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Syntheses of bottromycin derivatives *via* Ugi-reactions and Matteson homologations†

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New bottromycin derivatives have been prepared using flexible Ugi and Matteson reactions. The Ugi reaction allows the fast and direct assembly of sterically hindered peptide fragments, while the Matteson homologation is excellently suited for the stereoselective synthesis of unusual amino acids like β-methylphenylalanine. Some of the new compounds show excellent activity against *Streptococcus pneumoniae*.

Introduction

The rise of antimicrobial resistance (AMR) causes severe healthcare problems and, based on urgency and the need for new antibiotics, multidrug-resistant pathogens are classified as the highest priority by the World Health-Organisation (WHO).[1] Such pathogens can develop resistance towards almost all currently used antibiotics, so the development of new antimicrobial agents, with new modes of action, is highly desired.[2]

In 1957, Waisvisz *et al*. reported the isolation of a new peptide from the fermentation broth of *Streptomyces bottropensis* and called it bottromycin.[3] Biosynthetically, bottromycins are formed from ribosomally synthesized peptides *via* post-translationally modifications.[4] Mode of action (MoA) studies indicated that bottromycin inhibits protein biosynthesis by binding to the aminoacyl-tRNA binding site (A site) of the 50S ribosome.[5] This biological target would avoid the cross-resistance issue but, to date, has not been addressed by any other antibiotic. Therefore, bottromycin is effective against problematic bacterial strains such as vancomycin-resistant *Enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA).[4b]

Although, the bottromycins were discovered and described as early as the late 1950′s, only one total synthesis of bottromycin A_2 and analogues by Ōmura and Sunazuka *et al*. has been reported.[6] A reason for this may be several incorrect structures proposed during these early years.[7] The latest, and correct, proposal, as confirmed by the first total synthesis, was described by Shipper in 1983 (Fig. 1).[8]

Several research groups,[9] including our own,[10] have carried out synthetic studies towards the various bottromycin building blocks and proposed structures. Researchers at AiCuris GmbH (Germany) saponified the C-terminal methyl ester of biotechnologically produced bottromycin and converted the acid into several amides.[11] Further modifications on this C-terminal unusual amino acid were carried out by Ōmura and Sunazuka based on their previously developed total synthesis.[6] Structure–activity relation (SAR) studies on these different derivatives indicate that especially the β-substituted phenylalanine is required for high activity. Obviously, the methyl group on the phenylalanine influences the conformation of the side chain and probably of the whole molecule as indicated by ^{1}H-NMR.[6] Interestingly, while the C-terminal thiazolyl amino acid ester is not required for activity, the free amino acid or its complete removal render the derivatives almost inactive. However, replacing the amino acid by a simple benzylamide had no significant effect on the activity. Clearly, only an amide bond is required, and an aromatic substituent has a positive effect on the activity.

In the context of our interest in the total synthesis of natural products with anticancer[12] or antibiotic[13] activities, we were motivated to develop a flexible synthetic approach towards bottromycin derivatives (Fig. 2) which should allow us to readily modify the core structure of the molecule.

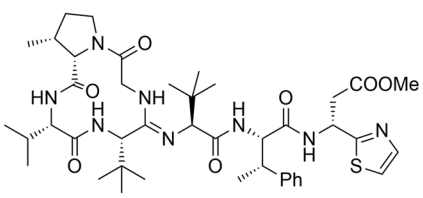

Fig. 1 Bottromycin A_2.

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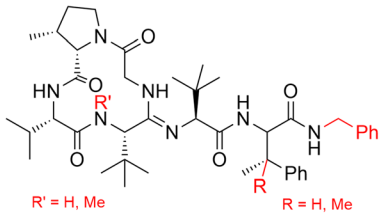


Fig. 2 Modification of bottromycin.

Results and discussion

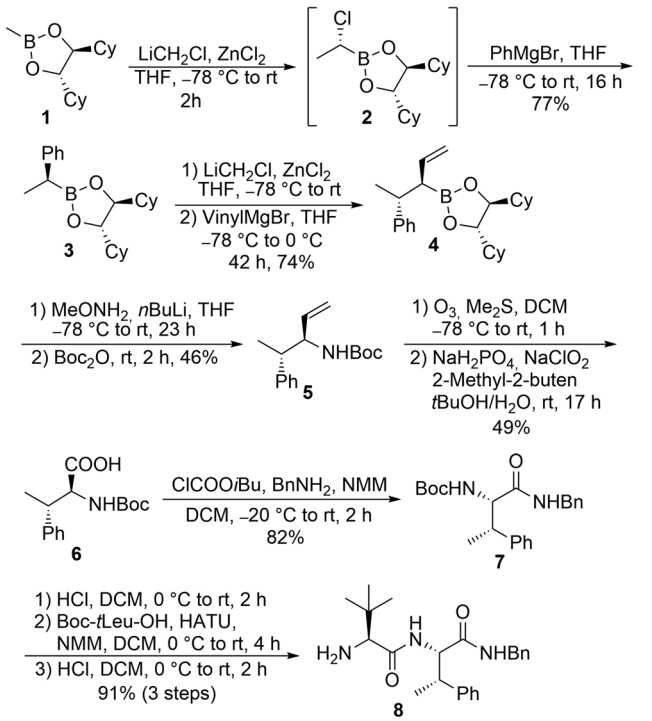
For a flexible synthesis of the side chain, we investigated two different approaches. First, a synthesis of the β-branched phenylalanine should give us the freedom to modify this amino acid. Because β-methylphenylalanine (MePhe) is incorporated not only in bottromycin but also several other natural products,[14] various syntheses for this building block already exist, although many are specific for this amino acid. Therefore, we decided to apply the Matteson homologation[15] which should give us a versatile and stereoselective means to modify every carbon atom of the amino acid side chain.

Starting from the known methyl boronic ester **1**,[15] addition of lithiated dichloromethane (DCM) (generated *in situ* by deprotonating DCM with LDA) provided the α-chloroboronic ester **2** in a highly stereoselective fashion (Scheme 1). Addition of phenylmagnesium bromide generated a boronate complex which underwent a 1,2-phenyl shift replacing the chlorine in an S_N2 fashion. The desired α-phenyl substituted boronic ester **3** was obtained as a single stereoisomer. Repeating the sequence using vinylmagnesium bromide in the second step gave access to allylboronate **4**.

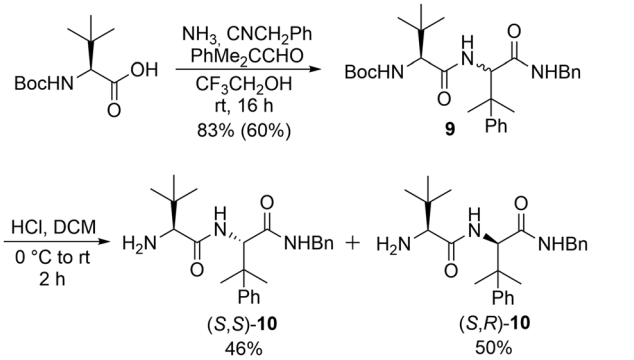
According to a protocol described by Morken, **4** was reacted with deprotonated *O*-methylhydroxylamine resulting in replacement of the boronic ester by an NH_2 group,[16] which was directly Boc-protected (**5**). Subsequent ozonolysis and Pinnick oxidation[17] gave the *N*-protected amino acid **6** which was converted into benzylamide **7**. Cleavage of the Boc protecting group, peptide coupling, and further *N*-deprotection gave the desired dipeptide **8**. In principle, by using other starting boronic esters or other aryl Grignard reagents, a wide range of derivatives should be accessible.

As a complementary and powerful protocol, we took advantage of the Ugi reaction[18] to generate the entire side chain in only one or two steps. In principle, enantiomerically pure 2-phenylpropionaldehyde can be used as the aldehyde component in this 4-component coupling for direct access to the bottromycin side chain. However, we decided to use achiral 2-methyl-2-phenylpropionaldehyde, generating a quaternary β-carbon (Scheme 2). Ugi reaction with Boc–*t*-leucine (Boc–Tle), ammonia, and benzyl isocyanide in trifluoroethanol generated the desired dipeptide **9** in high yield as a mixture of diastereomers and rotamers. Purification by flash chromatography provided a 1 : 1 diastereomeric mixture in 60% yield, which was subjected to *N*-Boc deprotection. The diastereomers of dipeptide **10** with the free amino terminus could be separated by reversed-phase chromatography or crystallisation. The crystals obtained were suitable for X-ray structure analysis which allowed us to determine the absolute configuration of the two diastereomers.

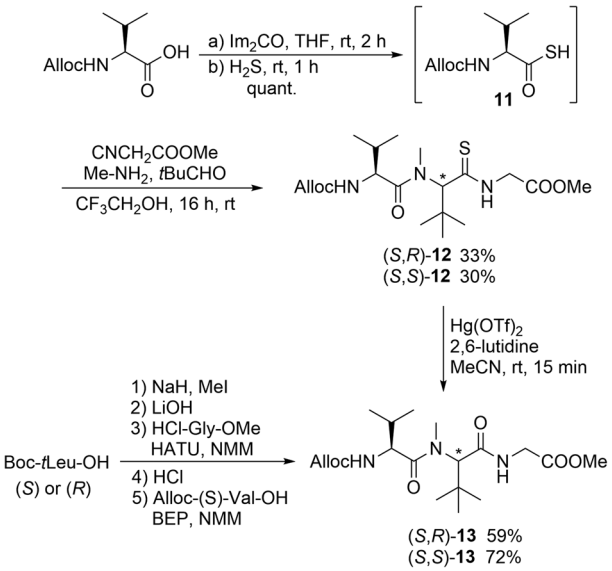
The Ugi reaction was also used to generate the sterically demanding peptide fragment applying our previously developed thio-Ugi approach (Scheme 3).[19] Alloc-protected valine was activated with carbonyldiimidazol (Im_2CO) to the corresponding imidazolide which was directly reacted with H_2S to afford the corresponding thioacid. Without purification, **11** was reacted with pivalaldehyde, methylamine, and the isocyanide obtained from glycine methyl ester. The reaction proceeded cleanly and the two diastereomers of **12** could be separ-



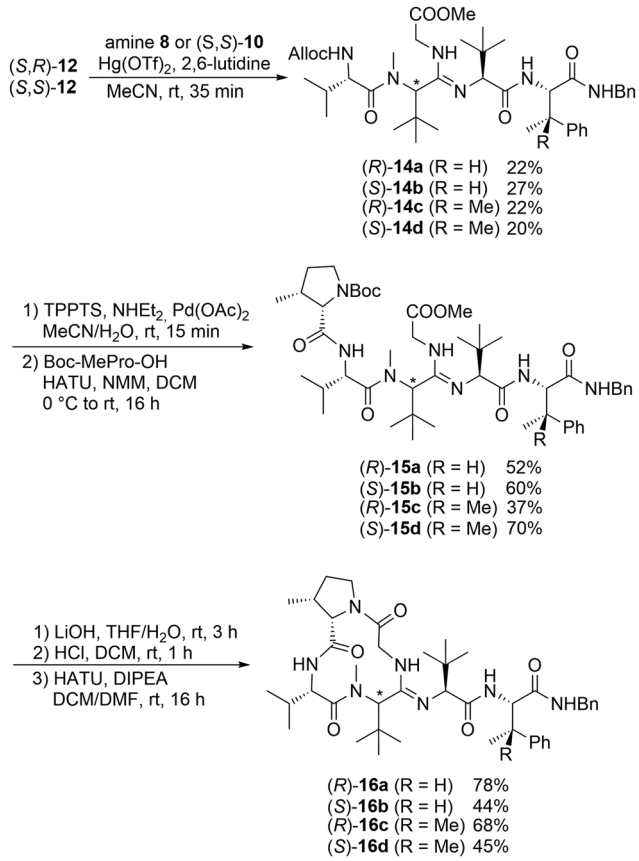
Scheme 1 Synthesis of dipeptide **8** *via* Matteson homologation. Abbreviations: NMM: *N*-methylmorphiline; HATU: [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexafluorphosphat].



Scheme 2 Synthesis and separation of diastereomers of dipeptide **10**.



Scheme 3 Synthesis and separation of diastereomers of thioamides **12** and elucidation of their configuration. Abbreviation: BEP: 2-bromo-1-ethyl pyridinium tetrafluoroborate.



Scheme 4 Synthesis of bottromycin derivatives **16**. Abbreviation: TPPTS: trinatrium-3,3′,3″-phosphintriyltribenzolsulfonat.

ated by flash chromatography. To determine the configuration of the newly formed stereogenic centre of the central amino acid, we took advantage of a side reaction generally observed later during the formation of the amidine. Hence, stirring the separated diastereomers in the presence of $Hg(OTf)_2$ and lutidine led to desulfuration and the formation of tripeptide **13**. The configurations of these tripeptides could be correlated with those obtained by standard peptide coupling reactions.

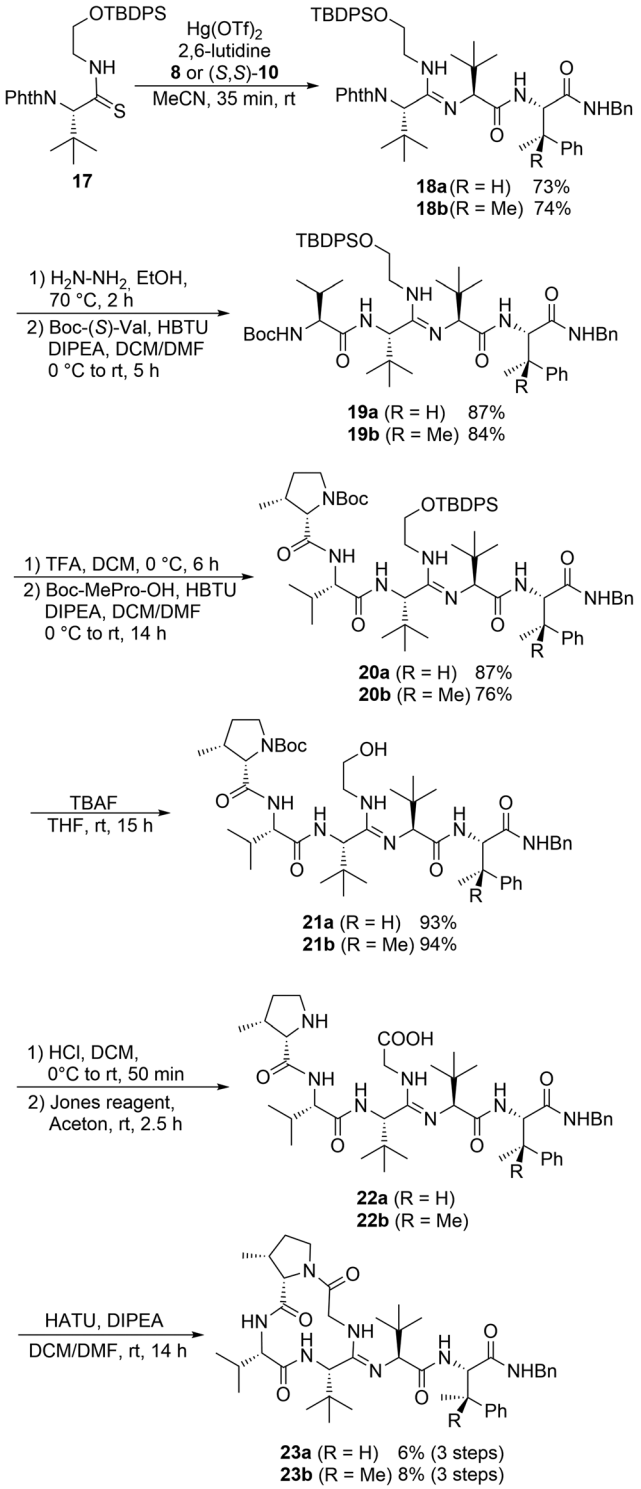
With thiopeptides **12** in hand, we next investigated the crucial amidine formation step (Scheme 4). Both diastereomers of **12** were reacted with dipeptide **8** in the presence of $Hg(OTf)_2$. Although the reaction is relatively fast, the desired amidines **14a** and **14b** were obtained in only moderate yields. A major side reaction was the desulfuration mentioned above, providing peptide **13**. Nevertheless, palladium-catalysed Alloc cleavage and coupling with *N*-Boc-protected 3-methylproline furnished linear peptides **15a** and **15b**, which were subjected to peptide cyclisation. The yield in the cyclisation step was significantly higher with the (*R*)-stereoisomer **16a** than the (*S*)-isomer **16b**. This is not surprising, as nearly all naturally occurring cyclotetrapeptides contain at least one (*R*)-amino acid. Such cyclic peptides generally show a *cis–trans–cis–trans* configuration of the cyclic peptide backbone.[20] This is why we incorporated *N*-methylated Tle opposite to the Pro in the ring, which should form a *cis*-amide bond more easily.

Based on these positive results, we applied the same reaction conditions to the coupling of **12** with the (*S*,*S*)-dipeptide **10**. The results obtained in the peptide coupling and cyclisation step to **16c** and **16d** were comparable. Here too, the (*R*)-Tle isomer **15c** resulted in a better cyclisation yield.

Unfortunately, the Ugi approach could not be applied to the synthesis of *N*-nonmethylated derivatives. Yields for the thio-Ugi reaction were comparable to those obtained with methylamine but the subsequent amidine formation clearly did not tolerate an NH amide bond between Val and Tle. No amidine formation was observed at all.

Therefore, we used the protocol described by Ōmura and Sunazuka in their bottromycin synthesis (Scheme 5). Coupling of thioamide **17** with our dipeptide **8** and (*S*,*S*)-**10** provided the desired amidines **18a** and **18b** in high yield. Cleavage of the phthaloyl protecting group and two subsequent peptide coupling steps gave access to the linear precursors **20a** and **20b**. The TBDPS ethers were cleaved, and the free primary alcohols were oxidised to the corresponding acids **22a** and **22b** which were then subjected to cyclisation. The yields obtained in both series were almost identical, including the cyclisation step. The cyclisation yields were unsatisfactory but not unexpected. In these cases, an all (*S*)-cyclopeptide **23** is sterically unfavourable. In addition, the *N*-methyl group that facilitates the formation of a *cis*-amide bond is absent and, in the linear peptide, the amide bond between the two sterically demanding amino acids Val and Tle is certainly *trans*.

The new bottromycin derivatives were evaluated for their biological activity towards *Mycobacterium tuberculosis* (Mtb) H37Ra and *Mycobacterium smegmatis* (Msm) mc^2155. Unfortunately, none of the *N*-methylated derivatives **16** showed



Scheme 5 Synthesis of bottromycin-derivatives **23**.

any significant activity (MIC >64 μG mL^{-1}), either against the *Mycobacterium* strains or any other Gram-positive or Gram-negative bacteria or yeasts and fungi. In contrast, some activity was observed for the two derivatives **23** with the unmodified peptide ring (Table 1). Although the activity against Mtb was

Table 1 Biological activities of new bottromycins **23**

	MIC (μG mL^{-1})		
Bacterial strain	$BotA_2$	**23a**	**23b**
M. tuberculosis H37Ra	1	16	32
M. smegmatis mc^2155	8–16	2	4
E. faecalis 20478		8	>64
E. faecium DSM-17050		32	>64
E. faecium 20477		2	16
S. pneumoniae DSM-11865		<0.03	0.5
S. pneumoniae 20566		<0.03	0.25

weaker than that of bottromycin A2, a higher activity was observed against Msm. This led us to investigate the activity against other strains that had not yet been tested. Derivative **23a** with the natural β-methylphenylalanine was approximately 10-fold more active than the double β-methylated derivative **23b**. The compounds proved to be particularly active against *Streptococcus pneumoniae*.

Conclusions

In conclusion, we have shown that bottromycins are readily accessible when applying Ugi reactions to generate larger fragments. Unfortunately, in SAR studies, no significant structural changes are tolerated on the peptide ring. *N*-Methylation facilitates cyclisation but, unfortunately, does not provide active derivatives. Matteson homologation was found to be a suitable tool for the stereoselective synthesis of β-methylated phenylalanine.

Data availability

The data supporting this article (copies of ^{1}H, ^{13}C NMR spectra and experimental details) have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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