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Total Synthesis of the Antifungal Depsipeptide Petriellin A

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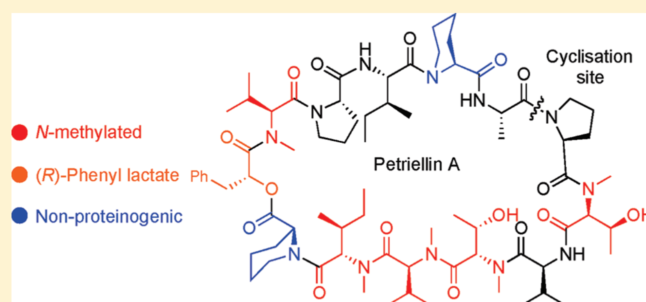
Supporting Information

ABSTRACT: We report the solid-phase total synthesis of the antifungal highly modified cyclic depsipeptide petriellin A. The synthesis confirms earlier reports on the absolute configuration of the natural product. The solid-phase approach resulted in a protected linear precursor, which was cleaved from the solid support prior to cyclization and final deprotection. Use of advanced coupling agents for several hindered amides was a feature of the synthesis. The natural product was prepared in overall 5% yield.



INTRODUCTION

Discovery and development of novel therapeutics is driven, in part, by the increase in pathogenic bacteria and fungi resistant to established drugs. Medicinal chemistry discovery has a focus on natural products displaying (particularly novel) biological activity.[1,2] These secondary metabolites, in some instances, have been useful as drugs, while others have served as lead compounds in drug development. While many different classes of metabolites have pharmacological importance, our research is directed toward modified amino acids that include *N*-methyl α-amino acids[3−6] and *β*-amino acids.[7−12] Furthermore, we are interested in the development of methodologies for the subsequent synthesis of bioactive peptides including such modified amino acids.[8,13] In particular, the present research focuses on total synthesis of the naturally occurring antifungal cyclodepsipeptide, petriellin A **1** (Figure 1).

Lee and Gloer and co-workers[14] isolated petriellin A **1** (Figure 1) from the organic extracts of an antagonistic coprophilous fungus, *Petriella sordida* (UAMH 7493). Petriellin A was found to have activity against *Ascobolus furfuraceous* (NRRL 6460) and *Sordaria fimicola* (NRRL 6459).[14] Structurally, petriellin A contains one α-D-hydroxycarboxylic acid and 12 L-amino acids, five of which are N-methylated. At the time of the initial communication, Lee and Gloer and co-workers[14] did not determine the absolute configuration of *N*-methylisoleucine and the two *N*-methylthreonine residues in the natural product. Clarification of the unknown stereochemistry was later communicated by Aurelio et al.[6,15] An attempted total synthesis of petriellin A via solution-phase chemistry was later reported by Aurelio et al.[13] The authors constructed the "northwestern" and "southeastern" halves of their planned linear precursor. However, the assembly of the acyclic depsipeptide was unsuccessful.

While solution-phase peptide techniques have been utilized extensively throughout the history of peptide synthesis and lead to the production of natural products,[16] many difficulties can accompany this approach. These difficulties are further exacerbated in the case of N-methylated peptides by the difficult coupling of secondary amines to form tertiary amide bonds. Accordingly, in planning the present total synthesis of petriellin A, earlier difficulties were taken into account and a solid-phase approach was chosen. Indeed, solid-phase peptide chemistry has difficulties of its own;[17] however, purification and isolation of intermediates is eliminated from the reaction course. Thus, it was anticipated that production of the linear precursor of petriellin A would be achieved in less time, and if difficulties were encountered in synthesizing the linear precursor or during the cyclization, a new approach could be investigated in a much shorter time frame. Furthermore, once the solid-phase technique was optimized and a total synthesis was achieved, this approach might be applied to the total synthesis of other similar depsipeptides of interest.

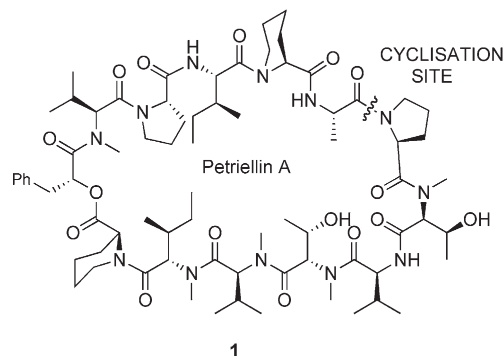


Figure 1. Cyclization site for solid-phase synthesis of petriellin A.

Prior to commencing the petriellin A solid-phase synthesis, a number of details were considered. In the first instance, only a

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small number of heavily N-methylated peptides had been synthesized by solid-phase peptide techniques.[19,20,22,23] This was attributed to the fact that resin-bound secondary amines have lower reactivities toward activated acids than analogous reactions in solution.[18] Some solid-phase syntheses of N-methylated peptides have been accomplished by utilizing coupling reagents that have shown success in solution. A successful solid-phase synthesis of cyclosporins was reported that employed 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) or *N*,*N*′-diisopropylcarbodiimide (DIC)/1-hydroxy-7-azabenzotriazole (HOAt) for the construction of tertiary amide bonds.[19] Other examples achieved *N*-methyl amide synthesis by symmetrical anhydride methods.[20] However, both of these approaches required multiple coupling cycles to obtain adequate coupling yields.

A reliable alternative to overcome the difficulties associated with the synthesis of N-methylated amide bonds was first reported by Gilon and co-workers.[21] Bis(trichloromethyl)-carbonate (BTC, or triphosgene) was added to an Fmoc-amino acid, yielding the corresponding acid chloride. This was immediately added to a peptidyl Rink amide 4-methylbenzhydrylamine (MBHA) resin at 50 °C, yielding a tertiary amide bond. Fmoc-amino acids bearing acid-labile side chains were found to be stable to these conditions. HPLC analysis established that the peptides were produced in high yields and purity without racemization when tetrahydrofuran (THF) was used as solvent. The absence of racemization was due to the high reactivity of the acid chloride compared to the slower oxazolone formation.[21] This approach was more versatile than methods that enlisted the use of premade Fmoc-amino acid chlorides, as some cannot be synthesized and others are not stable resulting in, for instance, oxazolone formation. Moreover, those with acid-labile side-chain protecting groups have a limited shelf life.

Applying the BTC coupling agent in the solid-phase synthesis of petriellin A was promising, particularly since total syntheses of omphalotin A[22] and cyclosporin O[23] using BTC have been reported. In the first instance, Jung and co-workers[22] conducted comparative N-methyl couplings on a trityl chloride resin, in which BTC was compared to dicyclohexylcarbodiimide (DCC), DIC/HOAt and tetramethylfluoroformamidinium hexafluorophosphate (TFFH). The highly acid-labile trityl chloride resin was used as a model, rather than the Rink amide resin originally employed by Gilon and co-workers,[21] due to the acid lability of N-methylated peptides.[24,25] In all cases BTC possessed superior coupling efficiencies; however, the original method[21] was found to be useless for the synthesis of larger peptides on the trityl chloride resin.[21] Under the original BTC-coupling conditions, premature cleavage of the growing peptide from the highly acid-labile trityl resin resulted. It was assumed that 2,4,6-collidine alone was not basic enough to neutralize the hydrogen chloride formed during the BTC activation and prevent it from cleaving the peptide from the resin. Jung and co-workers[22] eliminated this problem by pretreating the resin with diisopropylethylamine (DIPEA) and then adding the preformed Fmoc-amino acid chloride. The presence of DIPEA accelerated the reaction, and thus the elevated temperature employed in the original procedure[21] was no longer necessary and byproduct formation ceased. HPLC analysis confirmed the enantiomeric purity of the products was unaffected by these modifications.[23] It was decided that the modified BTC method of Jung and co-workers[22,23] would be utilized in the present synthesis of petriellin A.

The site chosen for cyclization was dictated by the sequence of the linear precursor. Tertiary amide bonds generally were discounted as possible cyclization sites. The junctions between the C- and N-termini of proline and the two neighboring non-N-methylated amino acids were two possible sites for cyclization. One was the amide bond connecting the C-terminus of proline to the N-terminus of isoleucine; the activated form of proline is known to resist racemization,[26] but the isoleucine side chain is sterically hindered. The other site was the C-terminus of alanine and the N-terminus of proline. Although cyclizing at this point would involve the formation of a tertiary amide bond, proline behaves more like a primary amine when reacting with activated acids. The alanine−proline junction was chosen as the preferred cyclization site due to the lower steric hindrance compared to isoleucine. As a consequence, the linear precursor would begin from the N-terminus of L-proline and finish at the C-terminus of alanine.

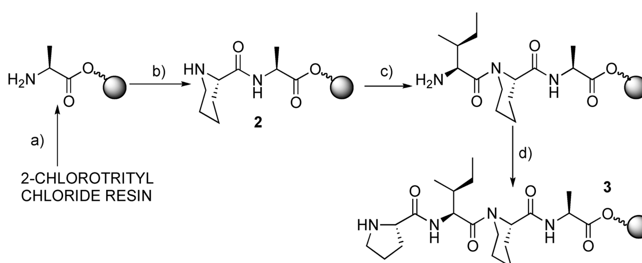
The final factor considered was the protecting group on the side chains of the two threonine residues. Conveniently, Fmoc-*N*-methyl-*O*-*t*-butyl-L-threonine was commercially available. However, the use of this *t*-butyl ether derivative would require establishing a trifluoroacetic acid (TFA) deprotection method that avoided TFA-induced amidolysis of the cyclization product. With this in mind, a series of acid stability tests were conducted on a sample of natural petriellin A isolated from *P. sordida*.[6] It was established that TFA-induced amidolysis was avoided, provided reduced temperatures were used during deprotection of the *t*-butyl ethers (vide infra).

RESULTS AND DISCUSSION

The construction of petriellin A began by loading Fmoc-L-alanine onto the 2-chlorotrityl chloride resin. After 2 h the unreacted resin sites were capped with methanol. The resin was found to have an amino acid loading of 0.52 mmol/1.00 g of resin. The Fmoc-protected peptidyl resin was treated with a solution of piperidine/1,5-diazabicyclo[5.4.0]undec-5-ene (DBU)/*N*,*N*-dimethylformamide (DMF) (2:2:96). The presence of the exposed primary amine was confirmed through a 2,4,6-trinitrobenzenesulfonic acid (TNBS) test on a small sample of the resin beads.

Fmoc-L-pipecolic acid was the next amino acid in the sequence. At the time only DL-pipecolic acid was available, and a

Scheme 1. Commencing Solid-Phase Synthesis of Petriellin A 1[a]



[a] Reagents and conditions: (a) (1) Fmoc-L-Ala-OH, DIPEA, CH_2Cl_2, 2 h; (2) MeOH; (3) piperidine, DBU, DMF. (b) (1) Fmoc-L-Pip-OH, HBTU, HOBt, DIPEA, DMF, 5 h; (2) piperidine, DBU, DMF. (c) (1) Fmoc-L-Ile-OH, HBTU, HOBt, DIPEA, DMF, 24 h; (2) piperidine, DBU, DMF. (d) (1) Fmoc-L-Pro-OH, HBTU, HOBt, DIPEA, DMF, 24 h; (2) piperidine, DBU, DMF.

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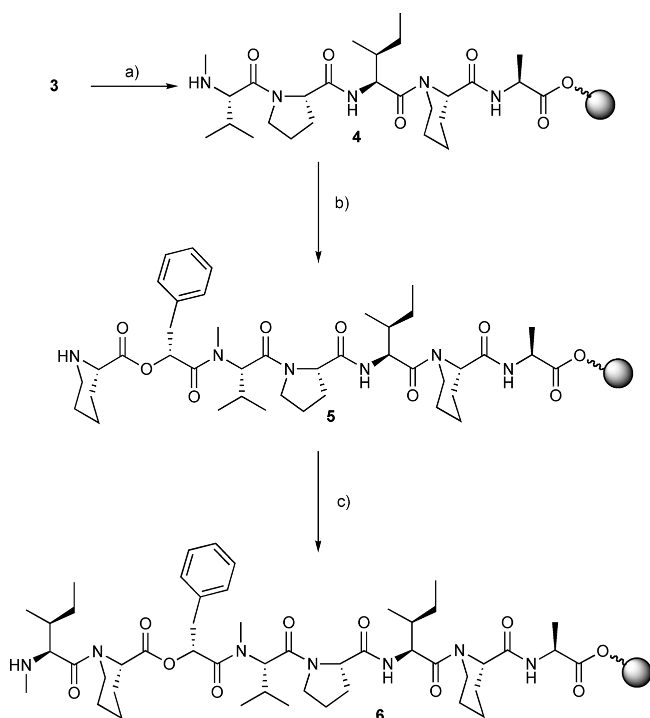
resolution by recrystallization of the tartaric acid salts was conducted. This purified L-pip-D-(−)-tartrate salt was treated with Fmoc-*O*-succinimide in the presence of base. Fmoc-L-pipecolic acid was isolated; its data were identical to previously reported material.[27] The protected pipecolic acid residue was pretreated with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU)/1-hydroxybenzotriazole (HOBt) in the presence of base and the solution was added to the resin-bound amino acid. Once this coupling cycle was complete, the resin was treated with base to afford resin-bound dipeptide **2** (Scheme 1). The exposed secondary amine group was verified by a chloranil test. Fmoc-L-isoleucine was next pretreated with the coupling reagent to form the activated ester and then it was immediately added to the resin. Base treatment of the resin followed and revealed the deprotected resin-bound peptide. Fmoc-L-proline was added to the resin and, again, the coupling was efficacious with HBTU/HOBt. Subsequent base treatment yielded the tetrapeptide **3** (Scheme 1).

Scheme 2[a]



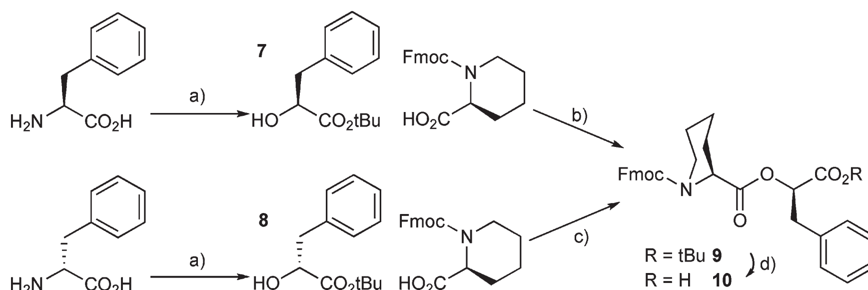
[a] Reagents and conditions: (a) (1) Fmoc-L-*N*-MeVal-OH, HBTU, HOBt, DIPEA, DMF, 18 h; (2) piperidine, DBU, DMF. (b) (1) Fmoc-L-Pip-D-PheLac-OH, BTC, 2,4,6-collidine, DIPEA, THF, 18 h; (2) repeat cycle; (3) piperidine, DBU, DMF. (c) (1) Fmoc-*N*-Me-L-Ile-OH, BTC, 2,4,6-collidine, DIPEA, THF, 24 h; (2) piperidine, DBU, DMF.

The resin-bound peptide **3** was treated with a preformed solution of Fmoc-*N*-methyl-L-valine HOBt ester (Scheme 2). Successive basic treatment revealed the resin-bound pentapeptide **4**. The α-hydroxy-D-phenyllactic acid residue was next in the sequence. However, this residue was converted to the didepsipeptide **9** (Scheme 3) by a Mitsunobu esterification and then added to the growing peptide. This approach was desirable because there are few sensitive resin tests for detecting unreacted resin-bound alcohols. It was initially considered that if the ester bond was made on the solid support, a sample of the growing peptide could be cleaved after the solid-phase esterification and analyzed by electrospray mass spectrometry (ES-MS). This would give an indication of whether the coupling had been successful. However, the α-hydroxycarboxylic acid present in petriellin A is D-configured. A Mitsunobu esterification in solution gives the product with inversion of configuration at the alcohol center. This approach allows the solution-phase synthesis to begin from the less expensive and readily available L-phenylalanine.

Accordingly, L-phenylalanine was diazotized to the corresponding α-acetoxycarboxylic acid[28] and subsequently converted to the *t*-butyl ester derivative[29] in 68% yield. The doubly protected intermediate was subjected to mild base hydrolysis, giving the alcohol **7** (Scheme 3). Mitsunobu esterification between Fmoc-L-pipecolic acid and the L-phenyllactate derivative **7** gave the depsipeptide **9** (Scheme 3). Initially, the esterification was conducted in THF, which resulted in low yields (40%). However, an improvement resulted when dichloromethane was used as the reaction medium (70%). For comparative purposes, D-phenylalanine was also diazotized and then treated with di-*t*-butyl dicarbonate to yield a doubly protected intermediate. Once the acetate group was hydrolyzed, a DCC-mediated coupling of the D-alcohol **8** and Fmoc-L-pipecolic acid gave the depsipeptide **9** in 56% yield (Scheme 3). The DCC coupling gave a reduced yield of the desired product with respect to the synthesis of the same compound by Mitsunobu esterification. Therefore, the economically favorable option is to commence the synthesis of the dipeptide **9** from L-phenylalanine.

The fully protected dipeptide **9** was subjected to a TFA-induced deprotection to expose the C-terminus (Scheme 3). The

Scheme 3. Assembly of Depsidipeptide 10[a]

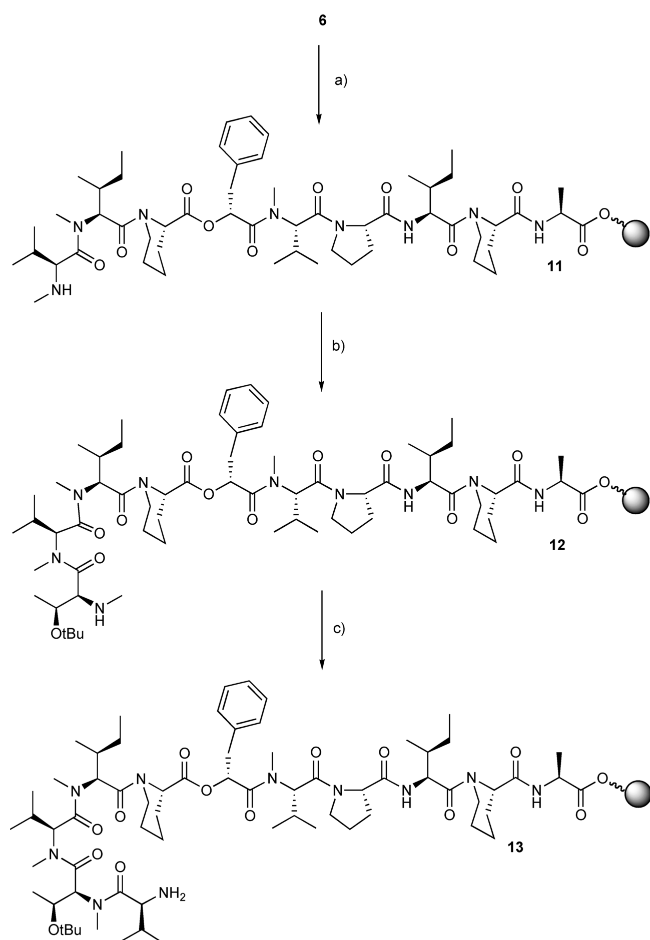


[a] Reagents and conditions: (a) (1) $NaNO_2$, CH_3CO_2H, H_2O; (2) $(Boc)_2O$, DMAP, *t*-BuOH; (3) K_2CO_3, CH_3OH, H_2O. (b) Ph_3P, DEAD, CH_2Cl_2. (c) DCC, DMAP, CH_2Cl_2. (d) TFA/CH_2Cl_2 (1:1).

crude acid **10** was sufficiently clean to use in the subsequent solid-phase synthesis. Pretreatment of the acid **10** with BTC gave the acid chloride, which was immediately added to the deprotected resin-bound pentapeptide **4** (Scheme 2). Immediately after an 18 h coupling cycle, a chloranil test indicated that a mixture of pentapeptide **4** and Fmoc-protected heptapeptide **5** was present. Another overnight BTC-mediated coupling of the activated depsipeptide **10** was successful in completing the synthesis of the resin-bound Fmoc-protected heptapeptide **5**. A small sample of the resin was treated with base and the deprotected heptapeptide **5** was cleaved from the resin. ES-MS analysis verified that the desired intermediate had formed ($[M + H]^+$ *m*/*z* 784 (100%)) with no deletion products present. The remaining resin-bound peptide was deprotected and a solution of the BTC-activated Fmoc-*N*-Me-L-isoleucine acid chloride was added. Analysis after 24 h indicated complete coupling had taken place and that the octapeptidyl resin **6** was present.

The peptidyl resin **6** was treated with base, and following this a solution of Fmoc-*N*-methyl-L-valine acid chloride was added (Scheme 4). A sample of the growing peptide was subjected to ES-MS analysis and the formation of the desired resin bound nonapeptide **11** was confirmed ($[M + H]^+$ *m*/*z* 1024 (50%)).

Scheme 4[a]



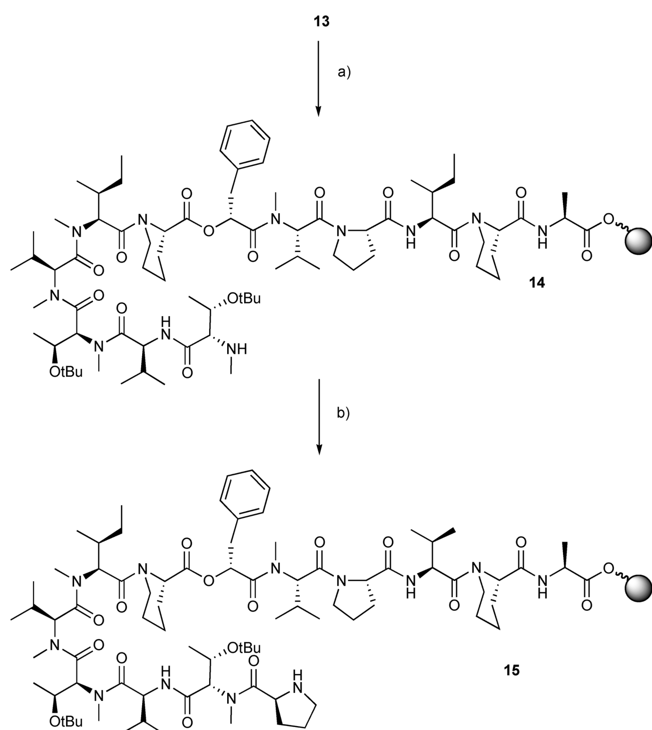
[a] Reagents and conditions: (a) (1) Fmoc-*N*-Me-L-Val-OH, BTC, 2,4,6-collidine, DIPEA, THF, 2 h; (2) piperidine, DBU, DMF. (b) (1) Fmoc-L-Thr(OtBu)-OH, BTC, 2,4,6-collidine, DIPEA, THF, 18 h; (2) piperidine, DBU, DMF. (c) (1) Fmoc-L-Val-OH, BTC, 2,4,6-collidine, DIPEA, THF, 24 h; (2) piperidine, DBU, DMF.

Fmoc-*N*-methyl-*O*-*t*-butyl-L-threonine was the next residue in the sequence of petriellin A. However, prior to committing to the use of this *t*-butyl ether derivative, the acid stability of petriellin A toward a 50% TFA solution was investigated. A sample of natural petriellin A[30] was obtained and analyzed by HPLC to establish purity. It was then used as a control. A solution of 50% TFA in dichloromethane was then added to a 1 mg sample of natural petriellin A for 1 h at room temperature. HPLC analysis of the TFA-treated sample and the control showed that significant degradation had occurred in the TFA-exposed petriellin A sample. Another experiment was conducted whereby a fresh petriellin A sample was treated with the same TFA solution; however, the temperature of the mixture was maintained below 0 °C. HPLC analysis showed that no degradation had occurred. At reduced temperatures it appeared that TFA-induced amidolysis was not occurring. Thus, the *t*-butyl ether protection could be employed on the threonine residues.

Accordingly, Fmoc-*N*-methyl-*O*-*t*-butyl-L-threonine was pretreated to form the acid chloride and the solution was added to the resin-bound peptide (Scheme 4). A chloranil test indicated that the nonapeptide was completely converted to the resin-bound decapeptide **12** and this was further verified by ES-MS analysis ($[M + H]^+$ *m*/*z* 1195 (30%)).

The next residue to be added to the growing peptide was valine (Scheme 4). In the first instance, a 2-h BTC-mediated coupling did not result in complete conversion to the undecapeptide intermediate **13** (Scheme 4). A second cycle mediated by DIC/HOAt was necessary for complete conversion. However, when the complete synthesis of petriellin A was later repeated, the formation of the undecapeptidyl resin was efficacious after

Scheme 5[a]



[a] Reagents and conditions: (a) (1) Fmoc-*N*-Me-L-Thr(OtBu)-OH, HBTU, HOBt, DIPEA, DMF, 18 h; (2) piperidine, DBU, DMF. (b) (1) Fmoc-L-Pro-OH, BTC, 2,4,6-collidine, DIPEA, THF, 18 h; (2) piperidine, DBU, DMF.

only one cycle when the first BTC-mediated coupling was left overnight rather than for 2 h.

The peptidyl resin was deprotected and a sample was subjected to the TNBS test. A dark red color indicated that the N-terminal protecting group had been removed. The second-to-last coupling involved another *N*-methylthreonine derivative. The amino acid was pretreated with HBTU, forming the HOBt activated ester, which was immediately added to the peptidyl resin **13** (Scheme 5). An ES-MS analysis on completion of the cycle gave a molecular ion peak at m/z 1466 ($[M + H]^+$, 90%) for the Fmoc-deprotected dodecapeptide **14**. The final residue to complete the linear precursor in the synthesis of petriellin A was Fmoc-L-proline. This coupling was accomplished by the BTC-acid chloride method. After an overnight cycle, ES-MS of a small sample of Fmoc-deprotected material gave a peak at m/z 1577 ($[M + H]^+$, 100%), confirming the successful synthesis of the resin-bound linear cyclization precursor **15**.

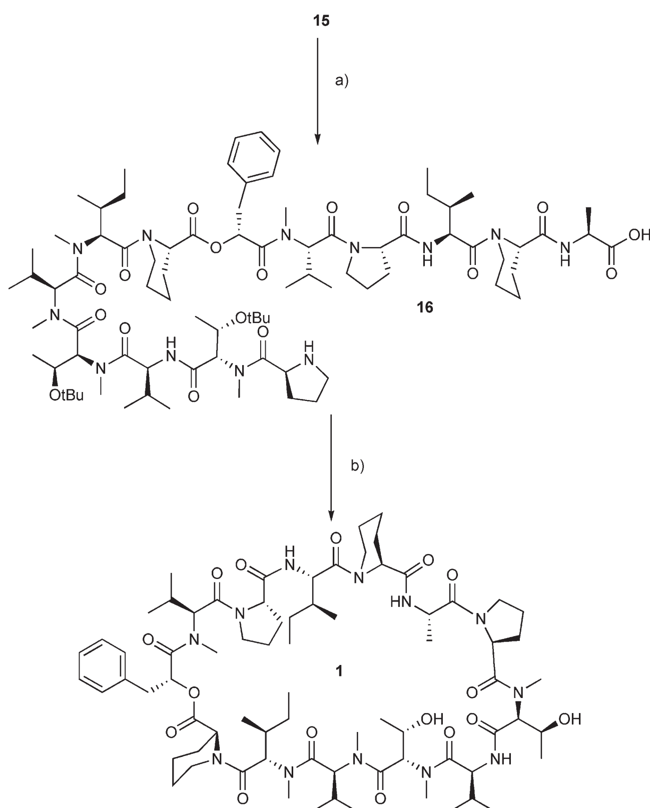
The remainder of the peptidyl resin was thus deprotected with a solution of piperidine and DBU in DMF (2:2:96) for 3 min. The resin was washed and dried. Cleavage of the linear precursor from the resin was effected by a solution of 1.5% TFA in dichloromethane. Upon addition of the cleavage solution, the resin beads immediately changed color from yellow to red, indicating the peptide was free from the resin. The solution was collected and immediately diluted with excess dichloromethane, to reduce the concentration of the acid solution. The solution was carefully removed under reduced pressure at room temperature. Caution was taken when the peptide was exposed to this acidic solution due to possible TFA-induced amidolysis observed with natural petriellin A (vide supra) and other peptides.[17,31,32]

The crude linear precursor was isolated as an oil. The material was dissolved in a solution of 50% acetonitrile/Milli-Q water and the mixture was freeze-dried overnight. A colorless solid was isolated in a moderate yield (58%, as calculated from the loading of the first residue). An HPLC analysis was conducted, which demonstrated that the linear precursor was sufficiently clean for further manipulations.

Cyclizations of large peptides are generally carried out at high dilutions. This technique is used to encourage the intramolecular reaction to take place rather than an intermolecular reaction. In the current cyclization, approximately 2 mL of solvent was used for every milligram of the linear precursor. The solvent used is another important consideration for the cyclization reaction. The solvent chosen influences the solution-phase conformation the peptide adopts and consequently directly influences the cyclization yield. There are no clearly defined parameters one can rely on in selecting a solvent. Petriellin A is heavily N-methylated and therefore there is already an increased risk of epimerization.[24,25] Dipolar aprotic solvents, such as DMF, were avoided since they further increase the risk of epimerization. A mixture of dichloromethane and acetonitrile in a ratio of 10:1 was selected, as this reaction medium has a low dielectric constant, reducing the risk of epimerization. Acetonitrile was included to aid in solubilizing the coupling reagents. Upon completion of the coupling reaction, the solvents were easily removed under reduced pressure.

Initially, trial cyclization–deprotection reactions were conducted and the products of these reactions were analyzed by comparative HPLC with a sample of the natural product. The two reactions, cyclization and deprotection, were conducted sequentially without isolation and analysis of the cyclic *t*-butyl-protected petriellin A intermediate. The reason for taking this approach was to reduce the handling of the intermediate and ultimately

Scheme 6. Resin Cleavage, Cyclization, and Deprotection To Form Petriellin A 1[a]



[a] Reagents and conditions: (a) 1.5% TFA in CH_2Cl_2. (b) (1) HBTU, DIPEA, CH_3CN/CH_2Cl_2 (1:9); (2) TFA/CH_2Cl_2 (1:1), 0 °C.

maximize the overall yield. The trial reactions were conducted on a 1 mg scale. The linear precursor **16** (Scheme 6) was dissolved in dichloromethane, and a solution of HBTU in acetonitrile and dichloromethane was added in the presence of base. After 1 h the solvent was removed under reduced pressure and the residue that remained was cooled in an ice–salt bath. In order to effect *t*-butyl ether deprotection, a solution of 50% TFA in dichloromethane was also cooled and then added to the cooled residue. The solution was left to stir on ice for 30 min and the temperature of the reaction was maintained below 0 °C. Following this, the solution was diluted with dichloromethane to a TFA concentration of no more than 0.5%. The solution was removed in vacuo at room temperature. The crude residue was analyzed by analytical HPLC. A peak with a retention time of 16.12 min was confirmed as the synthetic product after comparison to the retention time of the natural product. Once the synthetic peptide **1** (Scheme 6) had been identified, isolation of the desired peak was undertaken on an analytical scale. Once the purified synthetic peptide was isolated, analysis by ES-MS further confirmed the total synthesis of petriellin A.

A control trial cyclization–deprotection reaction was undertaken whereby no cooling was applied during TFA treatment. All other aspects of the trial reaction were identical to those described above. The crude material isolated was analyzed by analytical HPLC. As suspected, the peak at 16.12 min for the synthetic product was the minor component, as opposed to it being the major component when the TFA deprotection was

conducted below 0 °C. Furthermore, early-eluting byproduct peaks dominated the HPLC trace in the room-temperature experiment, suggesting formation of degradation products resulting from TFA-induced hydrolysis during the uncooled acidic treatment.

The coupling−deprotection reaction was repeated on a larger scale according to the first trial reaction described. A small sample of the crude material was first analyzed by analytical HPLC to ensure the presence of the synthetic product. After formation of the synthetic product was verified via analytical HPLC, purification and isolation by semipreparative HPLC was conducted on the remaining crude material. A major peak was detected and isolated at 28.4 min. ES-MS analysis confirmed the component isolated was the desired product ($[M + H]^+$ m/z 1433 (90%), [M + Na] m/z 1454 (90%)). The fractions isolated were combined and found to have a purity of 87.07% by analytical HPLC. The overall yield determined from the loading of the first residue was 5%.

The natural product has been analyzed extensively by NMR.[30] Therefore, ^{1}H NMR analyses were conducted on the synthetic product and compared to the literature. The aromatic region between 6.8 and 7.6 ppm and the region containing the α-protons between 4.5 and 6.0 ppm in ^{1}H NMR spectra of the synthetic product and the natural product were identical

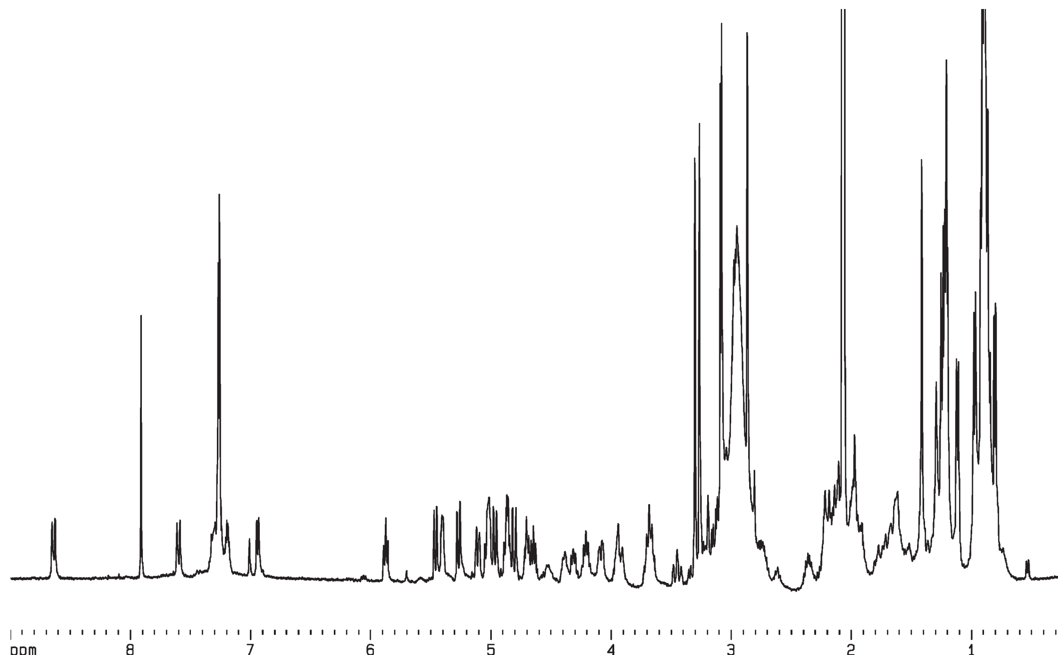


Figure 2. ^{1}H NMR spectrum of synthetic petriellin A **1** in acetone-d_6/deuteriochloroform (1:4).

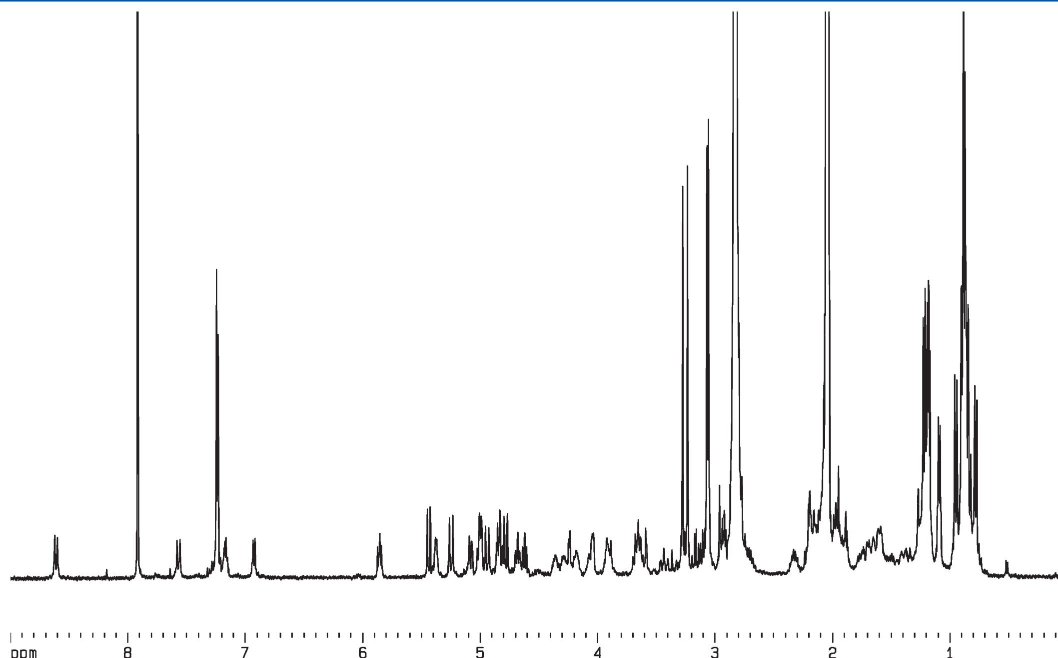


Figure 3. ^{1}H NMR spectrum of natural petriellin A 1 in acetone-d_6/deuteriochloroform (1:4).

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(Figures 2 and 3). The spectrum of the synthetic product does, however, have extra peaks at 1.4 and 3.0 ppm, which were attributed to solvent peaks from the deuterated solvent used for the NMR analysis. These comparisons confirmed the total synthesis of petriellin A by solid-phase chemistry.

CONCLUSIONS

Petriellin A was successfully synthesized by solid-phase peptide synthesis techniques in an overall yield of 5%, as determined from the loading of the first amino acid. The 2-chlorotrityl chloride resin was used to synthesize the peptide and the residues were incorporated as the Fmoc-amino acid derivatives. Solution-phase synthesis was applied in the production of the depsipeptide bond contained within petriellin A. The presynthesized depsipeptide was successfully incorporated by solid-phase techniques with no detection of any deletion products. Cleavage of the linear peptide by 1.5% TFA in dichloromethane did not result in any detectable TFA-induced amide bond hydrolysis. Most importantly, cleavage of the *t*-butyl protecting groups from the threonine residues in 50% TFA in dichloromethane at room temperature caused significant loss of the desired synthetic product. However, when the same TFA treatment was conducted below 0 °C, the yield of synthetic product increased significantly, as indicated by analytical HPLC. Furthermore, this reduced-temperature TFA deprotection technique may be useful in the synthesis of other heavily *N*-methylated peptides. The cyclization was conducted at high dilution, and analysis confirmed that the linear tridecapeptide intermediate underwent the desired intramolecular reaction, rather than an undesired intermolecular reaction.

EXPERIMENTAL SECTION

(*R*)-*N*-(9-Fluorenylmethoxycarbonyl)-(*S*)-pipecolinylphenyllactic Acid 10. Fmoc-L-pipecolic acid (1.4 g, 4.1 mmol), alcohol **7** (1 g, 4.5 mmol), and triphenylphosphine (TPP) (1.4 g, 5.4 mmol) were dissolved in dry CH_2Cl_2 (10 mL) under an atmosphere of N_2. The mixture was cooled to 0 °C, and diethyl azodicarboxylate (DEAD) (840 μL, 5.4 mmol) was added in three portions over 10 min. The reaction was stirred at 0 °C for 1 h and then at room temperature for 5 h. After this time the solvent was concentrated in vacuo and the residue was crystallized several times from diethyl ether. The filtrate was collected and again concentrated. The crude residue was purified by flash column chromatography eluted with 20% ethyl acetate/hexane to yield the depsipeptide **9** as a colorless oil (1.54 g, 69% yield). ^{1}H NMR (300 MHz, $CDCl_3$) (rotamers) δ 7.77−7.13 (13H, m), 5.16−4.85 (1H, m), 4.42−4.12 (3H, m), 3.13−3.01 (2H, m), 2.31−2.17 (2H, m), 1.60−1.20 (15H, m). ^{13}C NMR (75 MHz, $CDCl_3$) (rotamers) δ 173.1, 170.8, 170.6, 167.9, 167.8, 155.8, 155.0, 143.8, 143.7, 141.0, 136.5, 135.6, 135.4, 129.4, 129.1, 128.1, 128.0, 127.9, 127.4, 126.8, 126.6, 126.4, 124.8, 124.6, 119.7, 82.0, 73.9, 73.6, 71.0, 67.4, 54.3, 54.0, 46.9, 41.6, 41.3, 40.3, 37.0, 27.7, 27.6, 26.8, 26.4, 24.5, 24.3, 20.3, 20.1. The ester **9** was then dissolved in CH_2Cl_2 (3 mL) and TFA (3 mL). The mixture was left to stand for 30 min and then concentrated under reduced pressure from dichloromethane (10 mL × 3). Formation of the acid **10** was confirmed by ES-MS *m*/*z* 500.5 ([M + H]$^+$, 90%) and 522.4 ([M + Na], 100%). HRMS *m*/*z* for $C_{30}H_{30}NO_6$ [M + H]$^+$ calcd 500.2073, found 500.2059; *m*/*z* for $C_{30}H_{29}NNaO_6$ [M + Na]$^+$ calcd 522.1893, found 522.1874.

Loading of Resin. To the 2-chlorotrityl resin (ClTrt) (1.57 g, 1.57 mmol) was added dry CH_2Cl_2 (10 mL). In a separate vessel, Fmoc-L-alanine (311 mg, 1.0 mmol) was dissolved in DMF/CH_2Cl_2 (10 mL, 1:9). The amino acid solution was added to the resin, followed by the addition of DIPEA (1.03 mL, 6.0 mmol). The reaction was left to shake in a reaction vessel equipped with a sintered bottom for 2 h. MeOH (1.5 mL) was added and the mixture was shaken for a further 30 min. The resin was filtered and washed with DMF, CH_2Cl_2 and ether, successively. The resin was dried in vacuo overnight to yield 1.88 g, 52% (0.52 mmol/1 g) of loaded resin. The synthesis of petriellin A was performed on 400 mg, 0.21 mmol of preloaded ClTrt resin.

Fmoc Deprotection. Fmoc-peptidyl resin was washed and then shaken with piperidine/DBU/DMF (2:2:96) for 3 min. The resin was filtered and washed with the cleavage solution, followed by successive washing with DMF and CH_2Cl_2. Completion of the deprotection was confirmed by a positive TNBS test[33] for non-N-methylated amino acids and by a positive chloranil test[34] for N-methylated amino acids.

BTC-Mediated Couplings. To the semidry peptidyl ClTrt resin (0.21 mmol) that had been previously swollen with CH_2Cl_2 were added dry THF (1 mL) and DIPEA (287 μL, 1.6 mmol). In a separate reaction vessel, BTC (72 mg, 0.24 mmol) was dissolved in dry THF (2.5 mL, 96 mM), followed by addition of the Fmoc-amino acid (0.74 mmol). *sym*-Collidine (278 μL, 2.1 mmol) was added to the clear amino acid solution and a precipitate immediately formed. The amino acid solution was then added to the solution containing the resin beads and the reaction was shaken for 4−24 h. The peptidyl ClTrt resin was washed successively with DMF and CH_2Cl_2. Completion of the reaction was confirmed by a negative TNBS test[33] for non-N-methylated residues and the chloranil test[34] for N-methylated residues. In some cases further confirmation was gained through cleavage of the growing peptide from a small amount of ClTrt resin, followed by mass spectral analysis.

HBTU/HOBt-Mediated Couplings. To the semidry peptidyl ClTrt resin (0.21 mmol) that had been previously swollen with CH_2Cl_2 was added dry DMF (1 mL). In a separate vessel, the Fmoc-amino acid (0.74 mmol) was dissolved in a solution of HBTU (278 mg, 0.74 mmol) and HOBt (100 mg, 0.75 mmol) in DMF (3 mL). To this colorless solution was added DIPEA (287 μL, 1.7 mmol), and the mixture was left to stir for 5 min, until a yellow solution resulted. This solution was added to the peptidyl ClTrt resin and the reaction was shaken for 4−24 h. Completion of the reaction was confirmed by a negative TNBS test[33] for non-N-methylated residues and the chloranil test[34] for N-methylated residues. In some cases further conformation was gained through cleavage of the growing peptide from a small amount of ClTrt resin, followed by mass spectral analysis.

DIC/HOAt-Mediated Couplings. To the semidry peptidyl ClTrt resin (0.21 mmol) that had been previously swollen with CH_2Cl_2 was added dry DMF (1 mL). In a separate vessel, the Fmoc-amino acid (0.63 mmol) and HOAt (86 mg, 0.63 mmol) were dissolved in dry CH_2Cl_2/DMF (2.5 mL, 1:1). DIC (98 μL, 0.68 mmol) was added and the reaction was allowed to stir for 5 min. The amino acid solution was poured over the peptidyl resin and the reaction was shaken for 4−24 h. Completion of the reaction was confirmed by a negative TNBS test[33] for non-N-methylated residues and the chloranil test[34] for N-methylated residues. In some cases further conformation was gained through cleavage of the growing peptide from a small amount of ClTrt resin, followed by mass spectral analysis.

Cleavage of Linear Precursor from Resin. The peptidyl ClTrt resin (0.21 mmol) was treated with a 1.5% solution of TFA in CH_2Cl_2 (2 mL). Upon this addition, the resin beads immediately turned bright red in color and the cleavage mixture was filtered. The resin beads were washed with the cleavage mixture (3 mL × 2) and then with CH_2Cl_2 (10 mL × 2). The solution was reduced in vacuo and the residue was resuspended in HPLC-grade CH_3CN/Milli-Q H_2O (1:1). The mixture was freeze-dried overnight to yield a colorless solid (189 mg, 58%). A sample (20 μg) of the crude product was subjected to analytical reverse-phase HPLC on a C_{18} column (150 mm ×3.2 mm). The crude product was eluted with a gradient from 0 to 100% buffer B in buffer A

(see Supporting Information) over 20 min at a flow rate of 0.8 mL/min and detected at 254 nm. HPLC retention time was 15.5 min. The analytical trace showed the crude linear peptide to be sufficiently clean to proceed to the cyclization without HPLC purification. (Found: $[M + H]^+$ 1562.0275. $C_{82}H_{137}N_{12}O_{17}$ requires $[M + H]^+$ 1562.0225).

Cyclization and Deprotection (Synthesis of Petriellin A). The crude linear peptide (25 mg, 16 μmol) and HBTU (61 mg, 160 μmol) were dissolved in dry CH_3CN/CH_2Cl_2 (1:9) (50 mL, 1 mL/g). DIPEA (55 μL, 320 μmol) was added and the reaction was left to stir for 1 h under an atmosphere of nitrogen. The mixture was reduced in vacuo and the crude residue was cooled in a salt−ice bath. Meanwhile, a solution of 50% TFA/dry CH_2Cl_2 (5 mL) was prepared and cooled in liquid N_2. The cooled TFA mixture was poured onto the cooled crude peptide and allowed to stir for 30 min. The reaction mixture was diluted with CH_2Cl_2 (150 mL) and reduced in vacuo at room temperature. The crude product was dissolved in HPLC-grade 50% CH_3CN/Milli-Q H_2O (20 mL). A sample of this solution (20 μL) and a sample of natural petriellin A[30] were subjected to analytical reverse-phase HPLC on a C_{18} column (150 mm × 3.2 mm). The crude product and natural petriellin A were eluted with a gradient from 0 to 100% buffer B in buffer A over 20 min at a flow rate of 0.8 mL/min and detected at 220 nm. HPLC (synthetic and natural petriellin A)[30] retention time was 16 min. Once it was confirmed that the synthetic product was in the crude mixture, it was subjected to semipreparative reverse-phase HPLC on a C_{18} column (50 mm × 21.2 mm). The crude product was eluted with a gradient from 20% to 80% buffer B in buffer A over 30 min at a flow rate of 6 mL/min and detected at 220 nm. HPLC retention time was 28.4 min. The appropriate fractions were collected and freeze-dried overnight to yield a colorless white solid (2.1 mg, 9% from the linear precursor or 5% from loading of the first residue). Comparative analytical reverse-phase HPLC between the synthetic product (10 μg) and the natural product[30] (10 μg) was conducted on a C_{18} column (150 mm × 4.6 mm). The products were eluted with a gradient from 0 to 100% buffer B in buffer A over 25 min at a flow rate of 1 mL/min and detected at 220 nm. HPLC (synthetic and natural[30] petriellin A) retention time was 21.5 min. (Found: $[M + H]^+$ 1431.8813. $C_{74}H_{118}N_{12}O_{16}$ requires $[M + H]^+$ 1431.8867). ^1H NMR data were identical to the data previously reported for the natural product[30] (Figures 2 and 3).

■ ASSOCIATED CONTENT

S Supporting Information. General experimental methods and ^1H and ^{13}C NMR spectra for compounds **1**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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