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Synthesis and biological evaluation of moiramide B derivatives†

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Moiramide B is a peptide–polyketide hybrid with a bacterial origin and interesting antibiotic activity. Besides its structurally conserved peptide part, it contains a highly variable fatty acid side chain. We modified this part of the molecule by introducing a terminal alkyne, and we then subjected it to click reactions and Sonogashira couplings. This provided a library of moiramide B derivatives with high and selective *in vivo* activities against *S. aureus*.

Introduction

There is absolutely no question that the development of antimicrobial resistance is a global threat to humanity and that the development of new antibiotics with new modes of action is urgently needed to counter this danger. Broad-spectrum antibiotics are essential for the treatment of infections with often unknown pathogens, while narrow-spectrum antibiotics are predestined for the targeted treatment of a known pathogen, as collateral damage to the human microbiome can be avoided more easily.

In 1987, Komura *et al.* described the isolation of andrimid from *Enterobacter* sp.[1] A few years later, Andersen *et al.* isolated andrimid together with moiramides A–C (Fig. 1) from *Pseudomonas fluorescens*, with andrimid and moiramide B showing potent *in vivo* antibiotic activity.[2] The biosyntheses of these compounds occur in a nonribosomal peptide synthetase–polyketide synthase hybrid, generating the unsaturated fatty acid (green), the β-phenylalanine (red) and the unusual valinyl-succinimide unit (blue) from valine, glycine and malonyl-CoA.[3]

As an inhibitor of the carboxyltransferase component of acetyl-CoA carboxylase (ACC), moiramide B affects fatty acid biosynthesis in bacteria and thus their cell wall assembly.[4] The crystal structure of moiramide B bound to carboxyltransferase revealed that the succinimide head group tightly binds to the oxyanion hole of the enzyme in its enol or enolate form, while the valine-derived sidechain fills a hydrophobic pocket. The β-phenylalanine fragment increases the binding affinity by forming additional hydrogen bonds with the target. In contrast, the fatty acid unit does not bind strongly to the target but plays an important role in the *in vivo* activity, as it is likely responsible for the transport of the compound into the bacterial cell.[5]

It is therefore not surprising that this class of substances came to the attention of synthetic chemists. Two years after its isolation, Komura *et al.* accomplished the first total synthesis of andrimid. The succinimide unit was constructed by enolate alkylation of a valine-derived β-ketoamide, followed by ring closure.[6] In an alternative approach, Rao *et al.* acylated racemic 3-methylsuccinimide with the imidazolide of protected valine and separated the obtained diastereomers.[7] The first total synthesis of moiramide was reported by Davies *et al.* in 1996. The stereogenic center in the pyrrolidinedione fragment was constructed *via* an auxiliary-based enolate alkylation. After cyclization to the succinimide, the valinyl unit was introduced by acylation with the Boc-protected *N*-carboxyanhydride of L-valine (Boc-Val-NCA).[8] Very recently, we reported an alternative approach generating the succinimide subunit *via* a Pd-catalysed allylic alkylation.[9]

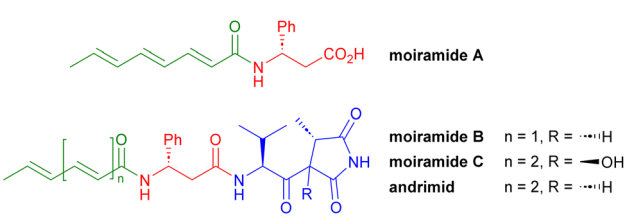

Fig. 1 Structures of moiramides A–C and andrimid.

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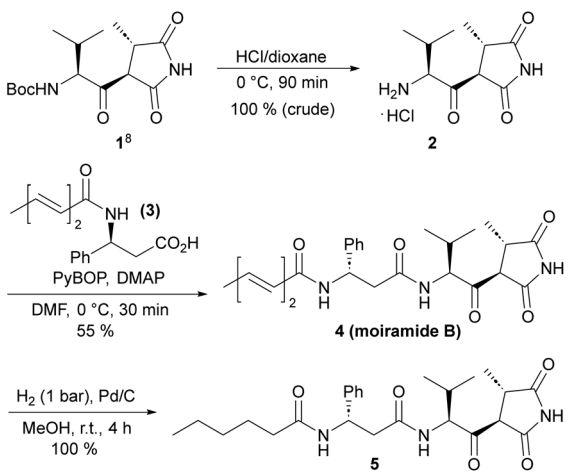
Since the first total syntheses of andrimid and moiramide B, several derivatives for structure–activity relationship (SAR) studies have been prepared by both chemical synthesis[4a,6,10] and combinatorial biosynthesis.[11] SAR studies showed that the succinimide fragment allows little variation, whereas the valine side chain can be replaced by other nonpolar groups. Substitution of the β-phenylalanine side chain with other aromatic residues is also possible.

In particular, the fatty acid unit is variable as long as apolar residues are employed. Therefore, this part of the molecule should be best suited for further derivatisations. Recently, moiramide B-based bivalent inhibitors of both the biotin carboxylase and the carboxyltransferase subunit of ACC have been reported, where the fatty acid moiety was replaced by a lipophilic linker.[12]

Results and discussion

For several years, our group has been involved in the total synthesis of natural products,[13] preferentially with anticancer[14] and antibiotic activities,[15] and we thus became interested in moiramides as well. As previous SAR studies on moiramide B and andrimid derivatives indicated, the fatty acid side chain is structurally highly variable. Therefore, we aimed to develop a synthesis that allows late-stage modifications of this subunit. For the construction of succinimide building block **1**, we used the established protocols by Davies *et al.*[8] The conversion of **1** into natural product **4** was accomplished by slightly modified literature procedures, that is, Boc deprotection to hydrochloride **2** and the PyBOP/DMAP-mediated coupling with acid **3** (Scheme 1). The first moiramide derivative, **5**, could be obtained in quantitative yield by catalytic hydrogenation of the fatty acid side chain.

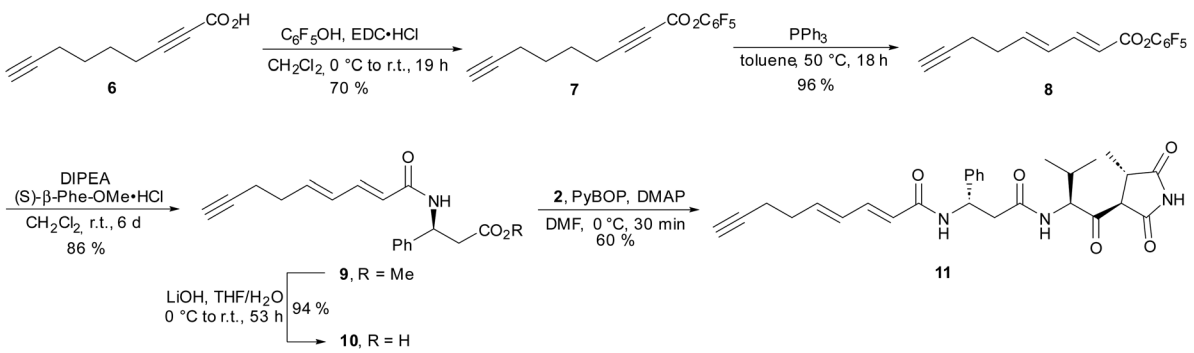


Scheme 1 Synthesis of moiramide B and its tetrahydro derivative **5**.

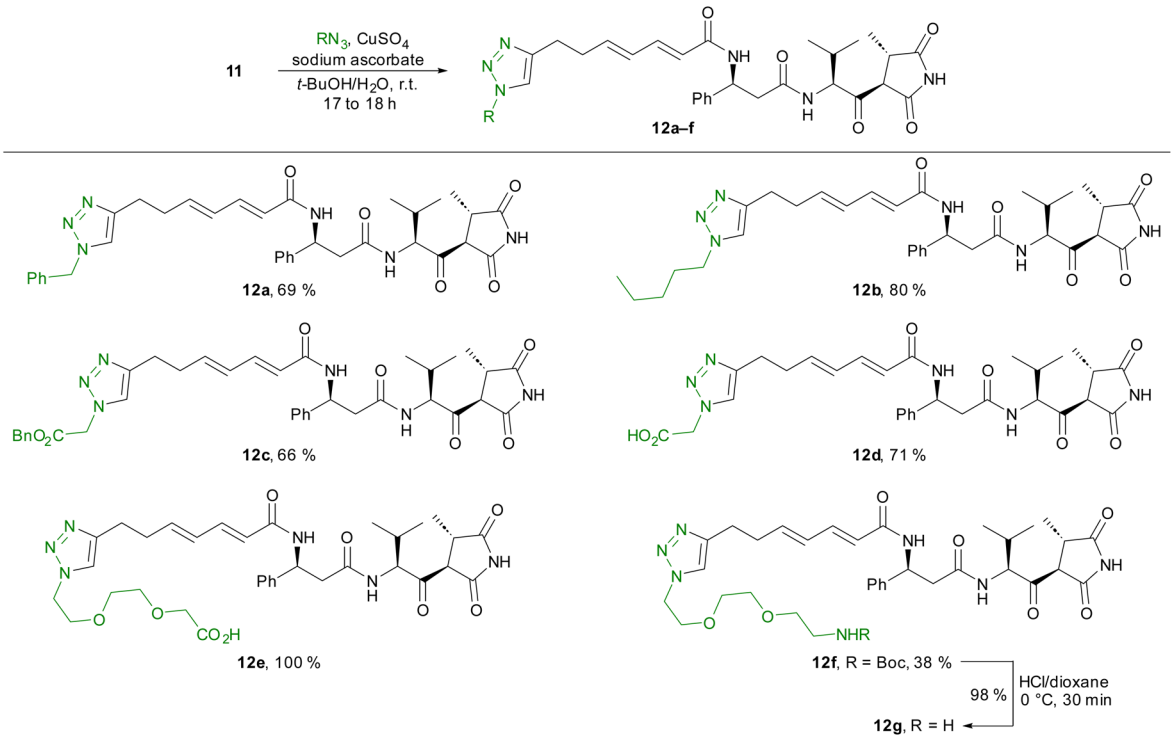
Next, we focused on the preparation of a modified fatty acid building block bearing a terminal alkyne (Scheme 2). Starting from nonadiynoic acid **6**, we prepared pentafluorophenyl ester **7**, which could be smoothly and selectively isomerized to dienoic acid ester **8** using triphenylphosphine as a catalyst.[16] Remarkably, the terminal alkyne remained unaffected under these conditions because the isomerization is limited to electron-deficient alkynes.[16a] The reaction of active ester **8** with (*S*)-β-phenylalanine methyl ester proceeded slowly but cleanly, yielding amide **9**. After saponification of the methyl ester, reaction of **10** with amine **2** provided moiramide derivative **11**.

Starting from compound **11**, we prepared several triazole derivatives **12a–f** using copper-catalysed azide–alkyne cycloadditions (CuAAC) using nonpolar (**12a–c**) as well as polar (**12d**) azides and linkers bearing terminal carboxylic acid or amino groups (**12e–g**) (Scheme 3).

As an additional modification, we subjected alkyne **11** also to Sonogashira couplings using various aryl iodides. If we used the classical conditions [$Pd(OAc)_2$/PPh_3/CuI or $PdCl_2(PPh_3)_2$/CuI as the catalyst and Et_2NH or NEt_3 as the solvent],[17] we observed no conversion, at least at room temperature. After increasing the reaction temperature to 50 °C, full consumption of the starting material was observed but we could isolate only trace amounts of the desired cross coupling products. Unfortunately, switching to a copper-free protocol (XPhos Pd G3 as the catalyst, Cs_2CO_3 as base and MeCN as the solvent)[18] did not increase the yield or the purity of the Sonogashira coupling products either. We assumed that the problems stemmed from the β-ketoamide moiety, which may be coordi-



Scheme 2 Synthesis of moiramide derivative **11** with a modified fatty acid bearing a terminal alkyne.



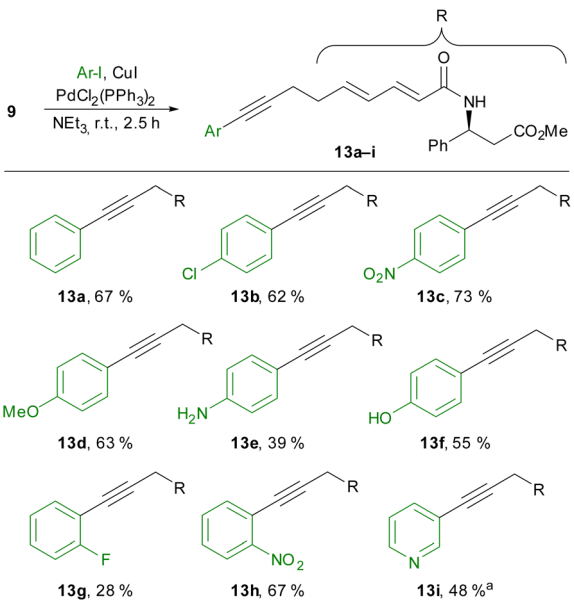
Scheme 3 Synthesis of triazole derivatives **12a–g** *via* CuAAC.

nating to the palladium or copper and/or leading to side reactions under the basic conditions.

To circumvent these difficulties, we then performed the cross-coupling reaction at an earlier stage without the β-ketoamide in place. While the conversion of carboxylic acid **10** was slow due to the formation of an insoluble ammonium carboxylate, the Sonogashira coupling of methyl ester **9** with several aryl iodides succeeded (Scheme 4). Unsubstituted iodobenzene (**13a**), as well as electron-poor (**13b–c**) and electron-rich (**13d–f**) *para*-substituted aryl iodides, could be employed in this reaction. A free aniline or phenol was also tolerated, but the yields decreased in these examples because of competing alkyne homodimerisation.

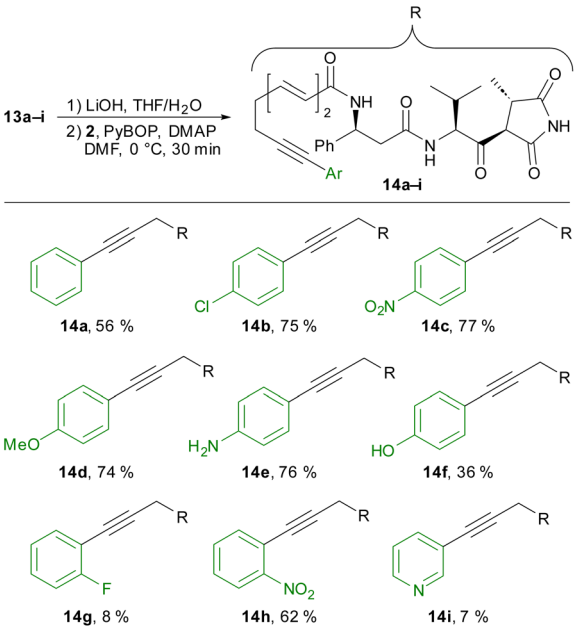
In principle, *ortho*-substituted aryl iodides could also be used (**13g,h**), although a rather low yield was obtained in the case of 1-fluoro-2-iodobenzene (**13g**). With 3-iodopyridine, we could also employ a heteroaryl iodide if the reaction temperature was increased to 60 °C (**13i**).

We completed the synthesis of moiramide derivatives **14a-I** by saponification of the methyl ester, followed by coupling with amine **2** (Scheme 5). In most cases, we obtained the desired coupling products in good yields. Notably, amide coupling in the presence of a free aniline succeeded without difficulty (**14e**). In contrast, crude product **14f** bearing a free phenol was initially impure and required further purification *via* preparative HPLC. In the case of *ortho*-fluorophenyl and 3-pyridyl derivatives **14g** and **14f**, the yields decreased significantly. Nevertheless, these products could also be isolated in sufficient quantity for biological testing.



Scheme 4 Sonogashira coupling of alkyne **9** with various aryl iodides. [a] The reaction was performed for 2 h at 60 °C.

Next, we measured the antibiotic activities of all compounds against *B. subtilis* and *S. aureus* as Gram-positive bacteria and *E. coli acrB* as Gram-negative bacteria (Table 1). Although moiramide B (**4**) was highly active against all bacterial strains tested, the MIC (minimum inhibitory concen-



Scheme 5 Synthesis of arylated moiramide derivatives **14a–i**.

Table 1 MIC [μg mL^{-1}] and IC_{50} [μg mL^{-1}] of the synthesized moiramide derivatives

compound	*S. aureus* Newman	*B. subtilis* [DSM-10]	*E. coli acrB* [JW0451-2]	HepG2
4 (moiramide B)	1–2	1–2	4	>37
5	4–8	16	32	>37
11	4	4–8	16–32	>37
12a	>64	>64	>64	>37
12b	>64	>64	>64	>37
12c	64	64	>64	>37
12d	>64	>64	>64	>37
12e	>64	>64	>64	>37
12f	>64	>64	>64	>37
12g	>64	>64	>64	>37
14a	1	2–4	>64	>37
14b	2	4	>64	>37
14c	2	8–16	>64	>37
14d	2	16	>64	>37
14e	1	8–16	64	>37
14f	1	4	32–64	>37
14g	2	8	64	>37
14h	2	8–16	32–64	>37
14i	8	>64	>64	>37

tration) values increased by a factor of 4–16 for the saturated derivative **5**. To our delight, alkynyl derivative **11** also displayed antibiotic activity against all three strains, although with increased MIC values compared with the natural product. Introducing a triazole moiety was poorly tolerated independent of the substituent on the triazole. None of the triazole derivatives showed any significant activity.

Better results were obtained with the aryl-substituted derivatives. For these compounds, the activities against Gram-positive bacteria were similar to those of the natural products and significantly higher than for the unsubstituted alkyne **11**.

The phenyl, *para*-amino and *para*-hydroxy derivatives, **14a**, **14e** and **14f**, respectively, were notable with MIC values of 1 μg mL^{-1} against *S. aureus*. All derivatives **14a–i** showed potent activity against *S. aureus*, and a little weaker activity against *B. subtilis*. Interestingly, little or almost no activities was observed against *E. coli*, in contrast to moiramid B. Also no activity was observed against a wide range of Gram-negative and -positive bacteria as well as fungi and yeasts such as *Candida albicans* (for details see ESI†). Additionally, none of the compounds tested showed cytotoxicity against HepG2 cells.

Conclusions

For the synthesis of moiramide derivatives, we prepared a central building block **9**, with a modified moiramide fatty acid side chain *via* triphenylphosphine-catalysed regioselective isomerisation. Starting from this building block, we prepared various moiramide derivatives in only a few steps. Triazole derivatives could be prepared from moiramide analogue **11** *via* CuAAC, but showed almost no antibiotic activity. In contrast, various aryl-substituted derivatives prepared *via* Sonogashira coupling were highly active and selective against *S. aureus*. This late-stage modification should allow the synthesis of moiramide B libraries to further optimize antibacterial activities and physicochemical properties.

Data availability

Copies of ^{1}H and ^{13}C NMR spectra, GC chromatograms and experimental details.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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