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Peptide Natural Products

Bioinspired Total Synthesis of Pyritide A2 through Pyridine Ring Synthesis

*Annie R. Hooper, Andraž Oštrek, Ana Milian-Lopez, and David Sarlah**

Abstract: Pyritides belong to the ribosomally synthesized and post-translationally modified peptide class of natural products that were recently genome-predicted and are structurally defined by unique pyridine-containing macrocycles. Inspired by their biosynthesis, proceeding through peptide modification and cycloaddition to form the heterocyclic core, we report the chemical synthesis of pyritide A2 involving pyridine ring synthesis from an amino acid precursor through aza-Diels–Alder reaction. This strategy permitted the preparation of the decorated pyridine core with an appended amino acid residue in two steps from a commercially available arginine derivative and secured pyritide A2 in ten steps. Moreover, the synthetic logic enables efficient preparation of different pyridine subunits associated with pyritides, allowing rapid and convergent access to this new class of natural products and analogues thereof.

Rapid advances in genomics have revolutionized every facet of biomolecular sciences, including the field of natural products.[1] Exploitation of genomic information, also known as genome mining, has caused a paradigm shift towards uncovering novel metabolites and discovering biosynthetic pathways and processes.[2] Compared to more traditional isolation methods, genome mining avoids rediscovery of known compounds and focuses on novel classes of metabolites that could not be produced under standard laboratory growth conditions.[3] In this regard, the most prolific discoveries have been associated with ribosomally synthesized and post-translationally modified peptides (RiPPs),[4] a family of natural products containing several dozen distinct compound classes with a diverse range of structures and biological activities.[5]

Unique additions to this class are the recently discovered pyridine-containing macrocyclic peptides, represented by

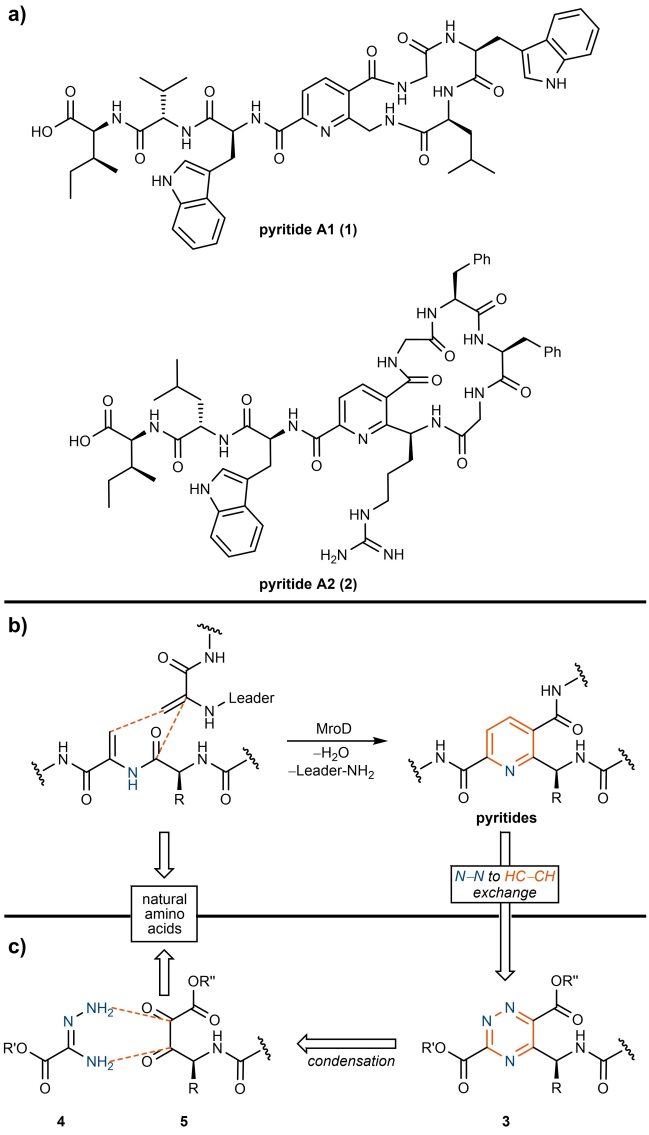
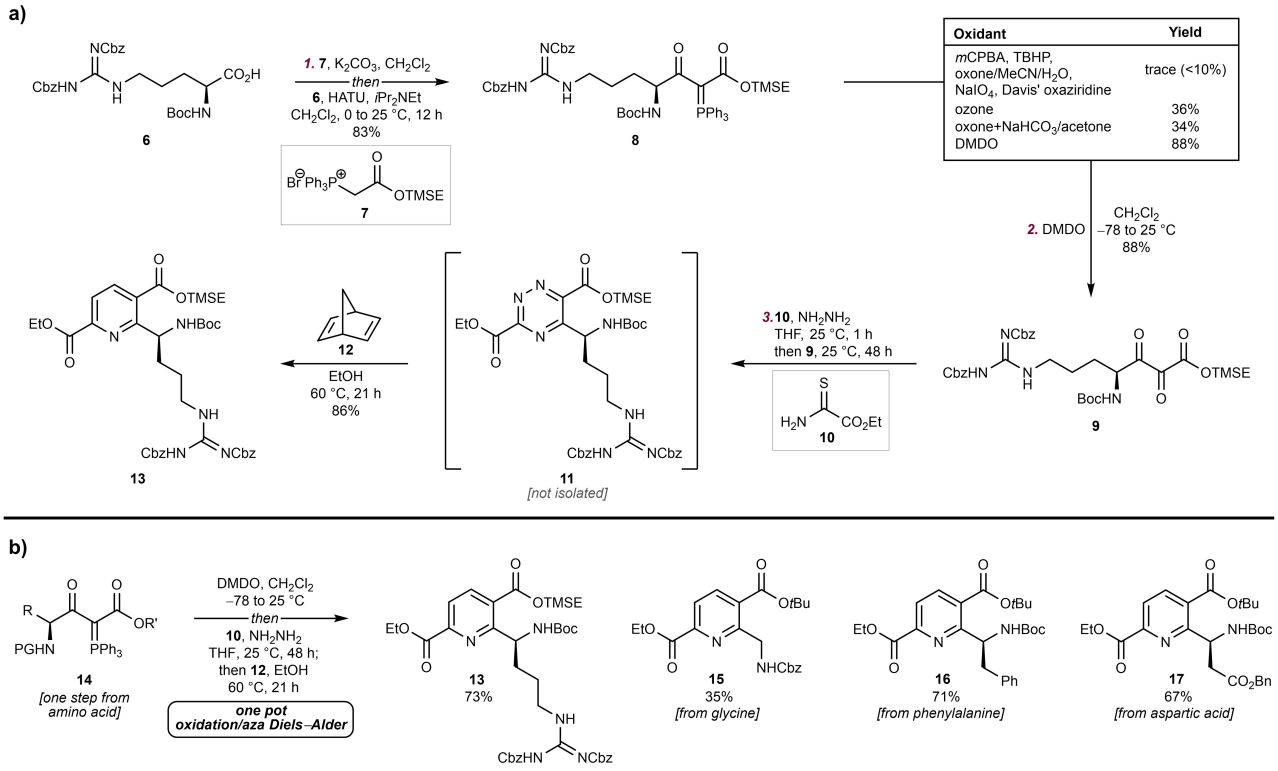


Figure 1. a) Structures of pyritides A1 (**1**) and A2 (**2**). b) Biosynthesis of pyritides involves MroD-mediated pyridine synthesis proceeding through formal cycloaddition of modified peptide residues. c) Retrosynthetic analysis of pyritides using aza-Diels–Alder cycloaddition to access trisubstituted pyridine core.

[*] A. R. Hooper, A. Oštrek, A. Milian-Lopez, Prof. D. Sarlah
Department of Chemistry and Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign
Urbana, IL 61801 (USA)
E-mail: sarlah@illinois.edu
A. Oštrek, Prof. D. Sarlah
Department of Chemistry,
University of Pavia
Viale Taramelli 12, 27100 Pavia (Italy)
E-mail: sarlah@unipv.it

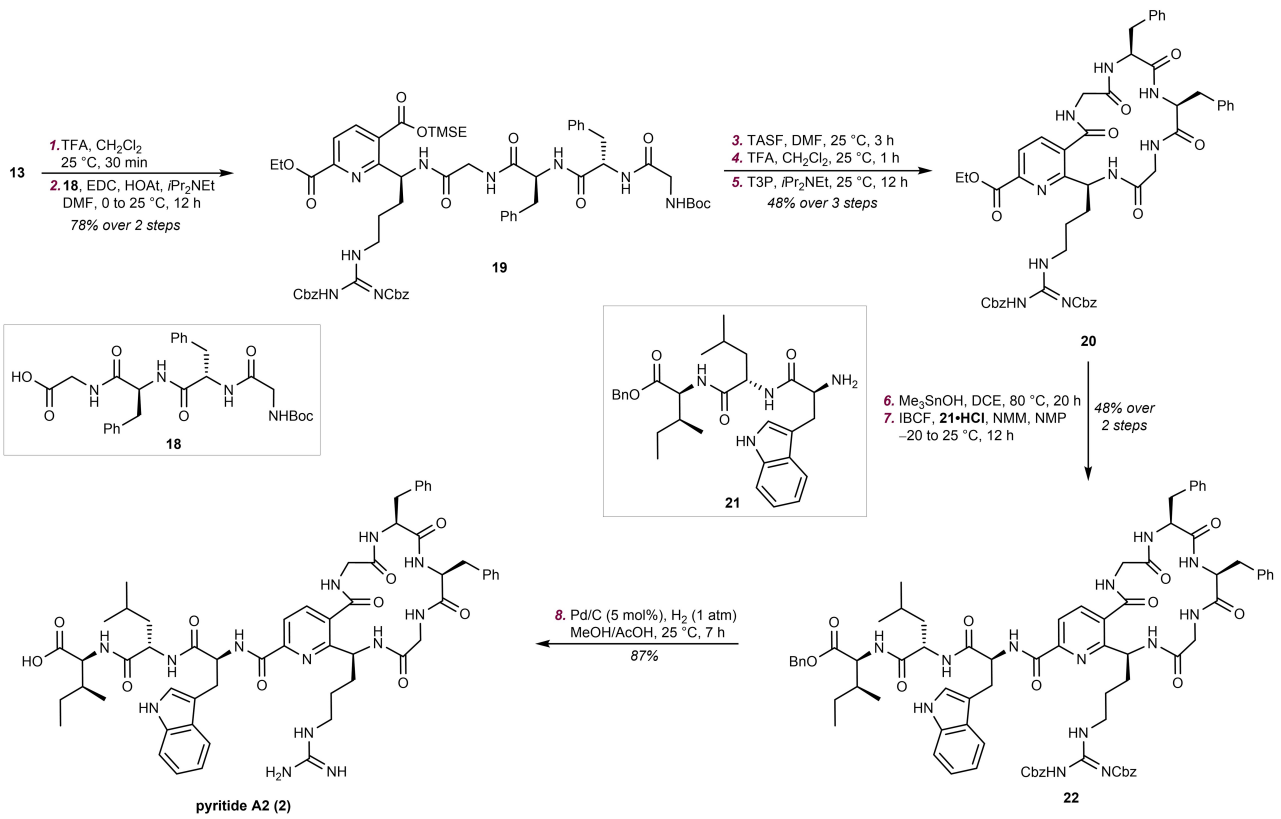


Scheme 1. a) Synthesis of pyridine **13** from arginine derivative **6**. b) Streamlined condensation/aza-Diels–Alder approach to several 2,3,6-trisubstituted pyridines related to pyritide class of natural products. Cbz = benzyloxycarbonyl, Boc = tert-butyloxycarbonyl, HATU = 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate, TMSE = 2-(trimethylsilyl)ethyl, *m*CPBA = *meta*-chloroperoxybenzoic acid, oxone = potassium peroxymonosulfate, DMDO = dimethyldioxirane.

the 14- and 17-membered pyritide A1 and A2 (**1** and **2**, Figure 1a).[6] Both pyritides **1** and **2** were identified through mining of the *Micromonospora rosaria* genome; however, they were not detected in isolates from the native producer. Extensive isolation efforts, including recombinant expression of key enzymes for chemoenzymatic synthesis, provided only milligram amounts of **1** and **2**. Our laboratory supported initial pyritide discovery studies by establishing an independent chemical synthesis of pyritide A1 (**1**), validating the structural assignment of this scarce natural product. However, our synthesis of **1** commenced from a 2,3,6-trisubstituted pyridine which prevented extension of this approach to members in this family containing a benzylic stereocenter. Herein, we report the total synthesis of pyritide A2 (**2**) through a general and convergent preparation of trisubstituted pyridine cores using natural amino acids.

The class-defining, post-translational modification for pyritide natural products is an enzyme-catalyzed formal [4+2]-cycloaddition that generates the pyridine core (Figure 1b).[6,7] The pyridine synthase, MroD, mediates the union of two dehydroalanine residues that concur with elimination of water and a leader peptide, ultimately resulting in the 2,3,6-trisubstituted pyridine-containing macrocycle. Though biogenetic pyridine formation is not feasible by chemical means, we envisioned a complementary synthesis that also involves the ring synthesis and direct utilization of α-amino acids (Figure 1c). Accordingly, the heterocyclic core could be derived from the 1,2,4-triazine **3** through an aza-Diels–Alder reaction that could convert the triazine to pyridine.[8] The triazine **3** could be traced back through condensation of amino acid-derived α,β-diketoester **5** and amidrazone **4**, making this strategy highly convergent and amenable to the preparation of many pyritide natural products, as analogous RiPPs have been proposed to arise from other bacterial species.[6]

In developing this synthetic plan, initial focus was placed on the preparation of a trisubstituted triazine from a suitable vicinal tricarbonyl system ultimately derived from readily available amino acids (Scheme 1). Since incorporation of amino acids in the synthesis of such intermediates is underexplored,[9] we conducted a series of prospecting experiments, including direct β-oxidation of α,γ-dicarbonyls and oxidation of the corresponding β-diazo or β-phosphorane intermediates,[10] to determine the most efficient way of installing or unmasking this sensitive motif (See Supporting Information for details). These studies revealed that diacylylide substrate **8**, prepared from the coupling reaction between suitably protected arginine **6** and the phosphorane derived from phosphonium salt **7**,[11] gave the most favorable outcome. Nevertheless, the conversion of **8** to tricarbonyl **9** proved challenging. Most established protocols for the oxidative cleavage of C=P bond, including the use of peroxides, oxone/H_2O, oxaziridines, gave either trace yields



Scheme 2. Completion of synthesis of pyritide A2 (**2**). TFA = trifluoroacetic acid, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOAt = 1-hydroxy-7-azabenzotriazole, DMF = *N*,*N*-dimethylformamide, TASF = tris(dimethylamino)sulfonium difluorotrimethylsilicate; T3P = propanephosphonic acid anhydride, DCE = 1,2-dichloroethane, IBCF = isobutyl chloroformate, NMM = *N*-methylmorpholine, NMP = *N*-methyl-2-pyrrolidone.

or resulted in decomposition of starting material (inset, Scheme 1a),[12] in line with reports that such tricarbonyl compounds are unstable under variety of reaction conditions.[13] Exposure to ozone delivered modest yield of product **9**;[14] however, further optimization efforts, even when utilizing indicators to control ozone equivalents, did not improve yields. Ultimately, we found that in situ generated DMDO gave a promising yield of 34 %, which was further improved to 88 % by using a freshly prepared solution.[15] With the desired precursor available, the key pyridine synthesis was explored next. Only a handful of reports describe triazine-based aza-Diels–Alder pyridine synthesis that can utilize an acetylene equivalent, such as 2,5-norbornadiene (**12**) and generate 2,3,6-trisubstituted pyridines.[16] Inspired by these reports, we sought to determine if such methodology could be translated to more elaborated substrates that previously reported, such as arginine-derived triazine **11**. Gratifyingly, upon exposure of tricarbonyl **9** to amidrazone, prepared from ethyl thioamido oxalate (**10**) and hydrazine, the desired triazine **11** was obtained and directly subjected to the aza-Diels–Alder by a solvent swap and addition of norbornadiene, delivering pyridine **13** in 86 % yield overall.

Given the rapid access to the heterocyclic core of pyritide A2 (**2**), we decided to further explore this chemistry, along with extending its generality to different amino acids (Scheme 1b). Due to the mild nature of diacyl phosphorane oxidation and benign byproducts (Ph_3PO and acetone), we were able to combine this step with the condensation/cycloaddition sequence, producing **13** in 73 % overall yield from phosphorane **8**. Moreover, this streamlined one-pot pyridine synthesis sequence was successfully translated to several other amino acids, namely glycine (**15**), phenylalanine (**16**), and aspartic acid (**17**) derivatives. All α-branched amino acid tricarbonyls **14** delivered the corresponding products **13**, **16**, and **17** as single consitutional isomers. On the other hand, glycine-derived precursor gave a separable mixture of desired pyridine **15** (35 %) and regioisomer (25 %, not shown). Notably, the pyridine **15** is the subunit of natural product pyritide A1 (**1**), while **16** and **17** present readily accessible pyritide core analogues.

With the arginine-derived pyridine core **13** in hand, the synthesis of macrocyclic portion of pyritide A2 (**2**) was undertaken next (Scheme 2). Thus, acid-mediated deprotection of the *tert*-butyloxycarbonyl group, followed by EDC/HOAt-mediated coupling with tetrapeptide **18** (see the Supporting Information for the synthesis of **18**), delivered linear peptide **19** and secured the full amino acid sequence of the macrocyclic portion of natural product. Concurrent acid-mediated deprotection of the 2-(trimethylsilyl)ethyl ester and amine *tert*-butyloxycarbonyl protecting groups did not occur as planned, as slow ester deprotection led to noticable decomposition of the substrate. Instead, a two-step protocol involving treatment with TASF and TFA delivered

the requisite amino acid substrate for macrocyclization. The formation of the 17-membered macrolactam proved more challenging than anticipated, as most benchmark reagents (HATU, PyBOP, Bop-Cl, and PyAOP) resulted in the formation of oligomers or negligible conversions to the desired macrolactam. Gratifyingly, we found that T3P gave the highest yield, providing macrocycle **20** in 48 % yield over the three steps from **19**.

With the macrocyclic core completed, the last task towards pyritide A2 (**2**) involved the installation of the side chain. Using standard conditions (NaOH or LiOH), the hydrolysis of ethyl ester was accompanied by significant deprotection of the guanidinyl benzyloxycarbonyl groups, which also occurred under milder conditions with $Ba(OH)_2$ or TMSOK.[17] Additionally, we explored methods for hydrolysis and direct amidation that are tailored for picolinate esters through chelating Lewis acid activation;[18] albeit, again we were faced with the lack of reactivity or with chemoselectivity issues involving arginine side chain deprotection. Gratifyingly, we found that application of Me_3SnOH[19] delivered the desired acid without any issues. Although the primary use of this reagent has been reserved for the selective methyl ester hydrolysis, its successful application with ethyl ester **20** is likely attributed to the assistance by the pyridine's nitrogen. The resulting acid was taken forward without purification and coupled with the tripeptide side chain **21•HCl**, delivering protected pyritide A2 (**22**) in 48 % yield over two steps. Final hydrogenolysis resulted in deprotection of the benzyl ester and release of the guanidine motif, securing pyritide A2 (**2**) in 87 % yield. The analysis of the reported ^{1}H NMR spectral data was in a good agreement with our synthetic sample. However, due to the challenges associated with the production and isolation of **2**, there was no ^{13}C NMR data available for comparison. Therefore, we obtained sub-milligram amounts of chemoenzymatically synthesized pyritide A (**2**) and further validated the authenticity of synthetic sample by HPLC co-injection as well as by comparison of MS/MS fragmentation patterns, confirming that both samples are indistinguishable (see the Supporting Information for details).

In summary, we accomplished the first synthesis of pyritide A2 (**2**), a member of a genome mining-discovered family of natural products characterized by a unique pyridine-containing macrocycle. Inspired by their biosynthesis, involving pyridine ring formation through post-translational modification of amino acids and cycloaddition, our approach involved elaboration of a suitably protected arginine derivative to vicinal tricarbonyl and subsequent pyridine synthesis through condensation and an aza-Diels–Alder reaction. Other salient features of this work include important lessons for macrocyclization and derivatizations of pyridine-containing peptides. This chemistry also enabled preparation of several different pyridines in two steps from commercial amino acids and can guide the synthesis of analogues associated with this class of compounds. Lastly, this work demonstrates the utility of chemical synthesis in providing practical access to predicted natural products,[20] which will hopefully inspire future studies of genome-mined natural products not readily produced by biological means.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Genome Mining · Macrocycles · Post-Translationally Modified Peptides · Pyridines · Total Synthesis

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