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Total Synthesis of Leupyrrin B_1: A Potent Inhibitor of Human Leukocyte Elastase

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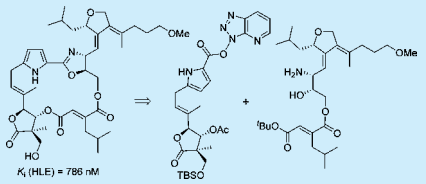
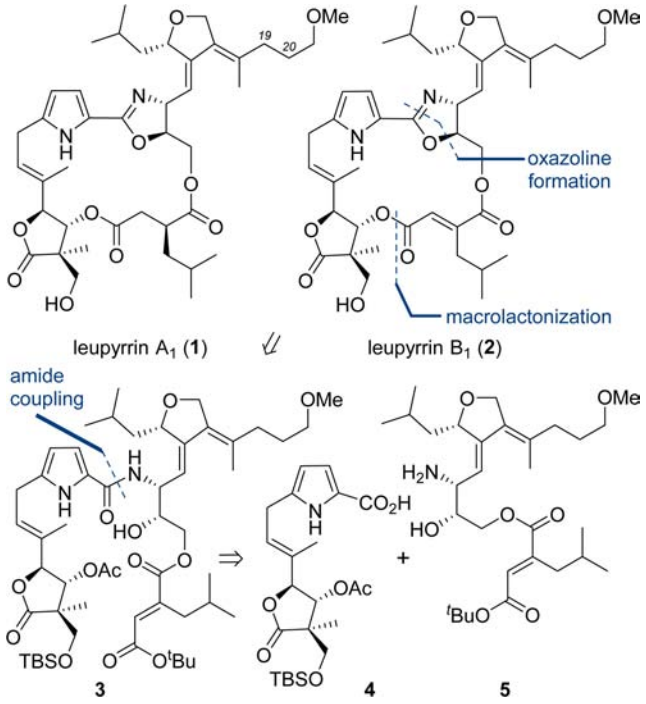
Supporting Information

ABSTRACT: The total synthesis of leupyrrin B_1 was accomplished by an expedient strategy that involves an optimized HATU-mediated amide coupling protocol of elaborate substrates. The generally useful procedure was also successfully applied in an improved total synthesis of leupyrrin A_1. Finally, leupyrrins A_1 and B_1 were evaluated toward a panel of proteases, and human leukocyte elastase was discovered as a molecular target of the leupyrrins.



Leupyrrins, which were first described by Höfle and Reichenbach as constituents of *Sorangium cellulosum*,[1] hold a special place among myxobacterial natural products,[2] as they originate from one of the most extraordinary and diverse biosyntheses reported for these gliding bacteria.[3−5] Furthermore, leupyrrins exhibit very potent antifungal activities, antiproliferative, and anti-HIV properties.[1,6] They efficiently inhibit DNA, RNA, and protein syntheses without disrupting other cellular systems.[1] However, a molecular target has not been defined to explain their remarkable bioactivities. A range of conventional targets have been excluded.[1] As shown in Scheme 1 for the most prominent representatives, leupyrrins A_1 (**1**) and B_1 (**2**), their unique architectures are characterized by an unusual 18-membered nonsymmetric macrodiolide core that incorporates a unique γ-butyrolactone, an oxazoline, and a pyrrole moiety and is connected to a side chain containing a singular dihydrofuran with two exocyclic alkylidene elements.[7] The leupyrrins possess up to seven stereogenic centers that were recently assigned by high-field NMR studies in combination with molecular modeling and derivatization.[8]

Scheme 1. Retrosynthetic Analysis of Leupyrrin B_1 (2)



The important biological properties of the leupyrrins with their natural scarcity, unique biosynthesis, and intriguing molecular architectures have attracted considerable interest of the synthetic community,[9] including one total synthesis of leupyrrin A_1 that was accomplished in our group.[8] Herein, we report the total synthesis of leupyrrin B_1 by a concise strategy that involves an improved HATU-mediated amide coupling protocol of two highly elaborate fragments, further application of this generally useful procedure in an improved total synthesis of leupyrrin A_1, and identification of a first molecular target of this unique class of myxobacterial natural products.

As outlined retrosynthetically in Scheme 1, our approach toward leupyrrin B_1 relied on a convergent assembly of two elaborate building blocks of similar complexity (i.e., the Eastern and Western subunits **4** and **5**). Fragment union was envisioned by a challenging amide coupling to give the fully functionalized backbone **3**, which should then be converted to **2** by oxazoline condensation and macrolactonization. While a similar strategy has recently been reported for leupyrrin A_1, this route was limited by a related but unfavorable amide coupling,[10] which could not be successfully applied to the synthesis of **2** (vide infra). Thus, an optimized amide coupling protocol to enhance the supply of these scarce metabolites for further biological investigations was required.

As shown in Scheme 2, two different olefination approaches to the diacid building block **14** were evaluated. While a Wittig

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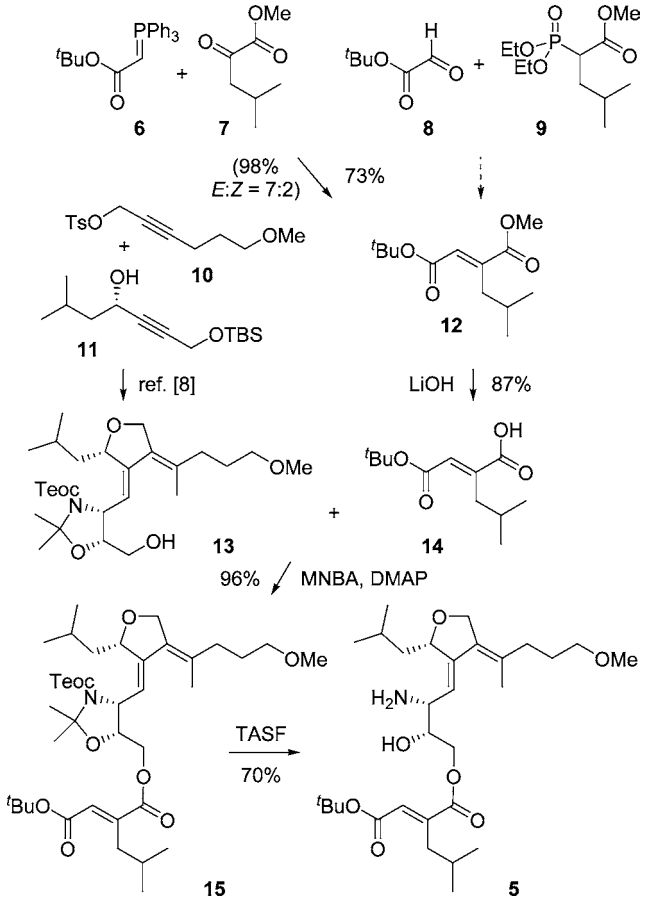
Scheme 2. Construction of the Eastern Fragment 5



reaction of ylide **6** with ketoacid **7** proceeded smoothly, giving the desired diester **12**[11] in high yield, only traces of **12** were observed in the HWE coupling of phosphonate **9**[12] with aldehyde **8**.[13] Careful saponification of **12** with LiOH then gave the desired monoacid **14** in 87% yield. The dihydrofuran coupling partner was obtained from alkynes **10** and **11** by a route previously developed in our group involving a one-pot zirconocene-mediated cyclization and regioselective opening sequence.[8,9c,d] Coupling of the terminal alcohol functionality of **13** with acid **14** proceeded in excellent yields following the Shiina protocol.[14] Finally, liberation of the vicinal aminoalcohol with TASF gave the desired Eastern fragment **5** in an effective manner.[15]

During subsequent efforts to apply a previously used protocol[8] for the key amide coupling of building blocks **4** and **5** (see Scheme 3), only very low yields of **3** (<20%) were obtained. Therefore, this coupling procedure was first optimized[8,10,16] by studying the model reaction of the simplified pyrrole carboxylic acid **16** with isopropylamine. As shown in Table 1, low yields were initially obtained following an original HATU protocol, involving 2.0 equiv of HATU and increased temperatures (entry 1).[8,16] During these studies, it became apparent that formation and isolation of the intermediate HATU derivative **17** proved to be difficult. In contrast, the coupling of separately prepared **17** with isopropylamine proceeded quantitatively. Hence, we focused on the initial formation of azabenzotriazole **17**.[17] Decreasing the reaction temperature dramatically increased the yield of **17** (entry 2), while an even improved conversion was observed by reducing excess HATU (entry 3). Under these

Scheme 3. Synthesis and Efficient Amide Coupling of the Western and Eastern Fragments 4 and 5

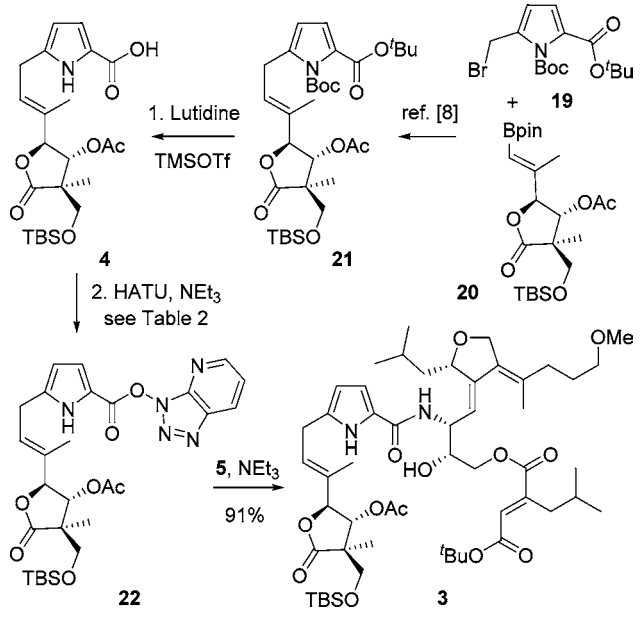
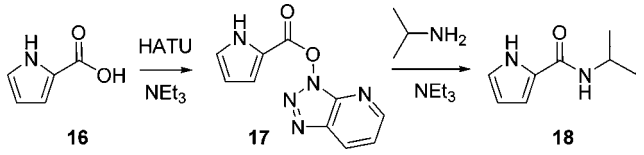


Table 1. Development and Optimization of Reaction Conditions for Coupling to the Simplified Amide 18[a]



entry	HATU (equiv)	temperature (°C)	yield (%)[b]
1	2.0	40	<20
2	2.0	−30	70
3	1.2	−30	90

[a]Reactions were performed in MeCN (0.04 M) and NEt_3 (2.0 equiv) at the indicated temperature. After 2 h, isopropylamine (1.0 equiv) was added and the mixture was stirred for 30 min at the same temperature. [b]Isolated yields over two steps.

optimized conditions, isolation of **17** and subsequent amide coupling to **18** proceeded smoothly in high yields (90%).

With this improved protocol in hand, we turned again our attention to the authentic fragments. As shown in Scheme 3, the synthesis of the required Western building block **4** involved a previously established sp^2–sp^3 coupling of **19** with **20**[8] and a subsequent simultaneous removal of the Boc- and *tert*-butyl ester group of resulting **21** under Lewis acidic conditions with TMSOTf. Next, it was intended to prepare azabenzotriazole **22**. However, initially, no turnover of **4** with HATU was observed at low temperatures (Table 2, entry 1). Also, heating the reaction resulted in only low yields, presumably due to decomposition (entry 2). It became apparent that isolation of the free acid **4** prior to the amide coupling was crucial for the conversion toward **22** as traces of lutidine appeared to inhibit the reaction. Consequently, after intermediate isolation of **4**, high yields of **22** (77% over two steps from **21**) could be obtained at low temperatures under the optimized conditions developed above (2.0 equiv of NEt_3, 1.2 equiv of HATU, entry 3). To the best of our knowledge, **22** represents the most advanced azabenzotriazole-activated substrate that was isolated and used in such a

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Table 2. Formation of Azabenzotriazole 22 from Acid 4[a]

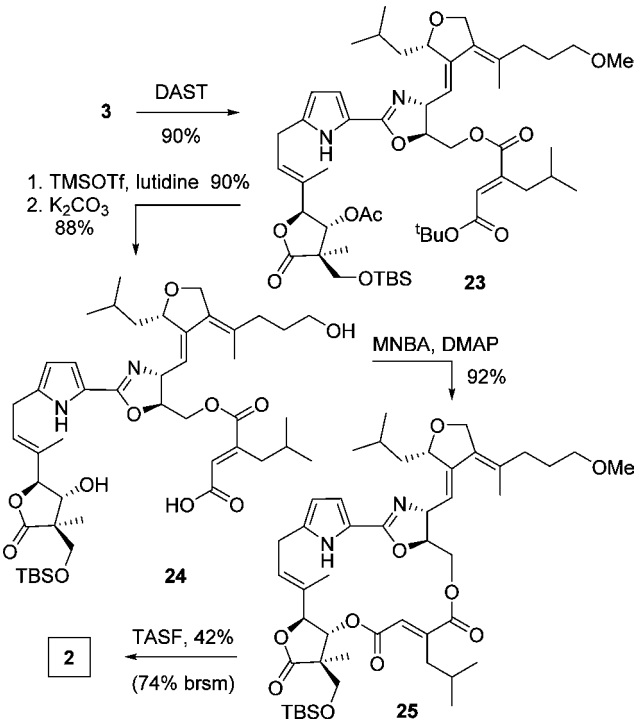
entry	temperature (°C)	source of 4	yield of 22 (%)[b]
1	−30	crude product used	0
2	40	crude product used	<10
3	−30	purified compound[c]	77

[a]Reaction step 2 was performed with **4**, in MeCN (0.04 M), NEt_3 (2.0 equiv), and HATU (1.2 equiv) under stirring for 2 h at the indicated temperature. [b]Isolated yield of **22** over two steps. [c]Intermediate isolation of **4** (91%).

complex target synthesis. With an efficient approach to **22** in hand, the amide coupling with **5** then proceeded smoothly at low temperature, giving the desired amide **3** in 91% yield. Notably, only 1 equiv of **22** was needed, in contrast to the original procedure that used an excess of **4**.[8,10]

As shown in Scheme 4, completion of the total synthesis of **2** was continued by cyclodehydration of **3** with DAST to give

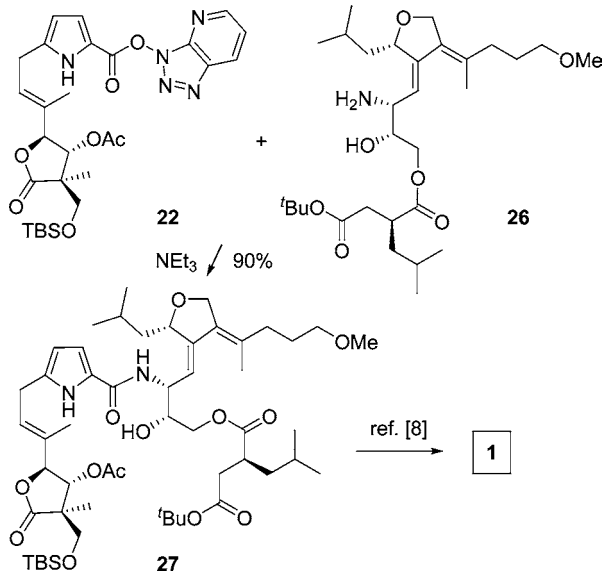
Scheme 4. Completion of the Total Synthesis of Leupyrrin B_1



oxazoline **23** in 90% yield.[18] Cleavage of the *tert*-butyl ester group was then accomplished by treatment with TMSOTf followed by careful saponification of the acetate group using potassium carbonate in a methanol/THF/water mixture, giving the *seco*-acid **24** in high yields over these two steps (90 and 88%). Utilizing the Shiina protocol[14] under high dilution conditions (0.001 M), excellent yields (92%) were obtained in the macrocyclization to **25**. Finally, removal of the remaining primary TBS group with TASF in acetonitrile appeared more difficult than in the total synthesis of leupyrrin A_1 (**1**). Under the same conditions (6.0 equiv of TASF over 5.5 h), only partial reaction (ca. 20%) was observed. Additional equivalents of TASF increased the conversion, but the extended reaction time also led to decomposition of the desired product. Therefore, the conditions were adjusted to gradual addition of 6.0 equiv of TASF over 2 h with only 30 min of additional reaction time before addition of saturated NH_4Cl solution. Under these conditions, leupyrrin B_1 (**2**) was obtained in acceptable 42% yield (74% brsm) with a fair amount (44%) of starting material. The spectroscopic data (^1H and ^{13}C NMR), specific rotation (synthetic: $[\alpha]_D^{20}$ = +3.0, *c* = 0.1, MeOH; natural: $[\alpha]_D^{20}$ = +2.7, *c* = 1.0, MeOH), and CD spectra of our synthetic material were in agreement with data acquired for an original sample of leupyrrin B_1, confirming its structural assignment.[19]

The general usefulness of the optimized amide coupling strategy was then demonstrated in an improved total synthesis of **1**. Following the procedure, again only 1 equiv of **22** and **26** was necessary to obtain amide **27** in excellent yield, which compares favorably to the previous route.[8,10] Notably, this increases the overall yield of the longest linear sequence to **1** from 6.3 to 8.2% (Scheme 5).

Scheme 5. Application of the Amide Coupling Protocol for an Optimized Total Synthesis of Leupyrrin A_1 (1)



With a reliable route to leupyrrins A_1 and B_1 in hand, efforts were then directed toward discovery of a molecular target. A panel of proteases was investigated as potential targets of leupyrrins A_1 and B_1. We evaluated the inhibitory effects of the two natural products against different proteases, that is, the human leukocyte elastase (HLE),[20] a serine protease, and the human cysteine proteases cathepsins B and L,[21] as well as bovine chymotrypsin[22] and bovine trypsin, both serine proteases.[23] These studies revealed that leupyrrins A_1 and B_1 represent potent HLE inhibitors with IC_{50} values of 1.10 ± 0.36 and 2.24 ± 0.36 μM. Under the assumption of a competitive mode of action, these data relate to K_i values of 124 ± 17 and 786 ± 125 nM for **1** and **2**, respectively (see Supporting Information). No effects against the other proteases were detected, demonstrating a distinct selectivity profile. Due to its involvement in inflammatory processes, such as in chronic obstructive pulmonary disease, HLE is considered to be a target for drug development.[24] Several types of low molecular weight inhibitors for HLE are known, mainly peptidic or heterocyclic structures with an electrophilic function, prone to covalently interacting with the active site serine residue of the protease.[24] Cyclic natural compounds, such as erythromycin,[25] and cyclic peptides and depsipeptides, such as brunsvicamides, lyngbyastatins, and scyptolins, have also reported as HLE inhibitors.[22,24,26] HLE exhibits a primary

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substrate specificity for small aliphatic residues also present in leupyrrins, which might explain their affinity for HLE.

In summary, the total synthesis of leupyrrin B_1 (**2**) was accomplished in 21 steps (longest linear sequence) from known compounds **10** and **11** with 4.0% overall yield. The synthesis unequivocally confirms the constitution and full stereochemistry of this macrodiolide. An important feature of our strategy to the leupyrrin class of natural products involves an optimized HATU-mediated amide coupling protocol relying on the isolation of a preactivated highly functionalized substrate. This generally useful procedure was successfully applied in an improved total synthesis of leupyrrin A_1. Expectedly, the reported HATU coupling protocol will be applicable in other complex syntheses. The routes reported herein were amenable for preparing significant amounts that allowed for biological evaluation and led to the discovery that human leukocyte elastase constitutes a potential molecular target of the leupyrrins. Present studies are now directed to explore the interaction of leupyrrins with elastase in more detail and to further elucidate the biological potential and extraordinary biosynthesis of this remarkable class of myxobacterial metabolites.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01724.

Detailed experimental procedures, characterization, and copies of ^{1}H and ^{13}C NMR spectra of new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(10) In our original procedure,[8] the HATU-mediated in situ activation and coupling of **4** with **26** required an excess (1.3 equiv) of the valuable Western fragment **4**. Otherwise, the yield dropped significantly.

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(15) Careful monitoring was required to minimize migration of the diacid to the secondary alcohol. Optimum conditions involved an excess of TASF at low temperatures to afford a 7:3 mixture favoring the desired ester **5**. The mixture proved inseparable and was hence directly used in the amide couplings. Separation was effected after synthesis of **23**.

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