boc deprotection afforded the dipeptide Leu−Val−OBn (**39**, Scheme 6). Exposure of **39** to Rajappa's commercially available dithioketene acetal **40**[33] in the presence of TsOH then delivered vinyl sulfide **41** as a single isomer of undetermined alkene stereochemistry. Treatment of **41** with $HgCl_2$ in aqueous MeCN served to hydrolyze the vinyl sulfide, revealing nitroacetamide **42**.

Our first attempt at performing the Knoevenagel condensation of aldehyde **37** and nitroacetamide **42** enlisted the combination of $TiCl_4$ and *N*-methylmorpholine (NMM) in THF,[34] a system that was effective in our synthesis of model radical conjugate addition substrates.[18] Unfortunately, aldehyde **37** was consumed, but coupling product **43** was not detected (Scheme 7). Numerous other conditions were screened (4 Å MS, $CHCl_3$, room temperature to reflux;[35] PPh_3, THF, reflux;[36] piperidine, HOAc, 4 Å MS, dioxane, reflux;[37] H_2O, room temperature to reflux;[38] KF−alumina, EtOH, room temperature to reflux[39]), but no desired product was formed. This failure to link building blocks **37** and **42** necessitated a redesign of our synthetic strategy.

We hypothesized that the wealth of functional groups present in aldehyde **37**, including numerous Lewis basic sites, might be interfering with the Knoevenagel condensation. Accordingly, we resolved to perform this reaction at an earlier stage of the synthesis. This decision spawned a left-to-right synthetic plan, which is summarized in Scheme 8. We reasoned that coupling of nitroacetamide **42** with the relatively simple C-6 formyl tryptophan **26** would proceed to afford α,β-unsaturated α-nitroamide **44**. A chiral Lewis acid-promoted radical conjugate addition would then provide acyclic peptide **45** after nitro reduction and coupling to pyroglutamic acid. Macrolactamization followed by attachment of the proline residue would generate cyclic peptide **46**, substrate for the indole−imidazole oxidative coupling reaction with dipeptide **16**. This transformation was valuable in the right-hand ring synthesis (see Scheme 5);[13] however, the steric hindrance and conformational constraints associated with imbedding the indole moiety in a macrocycle caused us to view this particular case with some trepidation. Nonetheless, if we were able to prepare octapeptide **47**, our earlier results (cf., Scheme 5) gave us confidence that macrolactamization between the Pro and Arg residues and subsequent deprotection would deliver **1** in good yield.

We had prepared C-6 formyl tryptophan **26** in three steps from the corresponding methyl ester **25** (Scheme 4), but we sought a more direct route to this compound. As shown in Scheme 9, it could be obtained in two steps from C-6 hydroxymethyl-substituted tryptophan **48**, a species whose synthesis was reported previously[12] by a route analogous to that shown for tryptophan **25** in Scheme 3. Removal of the TBS group from **48** was most easily accomplished by employing an AcOH:THF:H_2O (3:2:2) mixture.[40] DDQ-mediated oxidation of the primary benzylic alcohol[41] proceeded smoothly, providing the desired C-6 formyl tryptophan **26** in 89% yield from **48**.

The development of reliable conditions for the union of **26** and nitroacetyl-capped Leu−Val dipeptide **42** is summarized in Table 2. No reaction between the coupling partners was observed in the presence of $MgBr_2 \cdot OEt_2$[42] (entry 1). $TiCl_4$ and NMM in THF[18,34] provided the desired adduct **44**, but yields were low and inconsistent (entry 2). Addition of drying agents to the reaction mixture failed to improve the results (entry 3). When the condensation was conducted in Et_2O, a low yield of the product was obtained (27%), and substantial quantities of starting material were recovered due to poor solubility (entry 4). Although the yield was modest, the reaction was cleaner than those performed in THF. On the basis of this observation, we probed the efficacy of mixed solvent systems. Both 1:1 and 2:1 mixtures of THF and Et_2O afforded **44** in good yield (entries 5 and 6), with the latter system giving a slightly better result. Notably, **44** was produced as a single detectable alkene isomer. The alkene stereochemistry of **44** is assigned on the basis of the X-ray crystal structure of a simpler model compound that was produced under similar conditions.[43]

At this point, we examined the key radical conjugate addition. Our prior studies with simple substrates established the necessity of reducing the nitro group immediately after workup to prevent epimerization of the sensitive α-stereocenter.[18,43] To properly evaluate the stereoselectivity of radical conjugate additions to **44**, we required a nitro reduction protocol that was suitable for

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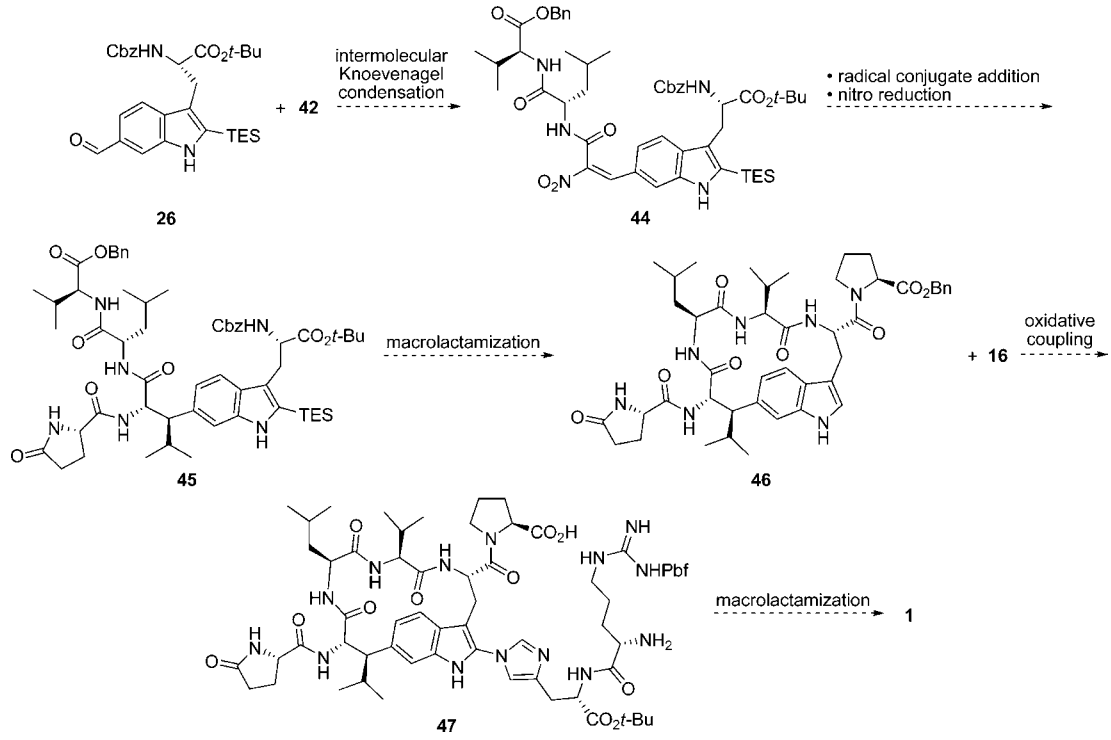
Scheme 8. Left-to-Right Synthetic Strategy



Scheme 9. Streamlined Preparation of C-6 Formyl Tryptophan **26**

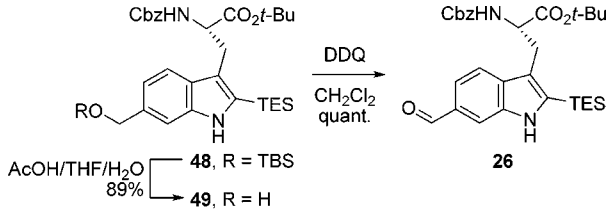
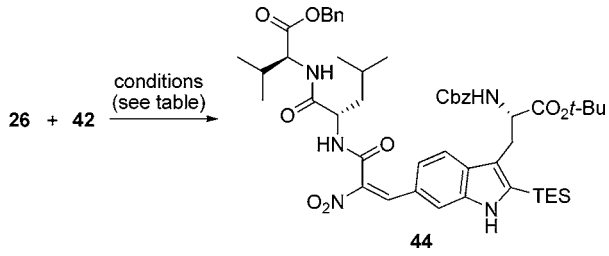


Table 2. Knoevenagel Condensation of **26** and **42**

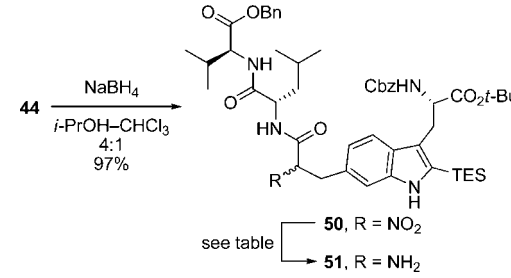


entry	conditions	yield (%)
1	$MgBr_2 \cdot OEt_2$, Et_3N, THF, or CH_2Cl_2	nr[a]
2	$TiCl_4$, NMM, THF	15−50
3	$TiCl_4$, NMM, 4 Å MS or $MgSO_4$, THF	15
4	$TiCl_4$, NMM, Et_2O	27[b]
5	$TiCl_4$, NMM, THF−Et_2O 1:1	65
6	$TiCl_4$, NMM, THF−Et_2O 2:1	68

[a] No reaction was observed. [b] Starting material was recovered (67% of **26**).

a complex substrate. Thus, we reduced the electron-deficient alkene of **44** with $NaBH_4$ and used the resulting alkyl nitro compound **50** to develop this reaction (Table 3). We began by testing In/HCl,[44] a reagent system that had proven valuable in the earlier model studies.[43] Unfortunately, both In/HCl and In/AcOH provided amine **51** in low yield (entry 1), and numerous byproducts were produced. The use of weaker acids

Table 3. Optimization of Nitro Reduction



entry	nitro reduction conditions	yield (%)
1	In, 6 N HCl or AcOH, THF−H_2O	18
2	In, HCO_2NH_4, MeOH	<10[a]
3	In, NH_4Cl, THF−H_2O	<10[a]
4	Zn, HCO_2NH_4, MeOH	23[a]
5	Zn, HCO_2NH_4, MeOH−CH_2Cl_2	13[a]
6	Zn, HCO_2NH_4, THF−H_2O	10[a]
7	Raney Ni, H_2 (1 atm)	nd[b]
8	$NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH	<10[a]
9	SmI_2, THF−MeOH 6:1	90

[a] The corresponding oxime was also obtained. [b] No product was detected.

(HCO_2NH_4[45] or NH_4Cl) afforded only trace quantities of **51**, and the corresponding oxime was obtained instead (entries 2 and 3). This observation concerned us, because generation of an oxime intermediate in the nitro reduction would render futile any attempts at controlling the α-stereochemistry. Substitution of Zn for In led to marginally higher yields of **51**, but oxime formation was still problematic (entries 4−6). Neither Raney

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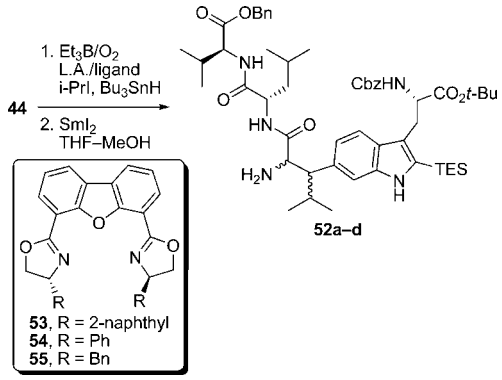
Table 4. Radical Conjugate Addition to **44**



entry	Lewis acid/ligand	equiv	a:b:c:d[a]
1	$Mg(NTf_2)_2$/**53**	2.0	1:2:1:1
2	$Mg(NTf_2)_2$/**54**	2.0	1:2:2:1
3	$Mg(NTf_2)_2$/**54**	5.0	2:2:2:1
4	$Mg(NTf_2)_2$/**55**	2.0	3:2:2:1
5	$Zn(OTf)_2$/none	2.0	1:3:2:1[b]
6	none/none		3:4:4:1

[a] Calculated from ^{1}H NMR spectra of the mixtures of **52a**−**d**. [b] When run on a preparative scale (0.4 mmol), 90% of a 1.0:2.9:2.0:1.2 mixture of **52a**−**d** was obtained.

Ni−H_2[46] nor $NaBH_4$−$NiCl_2\cdot 6H_2O$[47] yielded promising results (entries 7 and 8). At last, we discovered that employing SmI_2 in THF−MeOH[48] resulted in a facile, high-yielding reduction (entry 9). Amine **51** was isolated as a mixture of diastereomers due to the lack of selectivity in the 1,4-reduction of **44**.

With the availability of a high-yielding nitro reduction protocol, we investigated the radical conjugate addition to **44**. Previous studies conducted with β-aryl-substituted α,β-unsaturated α-nitroamides revealed that the chiral Lewis acid generated by complexation of $Mg(NTf_2)_2$ and DBFOX/Ph[49] (**54**, Table 4) produced the adducts with high ee's but low dr's.[43] Subsequently, we synthesized the second-generation ligands DBFOX/Nap (**53**) and DBFOX/Bn (**55**), which promoted the radical conjugate addition with slightly higher dr's.[50] On the basis of these findings, we tested ligands **53**−**55** in the conjugate addition of isopropyl radical to **44**, and the results are collected in Table 4. Unfortunately, each reaction was characterized by low levels of diastereoselectivity, and all four possible products **52a**−**d** (arranged in order of increasing polarity) were produced. Because of the multitude of Lewis basic sites in radical acceptor **44**, we conducted most of the reactions with 2 equiv of Lewis acid to ensure that sufficient complexation to the α-nitroamide moiety would occur. An increase in the amount of chiral Lewis acid to 5 equiv had a small impact on the product ratio, but did not yield a synthetically useful result (entry 3). In fact, the best selectivity for a single product, albeit modest, was achieved by employing substrate-directed stereocontrol with the achiral Lewis acid $Zn(OTf)_2$. In this case, a 1:3:2:1 ratio of products was obtained (entry 5). Interestingly, the reaction with no Lewis acid present afforded roughly equimolar amounts of **52a**−**c**, with **52d** as a minor product (entry 6).

We were disappointed by the performance of the chiral Lewis acids in the radical conjugate addition, yet intrigued by the result obtained with $Zn(OTf)_2$, because a major product (**52b**) was produced. Repeating this experiment on a preparative scale returned a very similar product ratio (1.0:2.9:2.0:1.2) in an excellent 90% yield. Accordingly, the radical conjugate addition and nitro reduction were each proceeding in ≥90% yield, and **52b** could be obtained in 36% yield from **44**. Thus, this process would provide a viable means of synthesizing **1** as long as the configuration of **52b** matched that of the natural product. Consequently, we resolved to determine the stereochemistry of this intermediate.

In 2006, Moody and co-workers reported the total synthesis of stephanotic acid methyl ester[10a] (**60**, Scheme 10), a derivative of the natural product stephanotic acid.[11] Importantly, **60** and the left-hand ring of **1** are extremely similar in structure; the only difference between the two species is the presence of an isoleucine residue in **60** at the site of the leucine residue in **1**. Because the configurations of **60** and the left-hand ring of **1** are identical, we hoped that a comparison of NMR data would reveal whether or not the main product of the radical conjugate addition possessed the requisite stereochemistry for elaboration into **1**. Accordingly, we synthesized the left-hand ring macrocycle from isomer **52b** as detailed in Scheme 10. The mixture of compounds **52a**−**d** was partially separable; the two minor isomers **52a** (least polar) and **52d** (most polar) could be removed via chromatography. As a result, the first two steps were performed on a 1.5:1 mixture of the two most abundant isomers from the radical conjugate addition. Coupling of amines **52b**,**c** with pyroglutamic acid provided pentapeptides **45b**,**c** in excellent yield (96%), but the mixture still could not be separated. However, after tandem Cbz/benzyl ester cleavage, the resulting peptides **56b** and **56c** were separable on SiO_2. Notably, isomerically pure **56b** was isolated in 31% overall yield from Knoevenagel condensation product **44**, as the excellent yields of the four intervening steps (radical conjugate addition, nitro reduction, peptide coupling, hydrogenolysis) compensated for the low diastereoselectivity of the radical conjugate addition.

In their total synthesis of **60**, Moody and co-workers conducted a macrolactamization at the site corresponding to the Leu−Val peptide bond in the left-hand ring of **1**. This reaction was plagued by epimerization;[10a] as a result, we decided to close our macrocycle at a different site. We were relieved to discover that cyclization of **56b** via Val−Trp amide bond formation was high-yielding and devoid of epimerization. Removal of the *tert*-butyl ester and triethylsilyl groups of macrocycle **57b** was then achieved by enlisting *B*-bromocatecholborane (BCB).[51] Finally, treatment of the resulting acid **58b** with $SOCl_2$ in MeOH delivered ester **59b**. Gratifyingly, the ^{1}H and ^{13}C NMR spectra of **59b** matched quite well with the published spectra of **60**.[52] This encouraging development gave us confidence that isomer **52b**, the major product of the radical conjugate addition, was of the proper configuration for conversion into **1**.

We also transformed compounds **52a**, **52c**, and **52d**, the minor isomers from the radical conjugate addition, into the corresponding cyclic peptides. Isomers **52a** and **52d** could be isolated

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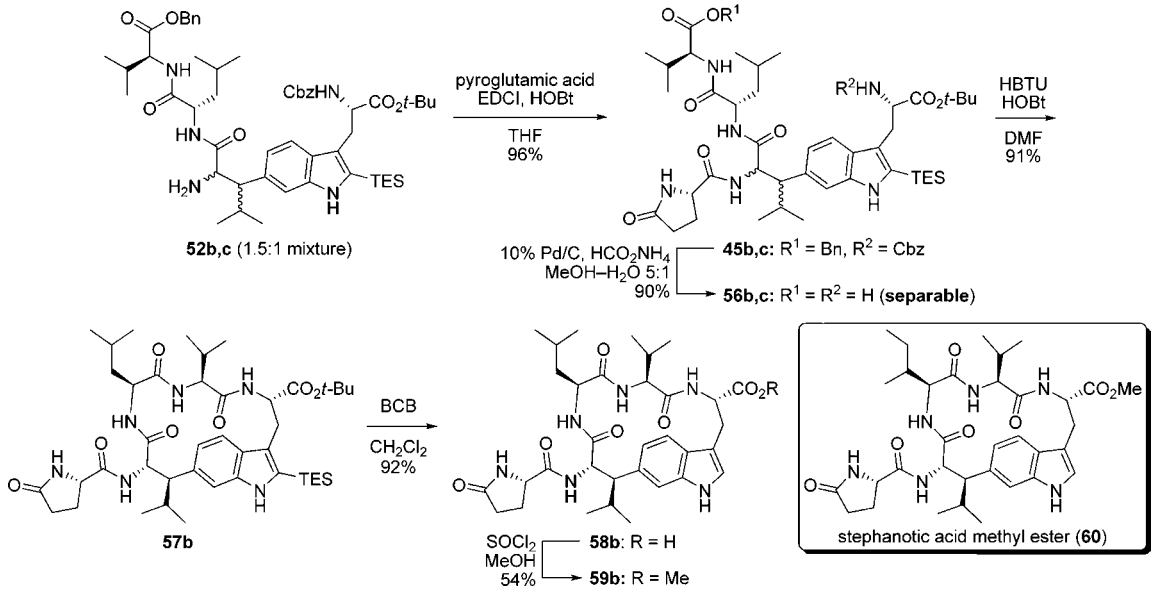
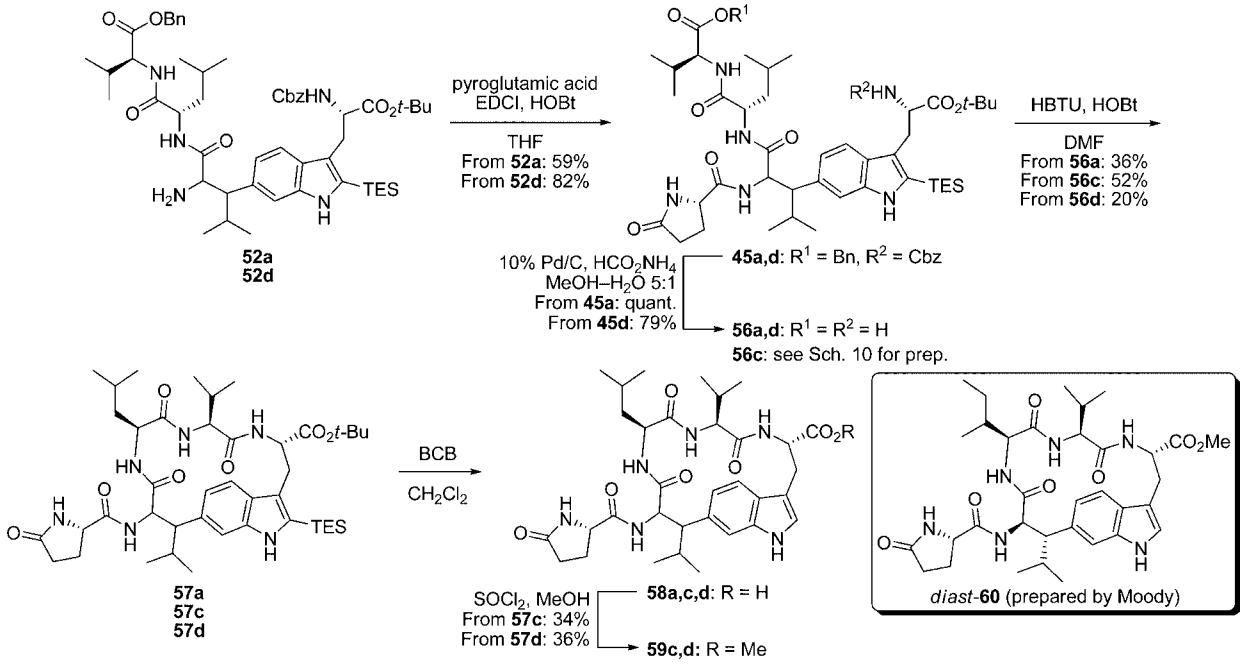
(49) (a) Kanemasa, S.; Oderaotoshi, Y.; Yamamoto, H.; Tanaka, J.; Wada, E.; Curran, D. P. *J. Org. Chem.* **1997**, *62*, 6454. (b) Iserloh, U.; Curran, D. P.; Kanemasa, S. *Tetrahedron: Asymmetry* **1999**, *10*, 2417. (c) Iserloh, U.; Oderaotoshi, Y.; Kanemasa, S.; Curran, D. P. *Org. Synth.* **2003**, *80*, 46.
(50) Banerjee, B.; Capps, S. G.; Kang, J.; Robinson, J. W.; Castle, S. L. *J. Org. Chem.* **2008**, *73*, 8973.
(51) Boeckman, R. K., Jr.; Potenza, J. C. *Tetrahedron Lett.* **1985**, *26*, 1411.
(52) See the Supporting Information for details.

Scheme 10. Synthesis of Macrocycle **59b** and Comparison to Stephanotic Acid Methyl Ester



Scheme 11. Synthesis of Other Diastereomeric Macrocycles

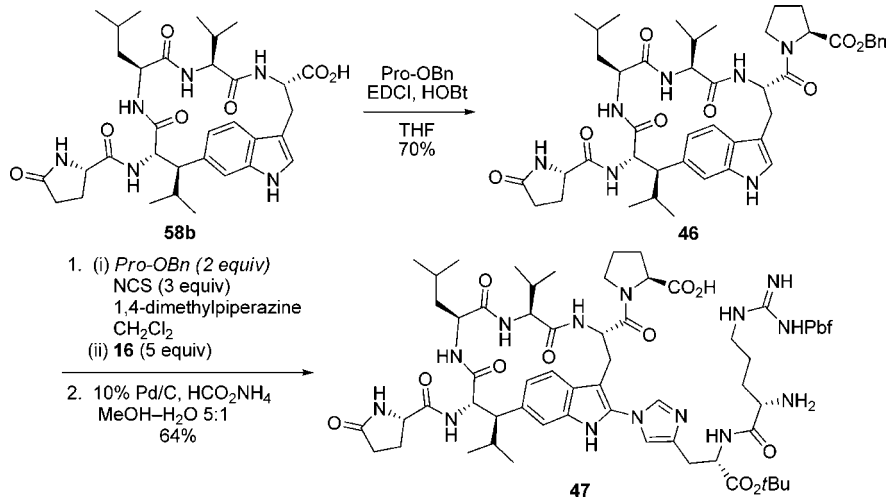


in pure form, so these compounds were individually processed into peptides **56a** and **56d** with yields similar to those obtained previously (Scheme 11; cf., Scheme 10). In contrast to the smooth cyclization of **56b**, macrolactamizations of **56a**, **56c** (obtained previously, see Scheme 10), and **56d** under identical conditions were low-yielding (20−52%). As a result of the poor macrolactamization yields, we are unable to rule out the possibility that epimerization of the activated carboxylate group may have produced epimers of **57a**, **57c**, and **57d** at the Val α-carbon. However, comparisons of spectral data to those published by Moody[10a] (vide infra) suggest that **57d** contains an L-Val rather than a D-Val residue and is not derived from epimerization during the peptide coupling.

As with **57b**, treatment of **57a**, **57c**, and **57d** with BCB afforded carboxylic acids **58a**, **58c**, and **58d**. However, the scarcity of the least abundant isomer **58a** combined with the low (unoptimized) yield of the esterification prevented us from isolating methyl ester **59a**. Fortunately, we did obtain sufficient quantities of **59c** and **59d** to compare their ^{1}H NMR spectra to those of **59b**, **60**, and *diast*-**60**, a diastereomer of **60** constructed by Moody and co-workers.[10a] These comparisons were informative, as the spectrum of **59d** matched closely with the spectrum of *diast*-**60**.[52] Thus, we believe that our "**d**" series of compounds possesses the same (2*R*,3*S*)-*β*-substituted leucine residue as Moody's *diast*-**60**. Moreover, the spectrum of **59c** did not match that of either **60** or *diast*-**60**.[52] The distinct nature of the ^{1}H NMR spectra of macrocycles **59b**−**d** and the clear similarities between **59d** and *diast*-**60** strengthened our belief that the most abundant "**b**" series of compounds was of configuration identical to that of **1**.

The stage was now set for annulation of the right-hand ring of celogentin C onto the left-hand ring. Coupling of acid **58b**

Scheme 12. Construction of Octapeptide **47** via Oxidative Coupling

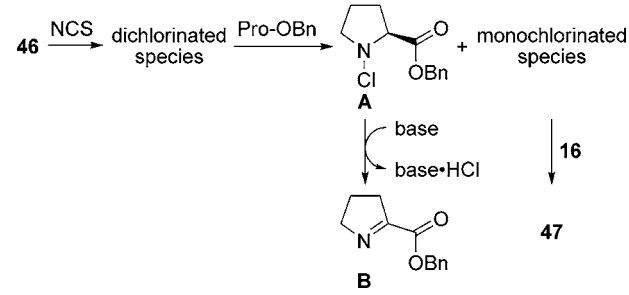


to Pro−OBn provided **46**, substrate for the crucial oxidative coupling reaction (Scheme 12). Disappointingly, hexapeptide **46** did not react with Arg−His dipeptide **16** under the conditions optimized for the coupling of Trp−Pro dipeptide **15** with **16** (see Scheme 5). Monitoring of the attempted coupling by mass spectrometry revealed that **46** was reacting with NCS to form a dichlorinated species instead of the required chloroindolenine, which incorporates only one chlorine atom. Additionally, comparison of a ^{1}H NMR spectrum of the crude reaction mixture to one of **46** showed clear differences in the signals emanating from the proline residue. On the basis of these data, we hypothesized that the chloroindolenine intermediate was undergoing a second chlorination, perhaps on the Trp−Pro tertiary amide. The dichlorinated species was unreactive, as increasing the temperature, time, or concentration of the reaction did not promote coupling with **16**.

Remarkably, a serendipitous discovery provided the solution to this problem. When a sample of **46** that was contaminated with unreacted Pro−OBn from the prior peptide coupling was subjected to the oxidative coupling conditions, the desired octapeptide was produced. Further experimentation confirmed the beneficial effect of Pro−OBn on the reaction. The optimized protocol involved stirring a solution of **46**, Pro−OBn (2 equiv), NCS (3 equiv), and 1,4-dimethylpiperazine in CH_2Cl_2 at ambient temperature for 6 h, then adding dipeptide **16** (5 equiv), and stirring for an additional 24 h. An excess of the imidazole component was required to ensure a reasonable reaction rate, but the presence of unreacted **16** complicated the product isolation. We found it most convenient to subject the crude oxidative coupling product to transfer hydrogenation, which cleaved both the Cbz and the benzyl ester protecting groups. The deprotected octapeptide **47** could then be isolated in 64% yield.

This intriguing result prompted us to investigate the role of Pro−OBn in the oxidative coupling. Mass spectrometric analysis of reactions conducted with added Pro−OBn indicated the presence of four noteworthy species: a monochlorinated adduct of **46** (presumably the desired chloroindolenine), a dichlorinated adduct of **46** (presumably the unreactive byproduct), a monochlorinated adduct of Pro−OBn, and the product of HCl elimination from this adduct. On the basis of this information, we formulated the preliminary mechanistic hypothesis summarized in Scheme 13. As in the reactions conducted without added Pro−OBn, hexapeptide **46** may be chlorinated by NCS at two different

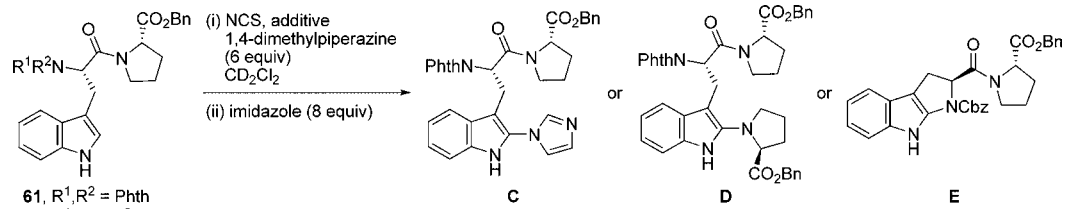
Scheme 13. Preliminary Mechanistic Rationale for Oxidative Coupling



sites (probably the indole C-3 atom and the Trp−Pro amide) with approximately equal rates. However, in the presence of the additive, the dichlorinated species could transfer the undesired chlorine atom to the nitrogen atom of Pro−OBn, generating the requisite monochlorinated species (i.e., chloroindolenine) along with *N*-chloroamine **A**. The chloroindolenine could then react with dipeptide **16** to form the coupled product, while elimination of HCl from **A** promoted by either 1,4-dimethylpiperazine, excess **16**, or Pro−OBn would produce imine **B**. Consequently, the unwanted chlorine atom of the dichlorinated intermediate would be sequestered as a hydrochloride salt of the base. In this scenario, both the nucleophilic nitrogen atom and the acidic α-hydrogen atom of Pro−OBn are essential structural features that enable its function as a scavenger of electrophilic chlorine.

The precious nature of hexapeptide **46** limited our ability to perform detailed mechanistic investigations with this compound. *N*-Phthaloyl Trp−Pro dipeptide **61**, which was originally used to develop the oxidative coupling conditions,[13] was readily available in our laboratory. In contrast to **46**, **61** underwent clean oxidative couplings with imidazole nucleophiles in the presence of 1 equiv of NCS and in the absence of Pro−OBn.[13] In these reactions, we did not observe dichlorinated intermediates. Apparently, the rate of the desired chlorination of **61** was quite rapid, so the undesired chlorination was not competitive. Thus, we had concerns regarding the suitability of **61** for probing the role of Pro−OBn in the oxidative coupling. Fortunately, dichlorination of **61** could be accomplished by employing excess NCS. We then embarked upon the mechanistic investigation summarized in Table 5 with the viability of **61** as a test substrate established.

Table 5. Investigation of Oxidative Coupling with Dipeptides **61** and **62**



61, R^1,R^2 = Phth
62, R^1 = H, R^2 = Cbz

C **D** **E**

entry	dipeptide	equiv NCS	additive (equiv)	product[a]
1	**61**	3	Pro−OBn (2)	**C**
2	**61**	2	Pro−OBn (2)	rec. SM (major), **C** (minor)
3	**61**	3	Pro-OBn (2)[b]	**D** + 2Cl (major), **C** + Cl (minor)
4	**62**	3	Pro−OBn (2)	**E** (major), **D** (minor)
5	**61**	3	Pro−OMe (2)	**C**
6	**61**	3	pyrrolidine (2)	**C** (major), **C** + Cl (minor)
7	**61**	3	none	**C** (47%)
8	**61**	3	pyrrolidine (2)	**C** (41%)
9	**61**	3	Pro−OBn (2)	**C** (68%)
10	**61**	3	Pro−OMe (2)	**C** (79%)

[a] Values in parentheses refer to isolated yields of product. [b] Added 3 h after addition of NCS and 1,4-dimethylpiperazine.

Oxidative couplings of **61** and its Cbz-protected congener **62** were conducted with excess imidazole (8 equiv) in CD_2Cl_2. The deuterated solvent was chosen to enable reaction monitoring by ^{1}H NMR. However, we found that more instructive data could be obtained by mass spectrometry (ESI-MS). Nonetheless, for consistency, CD_2Cl_2 was employed in all of the reactions listed in Table 5. Use of the conditions developed for oxidative coupling of **46** with **16** resulted in successful coupling of **61** and imidazole, providing adduct **C** (entry 1). Reducing the amount of NCS to 2 equiv gave predominantly starting material, demonstrating that an excess of NCS relative to Pro−OBn is required for oxidative coupling to occur (entry 2).

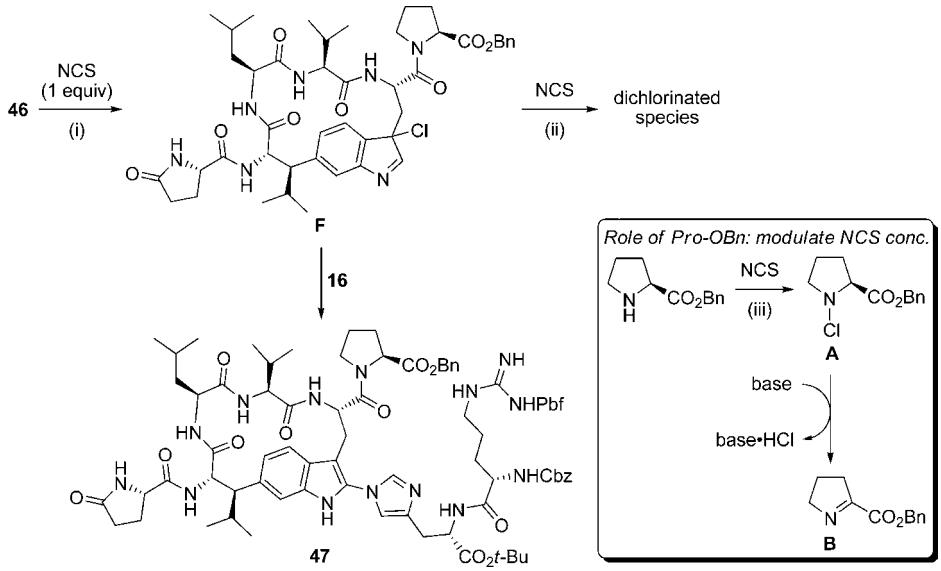
Our first indication that the hypothesis outlined in Scheme 13 was incorrect came when we waited 3 h after the start of the reaction to add Pro−OBn (entry 3). If the additive was indeed scavenging chlorine from the dichlorinated species, then its inclusion at the beginning of the reaction should not be essential. However, after addition of imidazole, **C** was not observed. A chlorinated version of **C** was detected by ESI-MS, but the major component of the reaction mixture was a dichlorinated adduct of **D**, in which Pro−OBn was functioning as a nucleophile rather than a chlorine scavenger. This instructive experiment revealed several important facts about the oxidative coupling reaction. First, in contrast to the situation with macrocyclic hexapeptide **46**, overchlorinated versions of dipeptide **61** are viable electrophiles. Second, Pro−OBn must be added to the mixture at the outset to exert a beneficial effect on the reaction; contrary to our earlier hypothesis, this additive is unable to remove chlorine atoms from dichlorinated species. Third, if Pro−OBn is added to the mixture after the NCS has been consumed by **61**, then it simply adds to the chloroindolenine intermediate, forming **D**.

In our early studies of the oxidative coupling, we employed the phthalimide-protected substrate **61** to prevent the Trp amine from cyclizing onto the chloroindolenine. Although the chloroindolenine derived from **46** was capable of cyclization via nucleophilic attack of the Trp−Val amide nitrogen, such a process was not observed. Presumably, the conformational constraints of the macrocycle of **46** prevent this undesired event from occurring. To determine if acyclic substrates possessing a monoprotected Trp amine could couple with imidazole, we subjected *N*-Cbz congener **62** to the standard conditions (entry 4). Tricyclic product **E** was obtained, along with a minor amount of Pro−OBn adduct **D**. Thus, double protection of the Trp amine is required for successful indole−imidazole oxidative couplings with acyclic substrates.

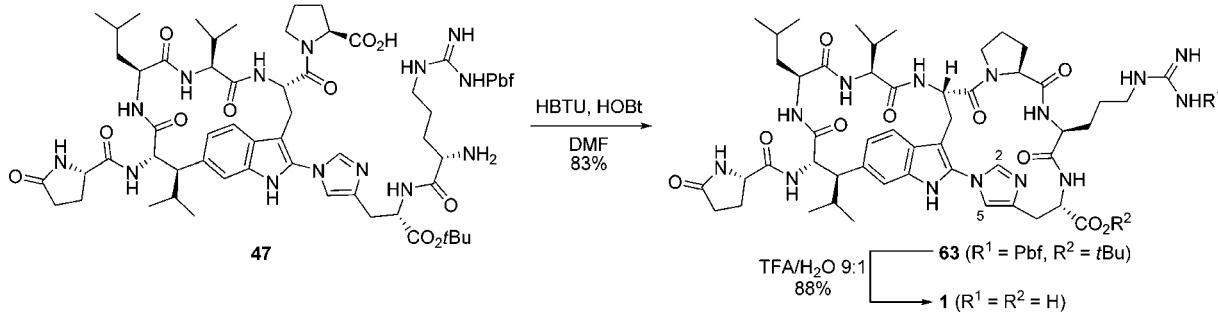
In an effort to determine the structural requirements of the additive, the reaction was conducted with Pro−OMe (entry 5) and pyrrolidine (entry 6) in place of Pro−OBn. In the former case, imidazole adduct **C** was produced, demonstrating that the benzyl ester of Pro−OBn is not essential. Interestingly, **C** was observed along with its chlorinated adduct in the latter reaction, suggesting that pyrrolidine may be a viable, albeit less effective, additive in the oxidative coupling. These results prompted us to obtain a quantitative measure of the effectiveness of various additives. Accordingly, the preparative-scale reactions listed in entries 7−10 were conducted. Surprisingly, adduct **C** was isolated in 47% yield in the absence of an additive (entry 7). Thus, with the simple substrate **61**, an additive is not required for production of the desired oxidative coupling product even when excess NCS is employed. Compound **C** was actually obtained in a slightly lower yield (41%) in the presence of pyrrolidine (entry 8), indicating that a cyclic secondary amine alone does not confer a beneficial effect on the reaction. In contrast, reactions conducted with Pro−OBn (entry 9) and Pro−OMe (entry 10) delivered **C** in 68% and 79% yield, respectively. Therefore, both Pro−OBn and Pro−OMe are useful additives in the oxidative coupling, with Pro−OMe being somewhat more effective.

The data in Table 5, especially the results shown in entry 3, prompted us to propose a new mechanism for the role of Pro−OBn in the oxidative coupling of **46** and **16**. A hypothesis that is consistent with our observations is presented in Scheme 14. It is likely that chloroindolenine **F** is produced by the reaction of hexapeptide **46** with 1 equiv of NCS (step i). This intermediate can react further with a second equiv of NCS, forming a dichlorinated species (step ii). Although we do not possess definitive data regarding the structure of the dichlorinated species, ^{1}H NMR spectra of crude reaction mixtures suggest that the proline residue is involved in the second chlorination. In the absence of Pro−OBn, the dichlorinated species predominates, and upon addition of dipeptide **16**, the desired product **47** is not generated. When Pro−OBn is present at the outset of the process, it can react with NCS directly to form *N*-chloroamine **A** (step iii). As in our prior proposal, elimination of HCl from **A** to give imine **B** sequesters an

Scheme 14. Revised Mechanistic Rationale for Oxidative Coupling



Scheme 15. Completion of Total Synthesis of **1**



electrophilic chlorine atom as an HCl salt. Apparently, the rates of steps i, ii, and iii are balanced such that chloroindolenine **F** predominates over the dichlorinated species when 3 equiv of NCS and 2 equiv of Pro−OBn are employed. Finally, coupling product **47** evolves from **F** upon addition of dipeptide **16**. Thus, the role of Pro−OBn is to modulate the concentration of NCS such that step ii is minimized and the amount of **F** is maximized.

To obtain further evidence in favor of this hypothesis, a solution of Pro−OBn in $CDCl_3$ was treated with NCS. This resulted in consumption of the starting material and formation of *N*-chloroamine **A** according to ESI-MS and ^{1}H NMR analysis. Next, addition of 1,4-dimethylpiperazine to the solution converted **A** into imine **B**. In addition to supporting our mechanistic proposal, this experiment highlights the critical roles of both the nucleophilic amine and the acidic α-hydrogen of Pro−OBn. Indeed, it is remarkable that such an exquisitely effective additive was discovered in serendipitous fashion.

At this stage, only two steps (macrolactamization and deprotection) were required to complete the total synthesis of celogentin C. In earlier model studies, formation of the isolated right-hand ring proved quite facile in the presence of HOBt and HBTU.[13] We were relieved to find that these conditions induced smooth cyclization of **47**, affording bicyclic octapeptide **63** in 83% yield (Scheme 15). Cleavage of both the Pbf and the *tert*-butyl ester protecting groups was then accomplished by the action of TFA, providing **1** in 88% yield. Importantly, and in harmony with prior observations regarding Pbf deprotections,[53] no alkylation of the Trp indole was observed. Moreover, chromatographic purification of **1** was unnecessary as long as its precursor **63** was rigorously purified.

Virtually all of the signals in the ^{1}H NMR spectrum of synthetic **1** precisely matched the published data for celogentin C.[1] However, the chemical shift of the imidazole H2 atom differed significantly, and a smaller deviation was exhibited by the imidazole H5 atom. We first considered the possibility that we had synthesized an unnatural atropisomer of celogentin C. However, diagnostic NOE correlations (indole NH/imidazole H2 and Trp β-H/imidazole H5) indicated that our synthetic compound was identical to the natural product in its orientation about the heterobiaryl axis.[54] Upon further investigation, we discovered that the chemical shifts of imidazole H2 and H5 were dependent on both temperature and concentration. The chemical shift of H2 decreased from 9.22 to 8.92 ppm as the temperature of a DMSO-d_6 solution of **1** was increased from 16 to 44 °C (Table 6). A less dramatic decrease was observed for H5. Interestingly, dilution of the solution by a factor of 20 caused a dramatic decrease in chemical shift (9.16 to 8.04 ppm for

(53) Fields, C. G.; Fields, G. B. *Tetrahedron Lett.* **1993**, *34*, 6661.
(54) Regarding the NOE correlations observed with natural celogentin C, the text of ref 1 is in error. The correct data can be located in Table 3 and on the NOESY spectrum given in the Supporting Information. This discrepancy caused us previously to erroneously conclude that our model right-hand ring of celogentin C was the unnatural atropisomer (see ref 13).

Table 6. Temperature Dependence of Chemical Shifts of Imidazole Hydrogens in **1**

temp (°C)	H2 (ppm)	H5 (ppm)
nat[a]	**9.41**	**7.79**
16	9.22	7.73
18	9.20	7.73
20	9.18	7.72
22	9.16	7.72
27	9.11	7.71
37	9.03	7.69
44	8.92	7.66

[a] Data from natural sample of **1** (ref 1).

Table 7. Concentration Dependence of Chemical Shifts of Imidazole Hydrogens in **1**

factor[a]	H2 (ppm)	H5 (ppm)
nat[b]	**9.41**	**7.79**
1.0	9.16	7.72
0.50	9.05	7.68
0.33	9.04	7.68
0.050	8.04	7.40

[a] Concentration of original solution (ca. 6 mg of **1** in 0.5 mL of DMSO-d_6) was set to 1.0. Subsequent dilutions are reported as a fraction of the original solution. [b] Data from natural sample of **1** (ref 1).

H2, 7.72 to 7.40 ppm for H5), as shown in Table 7. It is likely that the imidazole N3 atom of **1** participates in intermolecular hydrogen bonding and/or acid−base chemistry, and the temperature and concentration dependence of these phenomena are reflected in the chemical shifts of the neighboring H2 and H5 atoms. These observations gave us some comfort regarding the discrepancies between the NMR data for synthetic **1** and the data collected on the natural sample. Unfortunately, the reported chemical shift values for natural **1** were outside the ranges manifested by synthetic **1**. On the basis of the observed trends, it appeared that a colder or more concentrated sample of synthetic **1** would exhibit H2 and H5 chemical shifts equal to or greater than those recorded from natural **1**. However, the high freezing point of DMSO and the limited amount of synthetic **1** on hand both precluded us from verifying this hypothesis.

We reasoned that the imidazole signals in the ^{1}H NMR would also exhibit pH dependence. Indeed, addition of a small quantity of TFA (ca. 2 μL) to a solution of **1** in DMSO-d_6 at roughly the original concentration given in Table 7 caused a large downfield shift in both signals (9.53 ppm for H2, 7.83 ppm for H5). As a result, the range of chemical shifts observed for imidazole H2 and H5 in synthetic **1** at various temperatures, concentrations, and pH values (9.53−8.04 ppm for H2, 7.83−7.40 ppm for H5) now encompassed the reported chemical shifts of these hydrogen atoms in natural **1** (9.41 ppm for H2, 7.79 ppm for H5). This fact greatly increased our confidence that we had in fact synthesized celogentin C. The identity of our synthetic sample was then confirmed by HPLC coinjection with a sample of natural **1**.

The potent antimitotic activity of celogentin C prompted us to synthesize sufficient quantities of **1** for screening against the National Cancer Institute's panel of 60 cancer cell lines. In a single-dose assay conducted at 10 μM concentration of **1**, the cell lines exhibited a mean growth percent of 86%. While the overall result was disappointing, **1** demonstrated significant tumor growth inhibition in four cancer cell lines: the SR leukemia cell line (35% growth), the MDA-MB-435 melanoma cell line (23% growth), the HS 578T breast cancer cell line (30% growth), and the MDA-MB-468 breast cancer cell line (34% growth). Accordingly, the selective anticancer properties of celogentin C may be a subject worthy of future study.

Conclusions

We have achieved the total synthesis of celogentin C. Early efforts, which focused on a right-to-left approach to formation of the bicyclic framework of the natural product, were ultimately unsuccessful. Nevertheless, in the course of exploring this route, we discovered that the Braslau modification to the McFadyen−Stevens reaction[27,28] provided an effective solution to the reduction of an extremely unreactive methyl ester. It is likely that this heretofore underutilized transformation will find application in the reduction of other recalcitrant ester groups.

Ultimately, success was achieved after recourse to a left-to-right annulation strategy. The Leu−Trp side-chain cross-link, which is imbedded in the left-hand macrocycle, was constructed via a three-step protocol consisting of an intermolecular Knoevenagel condensation, a radical conjugate addition, and a nitro group reduction. The low diastereoselectivity obtained from the radical conjugate addition was largely offset by the excellent yield and concise nature of this sequence. The key Trp−His side-chain linkage was formed by an indole−imidazole oxidative coupling reaction that joined the intact left-hand ring with an Arg−His dipeptide. Interestingly, the success of this transformation depended on the use of Pro−OBn as an additive. Further experiments were supportive of a mechanistic hypothesis in which Pro−OBn reacts directly with NCS to form an *N*-chloroamine that collapses to an imine upon elimination of HCl. This process serves to moderate the concentration of NCS so that the desired chloroindolenine intermediate is favored at the expense of the unproductive dichlorinated species. The ability of proline esters to scavenge and sequester electrophilic chlorine atoms could prove to be useful in other synthetic endeavors.

Celogentin C was obtained in three straightforward steps after the oxidative coupling reaction, and sufficient quantities of **1** were produced to permit evaluation of its anticancer activity. In the course of comparing spectral data of synthetic **1** to the reported data for the natural product, we discovered that the chemical shifts of the imidazole hydrogen atoms were dependent on temperature, concentration, and pH. This behavior is presumably an indication of the participation of the imidazole moiety of **1** in intermolecular hydrogen bonding and/or acid−base chemistry. Our total synthesis of **1** is the first of a constituent of the celogentin/moroidin family of bicyclic peptides, and we believe that our route can be readily adapted to enable the preparation of other members of this interesting class of antimitotic compounds.

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Supporting Information Available: Experimental procedures, characterization data, and NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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