Review

Israel Journal of Chemistry

DOI: 10.1002/ijch.202000068

The Long, Long Way to Bottromycin

Uli Kazmaier*[a]

Dedicated to Prof. Barry M. Trost on the occasion of his 80th birthday.

Abstract: Although discovered already in the middle of last century, the bottromycins are a unique class of natural products, and a real challenge for all kind of researchers trying to get familiar with them. The structure elucidation was a tour de force and last over 50 years. Synthetic approaches were also painful and it was actually the first and so far only synthesis which confirmed/revised the previously proposed structures. Recent investigations on the biosynthetic pathway indicate that the bottromycins belong to the ribosomally synthesized and posttranslational modified peptides, but that also the biosynthesis does not proceed along the "usual way". This review will cover the development of bottromycin research from the beginning until today, with a focus on synthetic studies, total synthesis and modifications.

Keywords: amidines · antibiotics · bottromycin · cyclic peptides · natural products

1. Introduction

The bottromycins are an interesting class of rather unusual peptidic natural products, isolated already in the mid 1950s from the fermentation broth of a *Streptomyces* species found in the area of Bottrop, Germany. *Streptomyces bottropensis* produced a "new sulfur-containing" compound with interesting antibiotic activity toward Gram-positive pathogens.[1] It was observed that this compound inhibits protein synthesis in vivo and in vitro,[2] but the detailed mode of action was unclear for a long time.[3]

1.1 Structure Elucidation

Even more difficult was the structure elucidation of the bottromycins. First attempts to solve the structure were made by Waisvisz et al. in 1957 who assigned an empirical formula of $C_{38}H_{57\text{-}61}N_7O_{7\text{-}8}S$ for their "new sulfur-containing antibiotic".[1] Acidic hydrolysis provided six ninhydrin-positive substances, while two of them could be identified as glycine and valine. The other four components were unclear, but it was obvious, that the bottromycins must be peptides.[4] Mild alkaline hydrolysis provided a crystalline compound ($C_{37}H_{57}N_7O_7S$) which showed no antibiotic activity. Interestingly, the reaction with methanol under acidic conditions resulted in a recreation of the biological activity. Other alcohols gave also rise to similar active compounds. Obviously bottromycin contains an ester functionality which is important for the biological activity.[5] Upon acetylation of bottromycin, *e.g.*, with acetic anhydride two crystalline decomposition products were obtained. One of these compounds was identified as the dipeptide methyl ester **A** (Figure 1).[5]

In 1965 Nakamura et al. isolated closely related antibiotics from the strain Streptomyces No. 3668-L2, which they called bottromycin A and B.[6] Acidic hydrolysis provided a mixture of all-(S)-configured amino acids containing 3-methyl-phenylalanine (β-MePhe),[7] *tert.*-leucine (*t*-Leu), valine, β-(2-thiazolyl)-β-alanine (thia-β-Ala)[8] and glycine. Bottromycin A contained *cis*-3-methylproline (β-MePro), while proline was incorporated into bottromycin B.[3b] They postulated a linear *N*-acylated iminohexapeptide structure (**B**), a proposal which was revised after synthetic studies[9] as well as by nmr spectroscopic investigations by Takita et al. in 1976, which proposed a cyclic iminopeptide structure.[10] This proposal was verified by Schipper (1983)[11] and Kaneda (1991),[12] based on detailed nmr studies. According to them the bottromycins are cyclic tetrapeptides (**C**), connected to a tripeptidic side chain via an amidine structure. The different bottromycins differ only in the substitution pattern of the proline. The absolute configuration of all building blocks was finally determined by a total synthesis by the groups of Sunazuka and Ōmura in 2009, indicating that the original postulated all-(*S*)-configuration of the amino acids was wrong.[13] The *C*-terminal thia-β-Ala is not (*S*)- but (*R*)-configured (**D**). Shortly thereafter, Bugni et al. (2012) reported the structure and biosynthesis of bottromycin D, a bottromycin A derivative where the valine in the ring is replaced by an alanine.[14]

[a] *U. Kazmaier*
Institute for Organic Chemistry, Saarland University, P.O. Box 151150, 66123 Saarbrücken, Germany
phone: +49 6813023409
fax: +49 6813023409
E-mail: u.kazmaier@mx.uni-saarland.de

Israel Journal of Chemistry

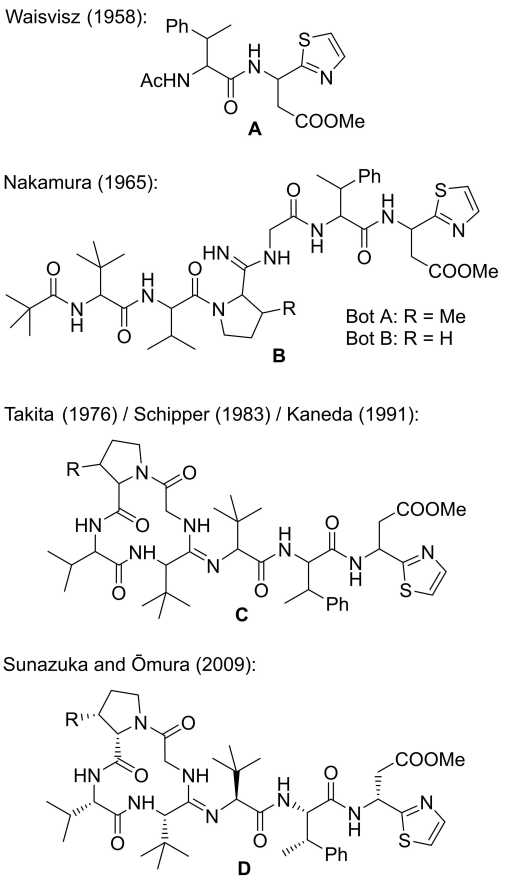


Figure 1. Proposed and final structures of bottromycins.

In the same year, the three-dimensional structure was described by Gouda et al. based on nmr data.[15] Obviously the side chain with the three C-terminal amino acids folds back to the tetrapeptide ring in such a way, that β-MePro and thia-β-Ala are located on one side of the molecule, what might be important for the binding toward the bacterial ribosome. Therefore, modifications at this positions should have an influence on the biological activity of bottromycins and derivatives.

1.2 Biosynthesis

The structure of the bottromycins is quite unique, not only because of the amidine moiety but also because of the high number of unusual amino acids. Like the structure, also the biosynthetic origin of the bottromycins was unclear for a long time. With respect to the rather unusual structural features one might assume synthesis via non-ribosomal peptide synthetases, a widespread bacterial pathway, but also a ribosomal pathway can't be excluded.[16] Early biosynthetic studies in the Arigoni group (1997) using isotope labeling experiments showed, that the "additional" methyl groups (Pro, Phe, 2 × Val) and on the *C*-terminal thia-β-Ala were incorporated from methionine.[17] The methylation occurs with retention of configuration and can be explained by radical SAM mechanism.[18] In 2012, four groups identified independently biosynthetic gene clusters (BGCs) in four different Streptomyces species.[14,19] They found that the BGCs encode for three radical SAM methyltransferases and propose a ribosomal peptide synthesis with subsequent posttranslational modifications, what is in agreement with the early feeding studies. Although such posttranslationally modified peptides (RiPPs)[16a] are widespread found in nature, also from the biosynthetical point of view the bottromycins are really unique. In general, RiPPs are formed from a larger ribosomally synthesized precursor peptide, consisting of a core peptide and a leader peptide, which is post-translationally modified by tailoring enzymes. But the bottromycins are the first RiPPs which are "born" without a leader peptide. Very recently, Koehnke et al. investigated in detail the enzymes involved in the biosynthetic pathway.[20]

2. Synthetic Approaches and Total Syntheses of Bottromycins

Before going into the total syntheses of the bottromycins, the syntheses of the building blocks, the unusual amino acids will be discussed. While *tert.*-leucine is commercially available, the other non-proteinogenic amino acids are not.

2.1 Syntheses of the Non-proteinogenic Amino Acids

2.1.1 (2S,3R)-3-Methylproline (β-MePro) and Derivatives

Uli Kazmaier studied chemistry at the University of Stuttgart where he obtained his PhD in 1990 while working with U. Schmidt on the synthesis of bottromycins. Afterwards he joined the groups of M. T. Reetz (Marburg) and B. M. Trost (Stanford) as postdoctoral fellow. In 1992, he moved to Heidelberg, starting his own scientific work under the mentorship of G. Helmchen. In 2000, he received a Novartis Chemistry Lectureship and in 2001 an offer for a full professorship at Saarland University.

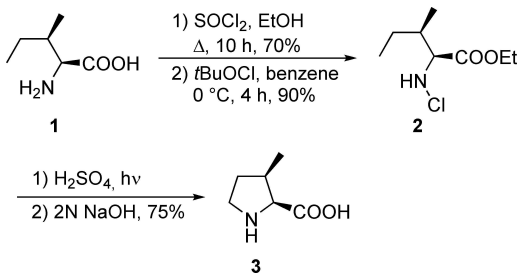
While bottromycin B contains a non-methylated proline in the tetrapeptide ring, (2*S*,3*R*)-β-MePro is incorporated into bottromycin A and D. A first stereospecific synthesis was reported by Titouani *et al.* in 1980.[21] Key step was a Hofmann-Löffler-Freitag reaction using (*S*)-*allo*-Ile (**1**) as a starting material (Scheme 1). Esterification and *N*-chlorination provided amino acid derivative **2**. Irradiation in sulfuric acid generated the δ-chlorinated amino acids *via* radical intermediates. Cyclization gave access to the desired amino acid **3** without affecting the asymmetric centers of the starting material. In an analogous fashion the (2*S*,3*S*)-stereoisomer was accessible from (*S*)-Ile.

Herdeis *et al.* reported the syntheses of both, the (2*S*,3*S*)- and the (2*S*,3*R*)-isomer of β-MePro from pyroglutaminol derivate **4**, a common building block for the synthesis of protease inhibitors (Scheme 2).[22] 1,4-addition of Me_2CuLi gave rise to the *trans*-configured product in good yield and as single stereoisomer.[23] To get also access to the required *cis*-isomer, **5** was subjected to elimination and subsequent catalytic hydrogenation of **6** from the least hindered face of the double bond provided **7** as a single diastereomer which was converted into **3** *via* standard operations.

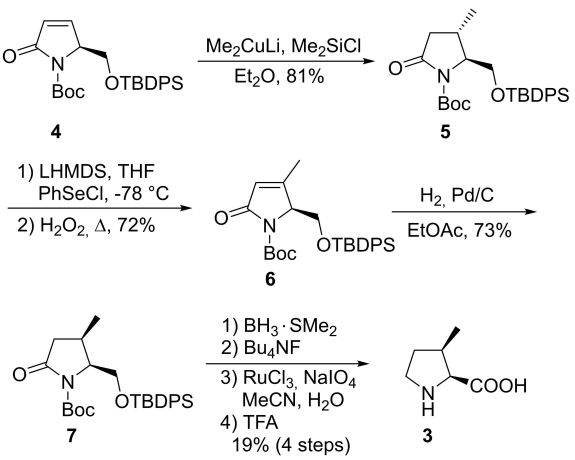
Karoyan and Chassaing used a 5-*exo* trig cyclization between a chelated zinc enolate formed from **8** and a non activated double bond (Scheme 3).[24] The reaction proceeded with chirality transfer from the chiral α-methylbenzyl group onto the C-2 carbon, and a chair-like transition state resulted in the formation of the *cis*-configured proline derivative **9**. Hydrolysis and catalytic hydrogenation provide proline ester **10**, which was saponified to the free amino acid **3**. This approach allows also the introduction of other substituents onto the β-position *via* coupling the zinc derivative **9** with electrophiles.[25]

Kamenecka *et al.* developed a protocol starting from commercially available 3-hydroxy-(*S*)-proline **11** (Scheme 4).[26] Esterification and *N*-tritylation provided hydroxyester **12** which was oxidized to β-ketoester **13** and further converted into enol triflate **14**. The regioselectivity of enolate formation was surprising: obviously the sterically demanding trityl protecting group protects the chiral α-carbon from deprotonation. Enol triflate **14** could be subjected to a wide range of palladium-catalyzed cross-coupling reactions allowing modification at the β-position. Cleavage of the trityl group and subsequent catalytic hydrogenation provided methyl ester **15** in moderate diastereoselectivity.

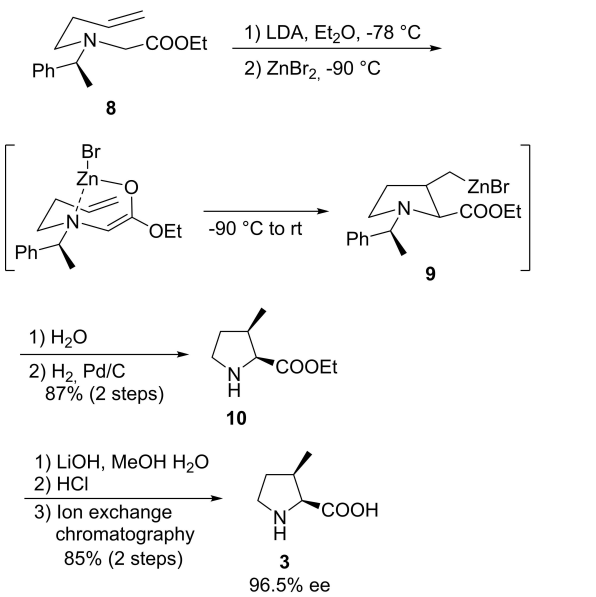
A synthesis of enantiomerically pure **3** based on a stereoselective cuprate addition as a key step was reported by Flamant-Robin *et al.*[27] Starting material was chiral oxazolidine **16**, easily accessible from Garner's aldehyde (Scheme 5). 1,4-addition of Me_2CuLi provided *syn*-**17** exclusively in high yield. Standard operations gave rise to amino acid derivative **18**, which was converted into **3**.



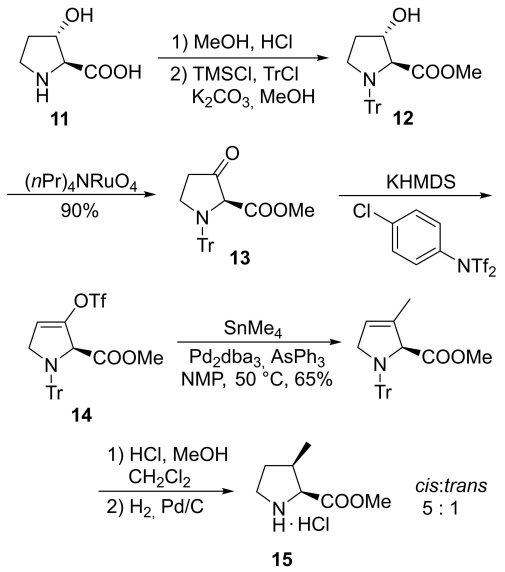
Scheme 1. Synthesis of (2*S*,3*R*)-β-MePro (**3**) *via* Hofmann-Löffler-Freitag reaction.



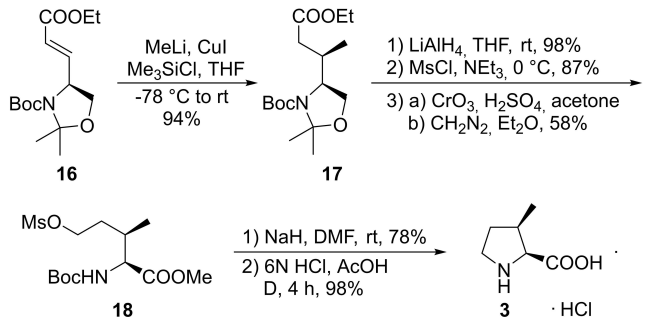
Scheme 2. Synthesis of (2*S*,3*R*)-β-MePro (**3**) *via* 1,4-addition/stereoselective hydrogenation.



Scheme 3. Synthesis of (2*S*,3*R*)-β-MePro (**3**) *via* chelate-enolate cyclization.



Scheme 4. Synthesis of (2*S*,3*R*)-β-MePro methyl ester (**15**) *via* Stille coupling.



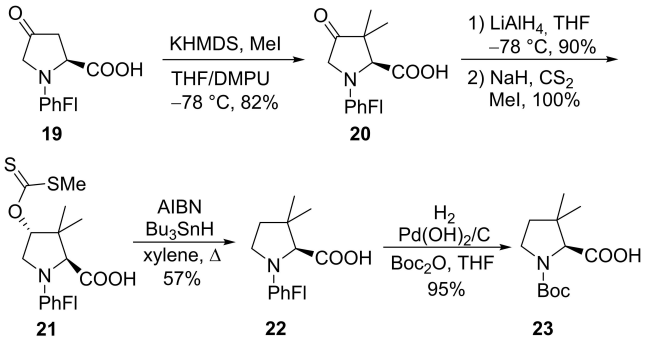
Scheme 5. Synthesis of (2*S*,3*R*)-β-MePro (**3**) *via* 1,4-addition/cyclization.

2.1.2 (2S)-3,3-Dimethylproline (β-Me$_2$Pro) And Derivatives

3,3-Dimethyl-(2*S*)-proline (β-Me$_2$Pro) is one of the unusual amino acids found in bottromycin C. A first enantioselective synthesis has been reported by Sharma and Lubell in 1996.[28] The regioselective enolization of 4-oxo-proline derivative **19** followed by alkylation with different alkyl halides, was used for the synthesis of a variety of proline derivatives (Scheme 6). Enolization with 4 eq. KHMDS and alkylation with methyl iodide provided **20** in excellent yield.

Subsequent reduction of the keto group and desoxygenation *via* radical reduction of the corresponding xanthate **21** gave rise to protected proline **22**. The 9-phenylfluorenyl (PhFl) protecting group could be removed together with the benzyl ether *via* hydrogenation using Pearlman's catalyst. Hydrogenation in the presence of Boc$_2$O provided *N*-Boc-protected β-Me$_2$Pro **23**.

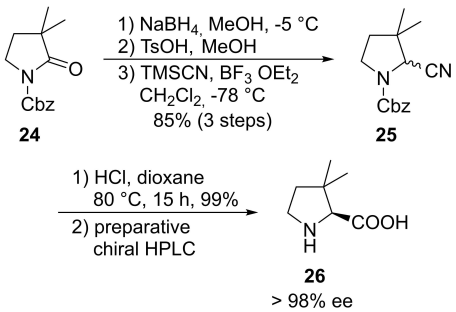
A synthesis of racemic β-Me$_2$Pro was reported by Medina *et al.* starting from *N*-protected pyrrolidinone **24**



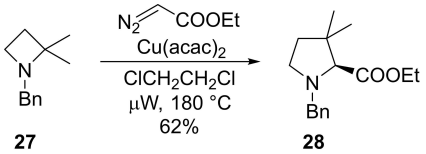
Scheme 6. Synthesis of Boc-(2*S*)-β-Me2Pro (**23**) *via* regioselective alkylation.

(Scheme 7).[29] The lactam was reduced with NaBH$_4$ to the hemiaminal. Its treatment with TsOH and MeOH and subsequent reaction with TMSCN in the presence of Lewis acid provided racemic nitrile **25**. Simultaneous removal of the *N*-protecting group and saponification of the nitrile gave access to amino acid **26** which could be separated into the enantiomers *via* chiral preparative HPLC.

Another interesting approach towards racemic β-Me$_2$Pro derivatives was reported by Bott et al (Scheme 8).[30] *N*-substituted azetidines **27** were heated with ethyl diazoacetate in the presence of Cu(acac)$_2$. *Via* Stevens [1,2]-shift a ring expansion occurred to the dimethylated proline **28**. Unfortunately, a separation of the enantiomer was not reported.



Scheme 7. Synthesis of (2*S*)-β-Me2Pro (**26**) *via* preparative HPLC.



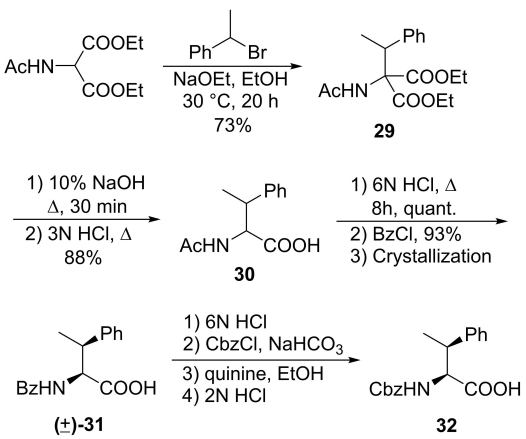
Scheme 8. Synthesis of *rac*-β-Me$_2$Pro derivative (**28**) *via* carbene insertion.

2.1.3 (2S,3S)-3-Methylphenylalanine (β-MePhe) and Derivatives

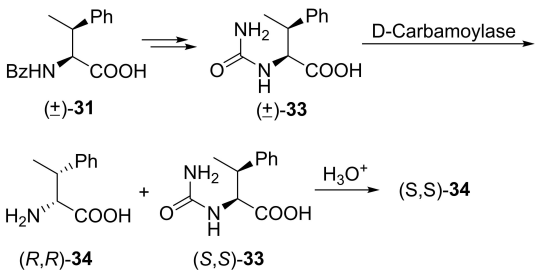
By far most investigations focused on the synthesis of this unusual amino acid because it also appears in some other natural products such as mannopeptimycin[31] or the isoleucyl-*t*-RNA-synthetase inhibitor SB-203208.[32]

In connection with one of the first synthetic studies towards bottromycins Kataoka *et al.* described the synthesis and optical resolution of β-MePhe *via* condensation of racemic 1-bromo-1-phenylethane with acetaminomalonate (Scheme 9).[33] Hydrolysis and decarboxylation of **29** provided a racemic mixture of diastereomers of **30**, which could be separated by fractional crystallization of the *N*-benzoyl derivatives. The pure diastereomers could be further separated into the enantiomers by treating the Cbz-protected-derivatives with quinine or quinidine. Thus, all four stereoisomers of **32** could be obtained in pure form.

Many attempts have been undertaken to separate the stereoisomers more easily using modern chromatographic techniques, e.g. *via* chiral stationary phases on HPLC,[34] capillary electrophoresis[34b,35] or anion exchange chromatography.[36]



Scheme 9. Synthesis of (2*S*,3*S*)-β-MePhe derivative (**32**) *via* crystallization.

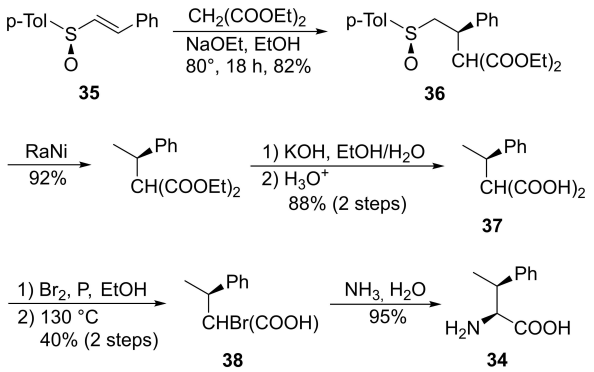


Scheme 10. Synthesis of (2*S*,3*S*)-β-MePhe (**34**) *via* enzymatic kinetic resolution.

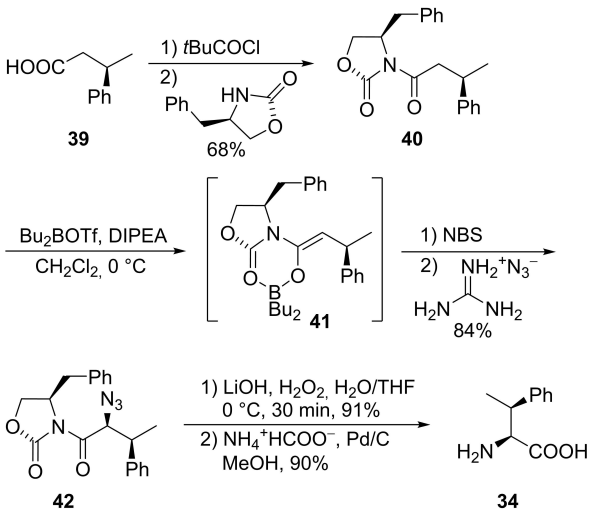
Ogawa *et al.* reported an enzymatic approach to (2*S*,3*S*)-β-MePhe (Scheme 10).[37] The racemic *N*-benzoylated amino acid **31** was converted into the *N*-carbamoyl-derivative **33**, which was subjected to enzymatic cleavage using D-Carbamoylase. While only the (2*R*,3*R*)-amino acid was slowly hydrolyzed, the remaining (2*S*,3*S*)-derivative was chemically hydrolyzed to the free amino acid **34**.

Tsuchihashi *et al.* reported the synthesis of (2*S*,3*S*)-β-MePhe from (*R*)-(1-phenylethyl)malonate **36**, which was obtained *via* Michael addition of malonate onto chiral vinyl sulfoxide **35** in a 8 : 2 diastereomeric ratio. Fractional crystallization provided pure **36** (Scheme 11).[38] Subsequent desulfurylation and saponification provided (*R*)-malonic acid **37**, which was subjected to α-bromination. Thermal decarboxylation provided crystalline carboxylic acid **38**, which after recrystallization was diastereomerically pure. Treatment with aqueous NH_3 finally provided the desired amino acid **34**.

Dharanipragada *et al.* described the first asymmetric synthesis of all four stereoisomers of β-MePhe (Scheme 12).[39] Commercially available racemic 3-phenylbutyric acid [(±)-**39**]



Scheme 11. Synthesis of (2*S*,3*S*)-β-MePhe (**34**) *via* Michael addition to chiral sulfoxide **35**.



Scheme 12. Synthesis of (2*S*,3*S*)-β-MePhe (**34**) using Evans-auxiliary.

was resolved into its enantiomers *via* fractional crystallization using (*S*)- and/or (*R*)-methylbenzylamine. Activation of the enantiomerically pure carboxylic acid **39** as mixed anhydride and coupling with the Evans-auxiliary[40] provided *N*-acyloxazolidinone **40**. Formation of the boron enolate **41** and subsequent bromination and azide formation *via* S_N2 displacement gave rise to azide **42**. Saponification allowed the recovery of the chiral auxiliary and the azido acid formed was reduced to the desired amino acid **34**.

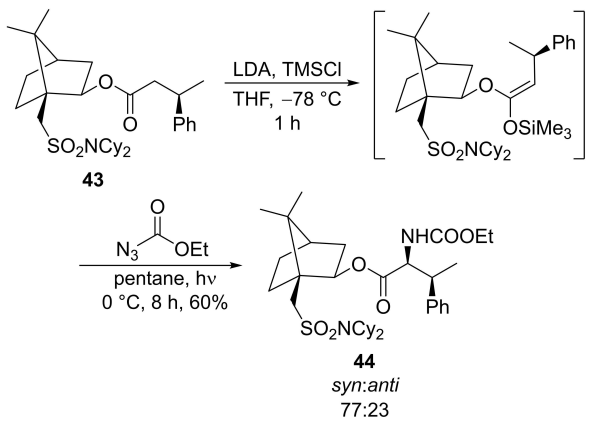
Another auxiliary-controlled approach was reported by Fioravanti *et al.* using Oppolzer's auxiliary (Scheme 13).[41] Kinetically controlled deprotonation/silylation of **43** gave rise to a (*E*)-silylketeneacetal preferentially,[42] which was subjected to amination. Its irradiation in the presence of ethyl azido formate provided **44** with moderate diastereoselectivity, but the diastereomers could easily be separated *via* flash chromatography. Hydrolysis under acidic conditions (6 N HCl) allowed the cleavage and recoverage of the chiral auxiliary.[42]

The groups of Pericas and Rieva developed a protocol using a Sharpless epoxidation as a stereo-controlling step (Scheme 14).[43] Asymmetric epoxidation of cinnamyl alcohol and regio- and stereospecific ring opening with a *N*-nucleophile provided starting material **45**. Selective protection of the primary OH-functionality and conversion of the secondary alcohol into the mesylate allowed the synthesis of aziridine **46** under basic conditions. Regioselective opening of the aziridine ring with Me_2CuLi, cleavage of the silyl protecting group and oxidation provided Boc-protected amino acid **47**. A similar approach was also applied for the synthesis of the (2*R*,3*R*)-enantiomer.[44]

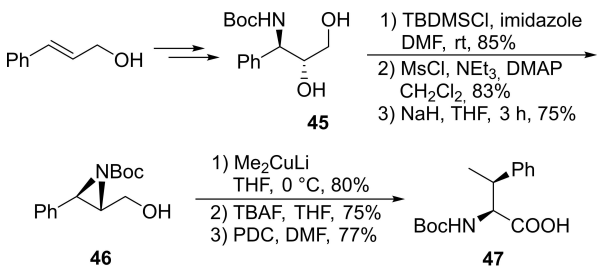
O'Donnell *et al.* reported an acyclic stereoselective boron alkylation as a key step (Scheme 15).[45] The protocol involved the reaction of an α-acetoxy derivative of the benzophenone imine of *tert*-butyl glycinate **48** with a chiral boron reagent **49** in the presence of cinchona alkaloids and LiCl. Depending on the alkaloid used, cinchonine (CnOH) or cinchonidine (CdOH), both α-stereogenic centers could be generated. It was assumed that the stereoselective protonation is controlled by complexation of the enolate to the alkaloid and delivery of the proton from the least hindered face.

The group of Turner developed a chemoenzymatic route towards enantiomerically pure β-MePhe derivatives, based on an oxidation-reduction sequence (Scheme 16).[46] A key feature was the combination of an enantioselective amino acid oxidase (AAO), which oxidizes the α-amino acid to the corresponding imine, together with a non-selective reducing agent, such as $BH_3 \cdot NH_3$, which effects reduction of the imine back to the starting material. Since the (2*R*,3*S*)-diastereomer of β-MePhe **52** can easily be obtained by asymmetric hydrogenation of the corresponding (*Z*)-dehydroamino acid **51** this protocol also allows the enantioselective preparation of the (2*S*,3*S*)-stereoisomer **34**.[47]

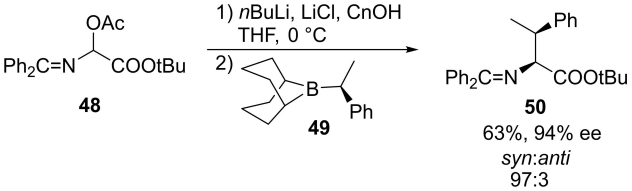
Doi *et al.* reported a phase transfer-catalyzed alkylation of glycinate Schiff base **53** with racemic 1-bromo-1-phenylethan (2 equiv) under the influence of chiral quaternary ammonium bromide **54** and 18-crown-6 (Scheme 17).[48]



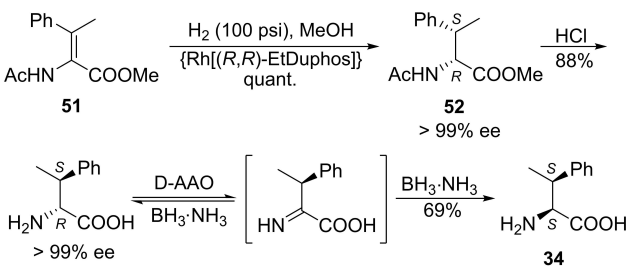
Scheme 13. Synthesis of (2*S*,3*S*)-β-MePhe derivative **45** using Oppolzer's auxiliary.



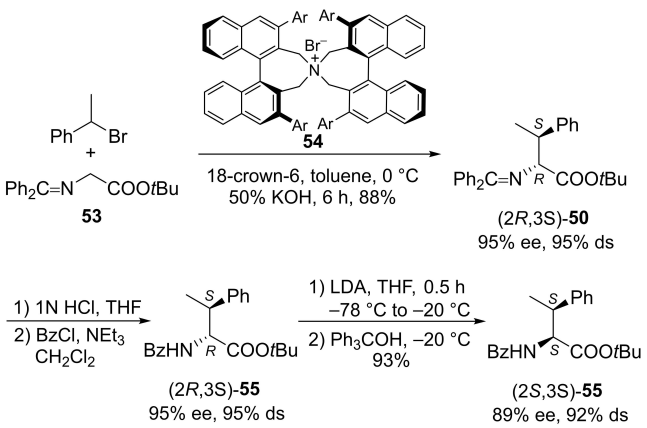
Scheme 14. Synthesis of (2*S*,3*S*)-β-MePhe derivative **45** *via* Sharpless epoxidation.



Scheme 15. Synthesis of (2*S*,3*S*)-β-MePhe derivative **50** *via* stereoselective protonation.



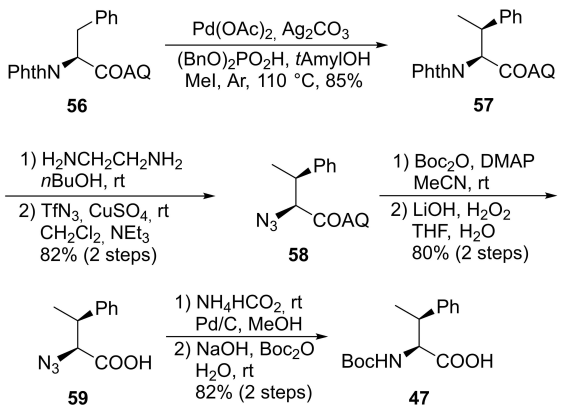
Scheme 16. Synthesis of (2*S*,3*S*)-β-MePhe **34** *via* enzymatic oxidation/reduction.



Scheme 17. Synthesis of protected (2*S*,3*S*)-β-MePhe **55** under asymmetric phase transfer conditions.

The chiral phase transfer catalyst can precisely discriminate not only the enantiofaces of the enolate but also the central chirality of the halide during the C–C-bond formation. Kinetic resolution provides the (2*R*,3*S*)-isomer of **50** in high yield, enantio- and diastereoselectivity. Replacing the imine by a *N*-benzoyl group (**55**) allowed the epimerization of the α-stereogenic center *via* deprotonation/protonation.

Zhang. *et al.* described the application of a palladium-catalyzed functionalization of C(sp^3)–H bonds in the synthesis of β-branched amino acids (Scheme 18). Using 8-aminoquinoline (AQ) as a directing group *N*-phthaloyl- (Phth-) protected Phe **56** was converted into fully protected β-MePhe derivative **57** in good yield and diastereoselectivity. The *N*-Phth group could be deprotected with ethylendiamine and reaction with TfN_3 gave access to azide **58**. Activation of the directing amide group with Boc_2O and subsequent treatment with $LiOH/H_2O_2$ provided azido acid **59**, which on hydrogenation and Boc-protection delivered **47**.



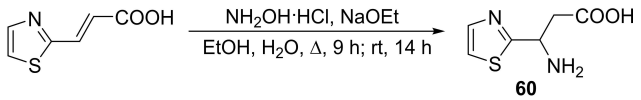
Scheme 18. Synthesis of (2*S*,3*S*)-β-MePhe derivatives **47** and **59** *via* C–H functionalization.

2.1.4 (S)- and (R)-3-(Thiazol-2-yl)-β-alanine (Thia-β-Ala)

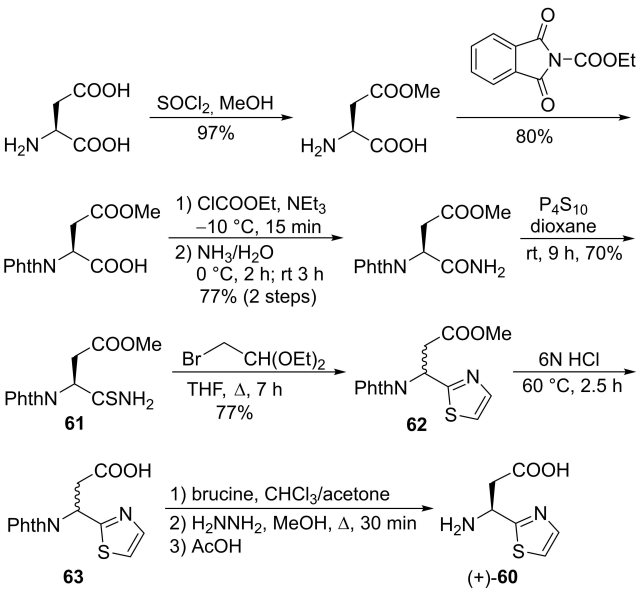
The *C*-terminal unusual thiazolyl amino acid was the last one whose configuration was determined. It required the total synthesis of the bottromycins to establish it definitely. The problems arose from the structure elucidation of bottromycin. By hydrolysis of the natural product with conc. HCl Waiswisz *et al.* obtained a "sulfur-containing amino acid", unfortunately showing no optical activity.[49] On the other hand, later on, Umezawa's group obtained an optically active amino acid ($[\alpha]^{18}_D$: +9) by hydrolyzing the antibiotic with acetic anhydride.[3b]

To determine the structure of the *C*-terminal amino acid Waiswisz prepared racemic thia-β-Ala **60** by addition of hydroxylamine towards β-2-thiazolacrylic acid, which was obtained by standard reactions (Scheme 19). Attempts to increase the yield failed, and the addition of NH_3 instead of NH_2OH resulted in the formation of the α-amino acid.[8]

Seto *et al.* tried to obtain optically active (*S*)-**60** starting from (*S*)-aspartic acid (Scheme 20).[8] The β-methyl aspartate was obtained with $SOCl_2$/MeOH, and after Phth-protection the amide was formed *via* the mixed anhydride method. Stirring with P_4S_{10} at room temperature in dioxane generated thioamide **61**, which was found to be unstable on silica gel. Thiazole formation was performed by condensation of **61** with bromoacetaldehyde or the corresponding acetal. Unfortunately,



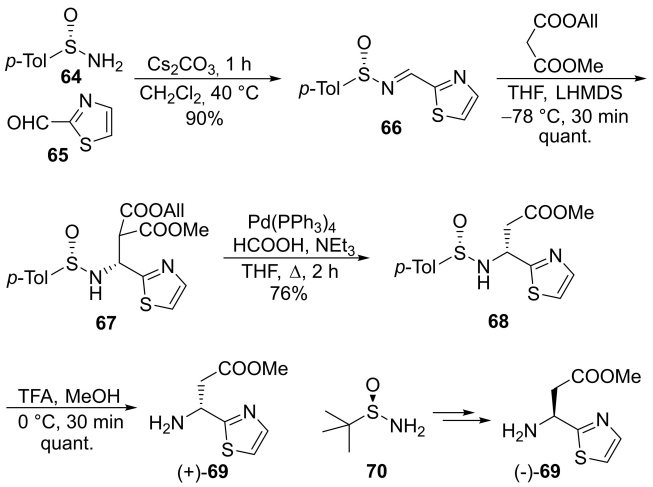
Scheme 19. Synthesis of racemic thia-β-Ala **60**.



Scheme 20. Synthesis of enantiomerically pure (+)-thia-β-Ala **60** *via* crystallization.

also the Phth-protected thia-β-Ala ester **62** was optically inactive. In an analogous way the corresponding *N*-Cbz-derivative could be obtained, also in racemic form. Obviously, complete epimerization occurred in the thiazole formation step. The racemic amino acid **63**, however, could be resolved into its antipodes by treating the Phth-derivative with brucine. Hydrazinolysis of **63** gave access to the optically active (+)-**60**, but with $[\alpha]^{17}_{D}$: +22,4. The optical rotation was much higher than the value reported by Umezawa. Obviously through their hydrolysis, also some epimerization occurred. Thus, the (+)-amino acid **60**, the constituent of bottromycin, was isolated in pure form. It was postulated to belong to the (*S*)-series based on optical rotatory dispersion (ORD). The thia-β-Ala derivatives prepared also lost their activity on heating to reflux in 6 N HCl for 8 h, while the same compounds were stable at room temperature or under slightly basic conditions.

The only so far enantioselective synthesis of enantiomerically pure (*S*)- and (*R*)-thia-β-Ala was reported by the groups of Sunazuka and Ōmura (Scheme 21).[13] They took advantage of the chiral sulfinamide chemistry developed by Davis and Ellman.[50] Condensation of (*S*)-*p*-toluene sulfinamide **64** with 2-formylthiazole **65** provided sulfinimine **66** in excellent yield. The subsequent Mannich reaction with allyl methyl malonate gave access to **67** as a 1 : 1 diastereomeric mixture. Palladium-catalyzed decarboxylation removed the epimeric stereogenic center and methyl ester **68** was obtained as a single diastereomer. Removal of the sulfinyl group afforded (+)-**69** in quantitative yield. X-ray structure analysis determined the absolute configuration to be (*R*). The opposite (*S*)-enantiomer was obtained in an analogous fashion from (*R*) sulfonamide **70**.



Scheme 21. Asymmetric synthesis of enantiomerically pure (+)-thia-β-Ala-OMe **69**.

2.2 Synthetic Studies towards Bottromycins

Because most structure proposals for the bottromycins were wrong for a long time, it is not surprisingly that so far only one total synthesis exists,[13,51] which also confirmed that the *C*-terminal thia-β-Ala is (*R*)-configured and not (*S*) as originally reported. Therefore, early synthetic work could not be successful because it was based on wrong assumptions, but it focused on the synthesis of the partial structure of this rather unique peptide. The first investigations were reported by Yamada *et al.* already in 1977.[52] Their synthetic route was based on the linear hexapeptide **71** proposed by Nakamura *et al.* (Figure 2)[3b,c,6]

Yamada *et al.* focused on the formation of the central amidine unit. Several amidines were prepared by condensation of protected amino acid imido esters with amino acid esters (Scheme 22).[53] Although, the desired amidine **72** could be obtained without problems, it was impossible to prolong the dipeptides at the *C*-terminus.

On activation, or even on standing under basic conditions, cyclization to the corresponding imidazolone **73** was observed. Therefore, they tried to generate the amidine unit of **76** by coupling two model tripeptide fragments, the tripeptide imido ester **74** and tripeptide **75** (scheme 23).[53] But interestingly, the pK_a-values of all synthesized amidines (pK_a ~9,3) were around

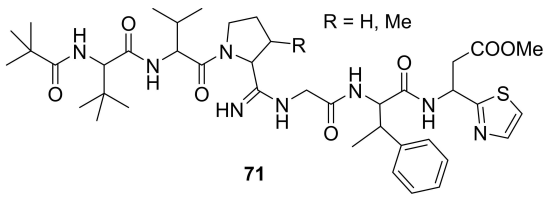
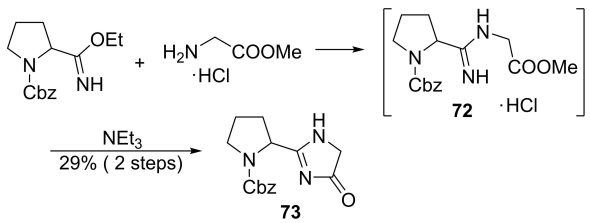
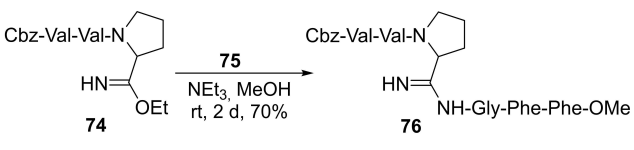


Figure 2. Structure of bottromycin (**71**) according to Nakamura *et al.*



Scheme 22. Formation of amidine **72** and imidazolone **73**.



75: H-Gly-Phe-Phe-OMe · HCl

Scheme 23. Formation of model amidine **76**.

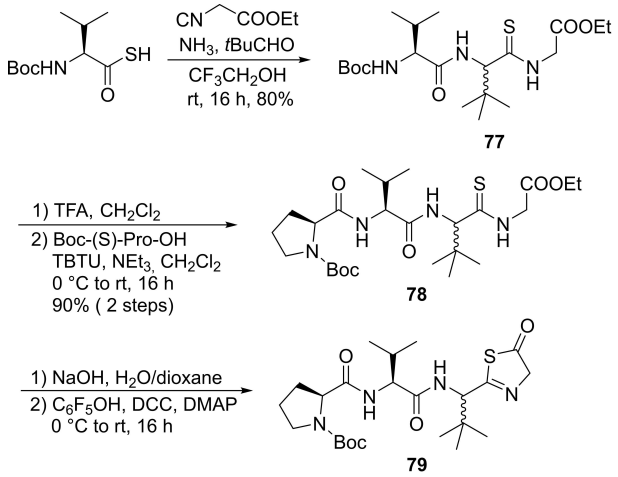
1 pK_a higher than in the natural product (~8.2), a first indication that the structure proposal might be not correct.

Based on the revised structures by Schipper[11] and Kaneda,[12] proposing a cyclic tetrapeptide with a tripeptide chain connected *via* an unusual amidine moiety, Kazmaier *et al.* focused on the synthesis of the corresponding peptide ring and the highly substituted amidine.[54] Key step of their approach was an Ugi-reaction using a protected thioamino acid and NH_3 as amine compound (Scheme 24). Although, Ugi reactions with NH_3 are often critical, giving a range of side products, with sterically demanding aldehydes good results were obtained.[55] With thiocarboxylic acids this approach allows the synthesis of endothiopeptides.[56] With isocyanoacetate the linear tripeptide **77** could be obtained, which was prolonged to the desired tetrapeptide **78** under standard conditions. Attempts to cyclize **78** or to connect the side chain *via* peptide coupling failed, because the thioamide underwent cyclization to the thiazolinone **79**, comparable to the imidazolone formation reported by Yamada.[53]

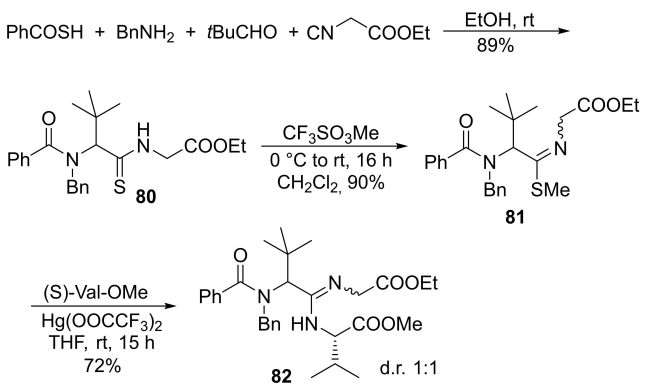
In parallel, to figure out if amidine formation is possible between sterically demanding amino acids thiopeptide **80** was synthesized in an analogous fashion (Scheme 25). Because attempts to couple **80** directly with amines failed, the thioamide was converted into the corresponding thioimidoester **81**, which could be reacted with valine methyl ester to **82** in the presence of $Hg(OOCCF_3)_2$. The diastereomers formed could be separated by flash chromatography, but unfortunately this protocol could not be applied to endothiopeptide **77**.

This caused a change in the strategy, replacing the intermolecular amidine formation *via* an intramolecular one by using the isocyanide of *t*-Leu-OMe (Scheme 26). The endothiopeptide **83** was obtained in high yield and could be prolonged on the *N*-terminus. *S*-Methylation and cyclization in the presence of $Hg(OOCCF_3)_2$ gave access to cyclic amidine **84**.

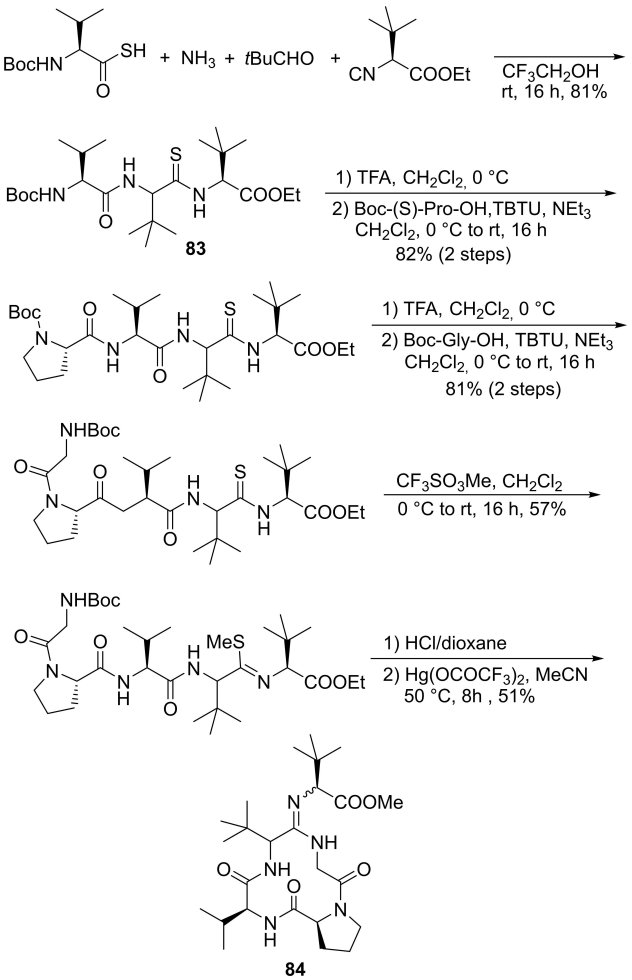
The amidine formation as key step was also investigated in detail by Ōmura and Sunazuka *et al.* during their synthesis of bottromycin A and B (Scheme 27).[51] They investigated the reaction of thioamide **85** with the tripeptide side chain **86**. While no reaction was observed in THF using NEt_3 as a base, in the presence of $Hg(OAc)_2$ unfortunately not the desired amidine **87** was obtained but the amide **88**. Better results were



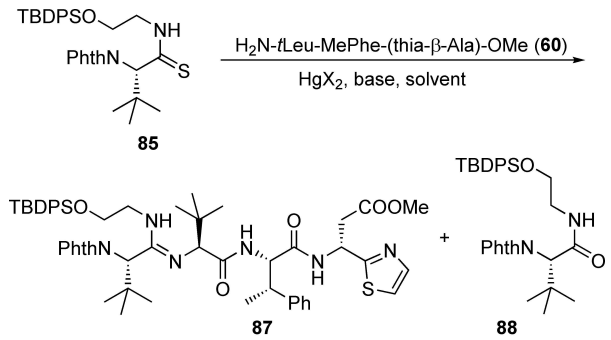
Scheme 24. Synthesis of endothiopeptides *via* Ugi reaction.



Scheme 25. Synthesis of amidine **82** *via* Ugi reaction



Scheme 26. Synthesis of cyclic amidine **84** *via* Ugi reaction.



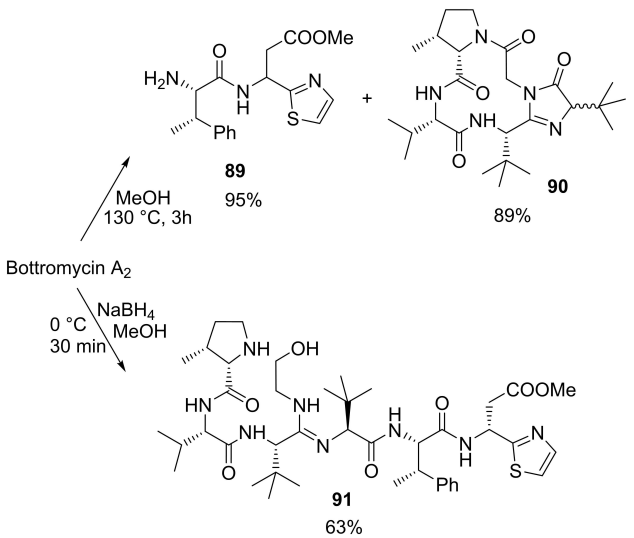
HgX_2	Base	Solvent	Yield 87	Yield 88
$Hg(OAc)_2$	NEt_3	THF	—	100
$Hg(OTf)_2$	2,6-lutidine	MeCN	96	—

Scheme 27. Synthesis of linear amidine **87**.

obtained with $HgCl_2$ and $Hg(OTf)_2$ as Lewis acids, and finally 2,6-lutidine as base in acetonitrile was the method of choice.

The same groups also preformed degradation studies of bottromycin obtained by fermentation (Scheme 28).[57] They subjected bottromycin A to pyrolysis in MeOH at 130 °C, resulting in a cleavage of the tripeptide side chain. Besides dipeptide **89** also a cyclic byproduct **90** was obtained as a diastereomeric mixture. Obviously, the epimerization of the *t*-Leu in the side chain occurred *via* the enol-form of imidazolone **90**. This could finally explain why the *t*-Leu obtained by total hydrolysis of the bottromycin has a lower optical rotation than synthetic enantiopure amino acid. Reduction of the natural product under mild conditions provided alcohol **91**, which could be used to investigate cyclization conditions.

Dipeptide **89** was also used to determine the configuration of the configurational labile amino acid thia-β-Ala (**69**).[8] Both

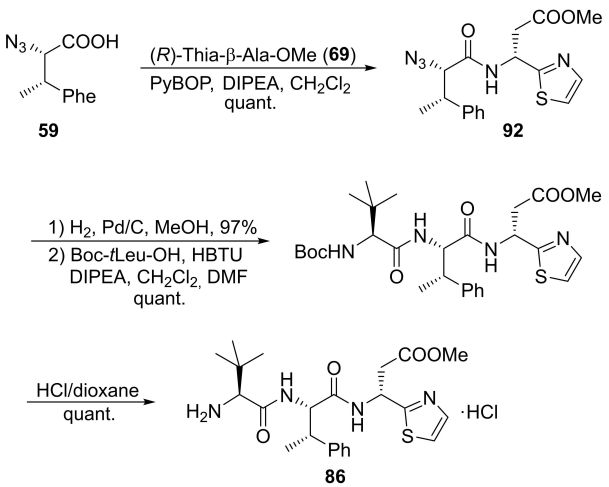


Scheme 28. Degradation of bottromycin A2.

enantiomers were synthesized *via* the sulfinamide protocol (Scheme 21) and subsequently coupled with azido-MePhe **59** (Scheme 29). Reduction of the azido group of **92** provided the two diastereomeric dipeptides **89**. Comparison of their ^1H NMR spectra with the spectrum of **89** obtained *via* pyrolysis clearly indicated that the (*R*)-isomer is incorporated into the bottromycins and that the original structure proposal (*S*) was wrong. Coupling of **89** with Boc-(*S*)-*t*-*Leu* and subsequent Boc-cleavage provided tripeptide **86**, which was used in the amidine formation experiments (Scheme 27).

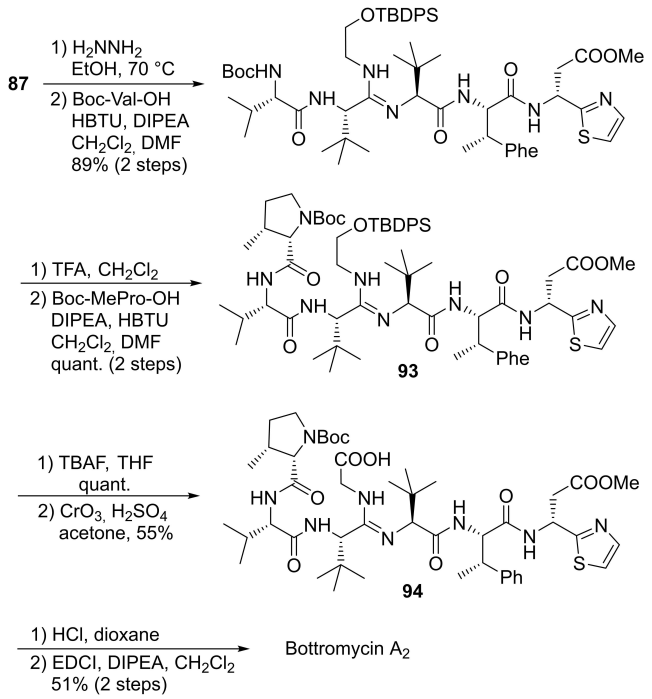
2.3 Total Syntheses of Bottromycin and Analogous

Based on their own synthetic studies and with all building blocks in hand Sunazuka and Ōmura *et al.* developed the first and so far only complete total synthesis of bottromycin (Scheme 30).[13,51] To extend the peptide chain, the Phth-group of amidine **87** was removed and the free amine was coupled with Boc-(*S*)-Val. Further prolongation gave rise to hexapeptide **93** which was subjected to desilylation and oxidation. These last two steps should be carried out on stage of the hexapeptide. Attempts to oxidize tetrapeptide **87** resulted in the formation of a diketopiperazine. The oxidation was the most critical step due to the nucleophilicity of the internal amidine. Thus all oxidation methods proceeding *via* an aldehyde intermediate failed, because this aldehyde was trapped by the amidine forming an imidazole. Only Jones oxidation was successful, providing an acceptable yield of the desired carboxylic acid **94**. The amidine also caused troubles in the final macrocyclization step, and the best results were obtained using EDCI/DIPEA in CH_2Cl_2, although here the yield of bottromycin was only moderate. This protocol was also used to generate some derivatives missing some β-methyl groups, such as bottromycin B_2 (Pro instead of MePro), or derivatives where β-MePhe was replaced by Phe [Phe–BotA_2 (**95**), PheBotB_2 (**96**)]. Their NMR spectra were rather

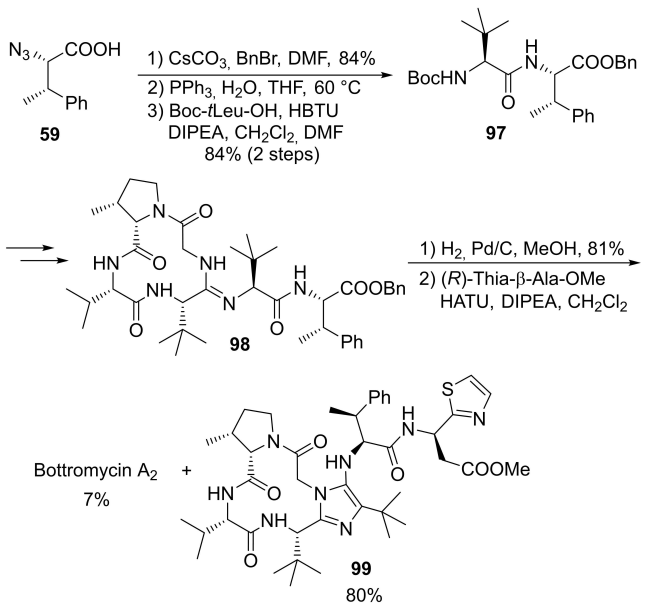


Scheme 29. Synthesis of tripeptide side chain **86**.

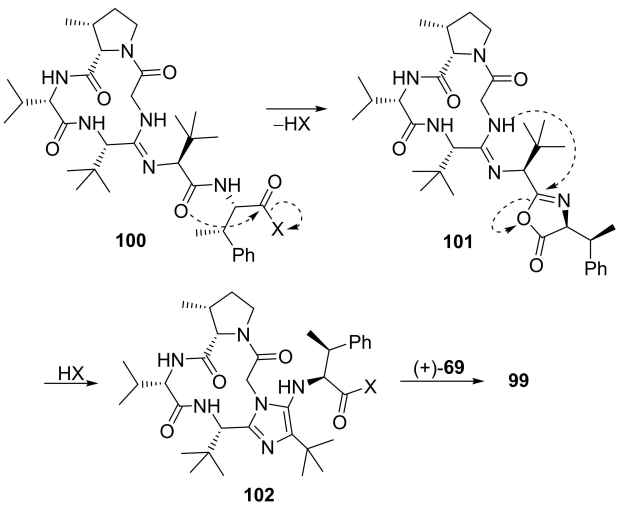
Israel Journal of Chemistry



Scheme 30. Total synthesis of bottromycin A2.



Scheme 31. Variable synthesis of bottromycin A2.



Scheme 32. Formation of side product **99**.

complicated (existence of conformers), what suggests that the methyl group of the β-MePhe is important for the three-dimensional structure of the bottromycins.

It was known from the early saponification experiments[5] that the methyl ester of the thia-β-Ala has a significant effect on the biological activity of the bottromycins in vitro and in vitro.[58] Therefore, Sunazuka and Ōmura also considered the synthesis of a bottromycin derivative missing the *C*-terminal amino acid, allowing modifications at this position in the last step by coupling a wide range of amines to the truncated hexapeptide.

Although this is a highly interesting approach, it was not as trivial as it looked like. Azido-MePhe **59** was converted into the corresponding benzyl ester and after reduction of the azide coupled to Boc-(*S*)-*t-Leu* (Scheme 31).

The dipeptide **97** was incorporated into bottromycin derivative **98** according to scheme 30. The benzyl ester could be cleaved easily to the carboxylic acid, the key intermediate for the synthesis of analogous. To proof the concept, the acid was coupled with (*R*)-thia-β-Ala [(+)-**69**] to the original natural product. The reaction proceeded smoothly, but bottromycin A_2 was only a side product. The major product was derivative **99**, containing an imidazole on the tetrapeptide ring.

Control experiments suggested that on activation of the carboxylic acid (**100**) attack of the adjacent amide bond occurs, generating an oxazolinone **101**, which is attacked by the amidine under formation of the imidazole (Scheme 32). The free carboxylic acid is activated again to **102**, which finally undergoes the amide bond formation to **99**. So far, HATU as coupling reagent gave the best yields for the bottromycin A_2 analogs **103** and was used for the incorporated of a range of amines, but the corresponding imidazole was the main product in all cases.

Further bottromycin derivatives were obtained by saponification of the natural product at the *C*-terminus and coupling the free acid with suitable nucleophiles. Miller *et al.* could show that some amide derivatives show better activity against *Staph. aureus* in mice than the natural product.[59] Researcher at AiCuris used this approach for the synthesis of "Weinreb-amide"-type amides by reaction with linear or cyclic *N,O*-dialkylhydroxylamines.[60] The groups of Ōmura and Sunazuka synthesized a range of different derivatives *via* the correspond-

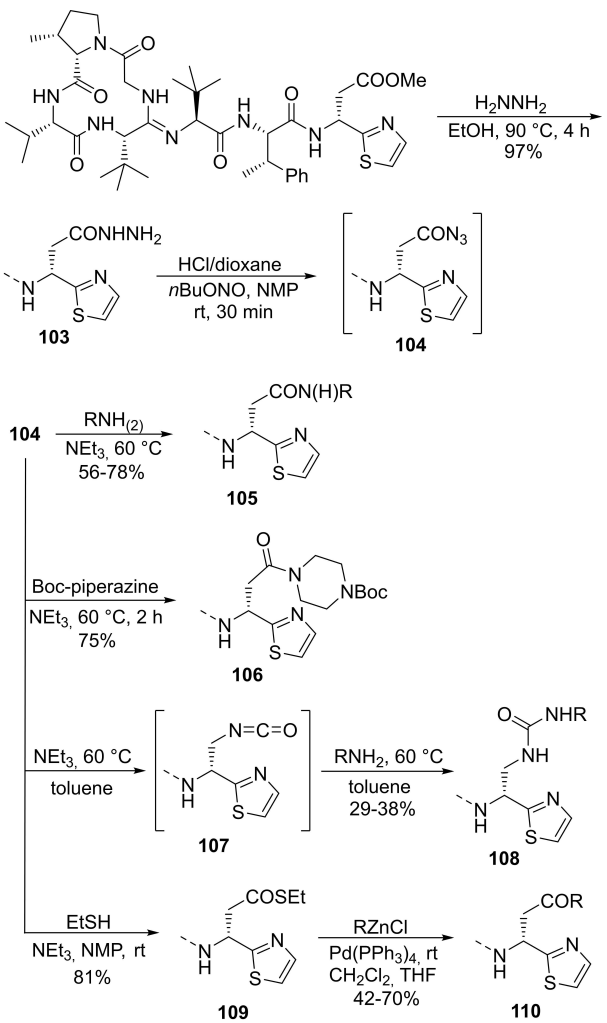
ing hydrazide **103** as common intermediate (Scheme 33).[58] Nitrosation of **103** gave rise to acyl azide **104** as an active intermediate which could be coupled with a range of amines to the corresponding amides **105**. Application of mono Boc-protected piperazine allowed further modification by replacing the Boc-protecting group of **106**. On the other hand, heating the acyl azide to 60 °C resulted in a Curtius rearrangement giving rise to an isocyanate **107**, which on treatment with amines provided ureas **108**. Reacting **104** with thiols gave rise to thioesters such as **109** which could be subjected to palladium-catalyzed cross couplings with organozinc reagents generating ketones **110**.

3. SAR Studies of Bottromycins and Derivatives

First biological data for the bottromycins A–C were reported by Nakamura *et al.*[3c] They determined the minimal growth inhibitory concentrations (MIC) towards a wide range of bacterial strains. The bottromycins showed strong inhibition against *Staphylococcus aureus, Bacillus subtilis and Mycobacteria* in a low or even sub-micromolar range. Although highly active *in vitro*, the bottromycins show no good *in vivo* efficiency because of their instability in oral and parenteral administration,[61] mainly because of the lability of the methyl ester under physiological conditions.[58] The bottromycin acid is more or less inactive. Therefore, Miller *et al.* prepared a range of amide derivatives and compared their *in vitro* and in vivo activity towards *Staph. aureus*.[59] Most compounds were active, but the primary and secondary amides were less active than the esters *in vitro*, but more active *in vivo*. Similar observations were made with the Weinreb amides of the AiCuris team.[60]

By far the most detailled SAR studies were reported by Ōmura and Sunazuka. A wide range of different derivatives were prepared from bottromycins obtained by fermentation (Scheme 33), but they also investigated some desmethyl derivatives, obtained by total synthesis (according to Scheme 30). The results of the SAR studies are summarized schematically in Figure 3.

The unusual methylation pattern has a significant effect on the bioactivity towards *Staph. aureus*. Bottromycin D, where the valine is replaced by a alanine was half as active as bottromycin A,[3c] while bottromycin B, missing the methyl group at the proline was 4-fold less active. Bottromycin C, the dimethylated analogue was comparable active as bottromycin A. The β-methyl group on the Phe seems to be essential, its removal causes a dramatic drop in activity. Obviously this methyl group influences the conformation of the side chain and controls the three-dimensional structure of the whole molecule, an assumption which is supported by ^{1}H NMR.[13,51] Linear peptides do not show significant activity, probably due to a wrong three dimensional conformation, clearly indicating that the cyclic peptide ring is essential.[51] Also no activity is observed for derivatives with a COOH-group at the *C*-terminus, or if the thia-β-Ala is missing completely. This might be caused by a drop in the hydrophobicity. Interestingly, incorporating the opposite (*S*)-isomer of thia-β-Ala has almost no effect on the activity. The thia-β-Ala is not essential at all for the activity, derivatives missing the acetate side chain or the thiazole unit are only slightly less active. Obviously only the amide functionality is necessary for good activities. The moderate *in vivo* activity of the ester in the natural products probably results from its low hydrolytic stability under physiological conditions and its cleavage towards the almost



Scheme 33. Synthesis of bottromycin A_2-analogs *via* hydrazide **103**.

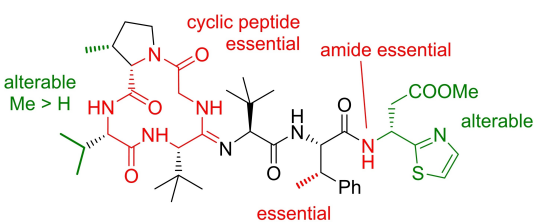


Figure 3. Summary of SAR for bottromycin derivatives.

inactive carboxylic acid. Although significant less active *in vitro*, better *in vivo* stabilities are observed for secondary aliphatic amides, while aromatic and tertiary amides as well as those with basic side chains are almost inactive.[59] Piperazino derivatives **106** and ureas **108** exhibit 4- to 32-fold weaker activity *in vitro*, but better stability.[58] Thioesters such as **109** are significantly more active than bottromycin A, but due to their great reactivity completely unstable e. g. in mouse plasma. Ketones **110** which cannot undergo hydrolysis are perfect stable and show activities comparable to bottromycin A_2 and vancomycin, but are also active against vancomycin resistant strains.

4. Summary

Although isolated and described already 65 years ago the bottromycins developed to a "nightmare" and a real challenge for scientists of almost all fields of natural product science. Even modern nmr techniques, developed during time, were not able to solve the structure of the bottromycins correctly. Total synthesis was required in order to determine the configuration of the last stereogenic center. From the first synthetic studies to the final total synthesis it took more than 50 years, until all obstacles could be abolished.

Acknowledgements

Open access funding enabled and organized by Projekt DEAL.

References

[1] J. M. Waisvisz, M. G. van der Hoeven, J. van Peppen, W. C. M. Zwennis, *J. Am. Chem. Soc.* **1957**, *79*, 4520–4521.
[2] N. Tanaka, K. Sashita, Y. Yamaguchi, H. Umezawa, *J. Biochem.* **1966**, *60*, 405–410.
[3] a) Y. C. Lin, N. Tanaka, *J. Biochem.* **1968**, *63*, 1–7; b) S. Nakamura, T. Chikaike, H. Yonehara, H. Umezawa, *Chem. Pharm. Bull.* **1965**, *13*, 599–602; c) S. Nakamura, T. Yajima, Y. C. Lin, H. Umezawa, *J. Antibiot.* **1967**, *20*, 1–5; d) T. Otaka, A. Kaji, *J. Biol. Chem.* **1976**, *251*, 2299–2306; e) T. Otaka, A. Kaji, *FEBS Lett.* **1981**, *123*, 173–176; f) T. Otaka, A. Kaji, *FEBS Lett.* **1983**, *153*, 53–59.
[4] J. M. Waisvisz, M. G. Vanderhoeven, J. F. Holscher, B. T. Nijenhuis, *J. Am. Chem. Soc.* **1957**, *79*, 4522–4524.
[5] J. M. Waisvisz, M. G. van der Hoeven, *J. Am. Chem. Soc.* **1958**, *80*, 383–385.
[6] a) S. Nakamura, T. Chikaike, H. Yonehara, H. Umezawa, *J. Antibiot.* **1965**, *18*, 60–61; b) S. Nakamura, H. Umezawa, *Chem. Pharm. Bull.* **1966**, *14*, 981–986.
[7] H. Arold, M. Eule, S. Reissmann, *Z. Chem.* **1969**, *9*, 447–448.
[8] Y. Seto, K. Torii, K. Bori, K. Inabata, S. Kuwata, H. Watanabe, *Bull. Chem. Soc. Jpn.* **1974**, *47*, 151–155.
[9] T. Yamada, K. Takashima, T. Miyazawa, S. Kuwata, *Bull. Chem. Soc. Jpn.* **1978**, *51*, 878–883.
[10] Y. Takahashi, H. Naganawa, T. Takita, H. Umezawa, S. Nakamura, *J. Antibiot.* **1976**, *29*, 1120–1123.
[11] D. Schipper, *J. Antibiot.* **1983**, *36*, 1076–1077.
[12] M. Kaneda, *J. Antibiot.* **1992**, *45*, 792–796.
[13] H. Shimamura, H. Gouda, K. Nagai, T. Hirose, M. Ichioka, Y. Furuya, Y. Kobayashi, S. Hirono, T. Sunazuka, S. Omura, *Angew. Chem. Int. Ed.* **2009**, *48*, 914–917.
[14] Y. Hou, M. D. B. Tianero, J. C. Kwan, T. P. Wyche, C. R. Michel, G. A. Ellis, E. Vazquez-Rivera, D. R. Braun, W. E. Rose, E. W. Schmidt, T. S. Bugni, *Org. Lett.* **2012**, *14*, 5050–5053.
[15] H. Gouda, Y. Kobayashi, T. Yamada, T. Ideguchi, A. Sugawara, T. Hirose, S. Omura, T. Sunazuka, S. Hirono, *Chem. Pharm. Bull.* **2012**, *60*, 169–171.
[16] a) P. G. Arnison, M. J. Bibb, G. Bierbaum, A. A. Bowers, T. S. Bugni, G. Bulaj, J. A. Camarero, D. J. Campopiano, G. L. Challis, J. Clardy, P. D. Cotter, D. J. Craik, M. Dawson, E. Dittmann, S. Donadio, P. C. Dorrestein, K. D. Entian, M. A. Fischbach, J. S. Garavelli, U. Goransson, C. W. Gruber, D. H. Haft, T. K. Hemscheidt, C. Hertweck, C. Hill, A. R. Horswill, M. Jaspars, W. L. Kelly, J. P. Klinman, O. P. Kuipers, A. J. Link, W. Liu, M. A. Marahiel, D. A. Mitchell, G. N. Moll, B. S. Moore, R. Muller, S. K. Nair, I. F. Nes, G. E. Norris, B. M. Olivera, H. Onaka, M. L. Patchett, J. Piel, M. J. T. Reaney, S. Rebuffat, R. P. Ross, H. G. Sahl, E. W. Schmidt, M. E. Selsted, K. Severinov, B. Shen, K. Sivonen, L. Smith, T. Stein, R. D. Sussmuth, J. R. Tagg, G. L. Tang, A. W. Truman, J. C. Vederas, C. T. Walsh, J. D. Walton, S. C. Wenzel, J. M. Willey, W. A. van der Donk, *Nat. Prod. Rep.* **2013**, *30*, 108–160; b) J. B. Hedges, K. S. Ryan, *Chem. Rev.* **2020**, *120*, 3161–3209; c) C. T. Walsh, R. V. O. Brien, C. Khosla, *Angew. Chem. Int. Ed.* **2013**, *52*, 7098–7124.
[17] J. Kellenberger, *Ph.D. thesis, ETH* **1997**.
[18] H. Mosimann, B. Krautler, *Angew. Chem. Int. Ed.* **2000**, *39*, 393-++.
[19] a) W. J. K. Crone, F. J. Leeper, A. W. Truman, *Chem. Sci.* **2012**, *3*, 3516–3521; b) J. P. Gomez-Escribano, L. Song, M. J. Bibb, G. L. Challis, *Chem. Sci.* **2012**, *3*, 3522–3525; c) L. Huo, S. Rachid, M. Stadler, S. C. Wenzel, R. Mueller, *Chem. Biol.* **2012**, *19*, 1278–1287.
[20] a) L. Franz, S. Adam, J. Santos-Aberturas, A. W. Truman, J. Koehnke, *J. Am. Chem. Soc.* **2017**, *139*, 18158–18161; b) G. Mann, L. J. Huo, S. Adam, B. Nardone, J. Vendome, N. J. Westwood, R. Muller, J. Koehnke, *ChemBioChem* **2016**, *17*, 2286–2292; c) A. Sikandar, L. Franz, S. Adam, J. Santos-Aberturas, L. Horbal, A. Luzhetskyy, A. W. Truman, O. V. Kalinina, J. Koehnke, *Nature Chem. Biol*; d) A. Sikandar, L. Franz, O. Melse, I. Antes, J. Koehnke, *J. Am. Chem. Soc.* **2019**, *141*, 9748–9752.
[21] S. L. Titouani, J. P. Lavergne, P. Viallefont, R. Jacquier, *Tetrahedron* **1980**, *36*, 2961–2965.
[22] C. Herdeis, H. P. Hubmann, H. Lotter, *Tetrahedron: Asymmetry* **1994**, *5*, 351–354.
[23] a) C. Herdeis, H. P. Hubmann, *Tetrahedron: Asymmetry* **1992**, *3*, 1213–1221; b) C. Milne, A. Powell, J. Jim, M. Al Nakeeb, C. P. Smith, J. Micklefield, *J. Am. Chem. Soc.* **2006**, *128*, 11250–11259.
[24] P. Karoyan, G. Chassaing, *Tetrahedron Lett.* **1997**, *38*, 85–88.
[25] J. Quancard, A. Labonne, Y. Jacquot, G. Chassaing, S. Lavielle, P. Karoyan, *J. Org. Chem.* **2004**, *69*, 7940–7948.
[26] T. M. Kamenecka, Y.-J. Park, L. S. Lin, T. Lanza, W. K. Hagmann, *Tetrahedron Lett.* **2001**, *42*, 8571–8573.
[27] C. Flamant-Robin, Q. Wang, A. Chiaroni, N. A. Sasaki, *Tetrahedron* **2002**, *58*, 10475–10484.
[28] R. Sharma, W. D. Lubell, *J. Org. Chem.* **1996**, *61*, 202–209.

[29] J. R. Medina, C. W. Blackledge, K. F. Erhard, J. M. Axten, W. H. Miller, *J. Org. Chem.* **2008**, *73*, 3946–3949.
[30] T. M. Bott, J. A. Vanecko, F. G. West, *J. Org. Chem.* **2009**, *74*, 2832–2836.
[31] S. Fuse, H. Koinuma, A. Kimbara, M. Izumikawa, Y. Mifune, H. He, K. Shin-ya, T. Takahashi, T. Doi, *J. Am. Chem. Soc.* **2014**, *136*, 12011–12017.
[32] C. S. V. Houge-Frydrych, M. L. Gilpin, P. W. Skett, J. W. Tyler, *J. Antibiot.* **2000**, *53*, 364–372.
[33] Y. Kataoka, Y. Seto, M. Yamamoto, T. Yamada, S. Kuwata, H. Watanabe, *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1081–1084.
[34] a) M. Alias, M. P. Lopez, C. Cativiela, *Tetrahedron* **2004**, *60*, 885–891; b) N. Grobuschek, M. G. Schmid, C. Tuscher, M. Ivanova, G. Gubitz, *J. Pharm. Biomed. Anal.* **2002**, *27*, 599–605; c) L. M. Nogle, C. W. Mann, W. L. Watts, Y. R. Zhang, *J. Pharm. Biomed. Anal.* **2006**, *40*, 901–909; d) A. Peter, G. Toth, G. Torok, D. Tourwe, *J. Chromatogr. A* **1996**, *728*, 455–465; e) G. Torok, A. Peter, D. W. Armstrong, D. Tourwe, G. Toth, J. Sapi, *Chirality* **2001**, *13*, 648–656; f) E. Vekes, G. Torok, A. Peter, J. Sapi, D. Tourwe, *J. Chromatogr. A* **2002**, *949*, 125–139.
[35] a) I. Ilisz, G. Fodor, R. Ivanyi, L. Szente, G. Toth, A. Peter, *J. Chromatogr. B* **2008**, *875*, 273–279; b) C. Jiang, D. W. Armstrong, A. W. Lantz, A. Peter, G. Toth, *J. Liq. Chromatogr. Relat. Technol.* **2007**, *30*, 1421–1436.
[36] R. Torok, R. Berkecz, A. Peter, *J. Sep. Sci.* **2006**, *29*, 2523–2532.
[37] J. Ogawa, A. Ryono, S. X. Xie, R. M. Vohra, R. Indrati, M. Akamatsu, H. Miyagawa, T. Ueno, S. Shimizu, *J. Mol. Catal. B* **2001**, *12*, 71–75.
[38] G. Tsuchihashi, S. Mitamura, K. Ogura, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2167–2168.
[39] a) R. Dharanipragada, E. Nicolas, G. Toth, V. J. Hruby, *Tetrahedron Lett.* **1989**, *30*, 6841–6844; b) R. Dharanipragada, K. Vanhulle, A. Bannister, S. Bear, L. Kennedy, V. J. Hruby, *Tetrahedron* **1992**, *48*, 4733–4748.
[40] a) D. A. Evans, J. A. Ellman, R. L. Dorow, *Tetrahedron Lett.* **1987**, *28*, 1123–1126; b) D. A. Evans, A. E. Weber, *J. Am. Chem. Soc.* **1986**, *108*, 6757–6761.
[41] S. Fioravanti, M. A. Loreto, L. Pellacani, F. Sabbatini, P. A. Tardella, *Tetrahedron: Asymmetry* **1994**, *5*, 473–478.
[42] W. Oppolzer, R. Moretti, *Tetrahedron* **1988**, *44*, 5541–5552.
[43] E. Medina, A. Moyano, M. A. Pericas, A. Riera, *J. Org. Chem.* **1998**, *63*, 8574–8578.
[44] M. Pasto, A. Moyano, M. A. Pericas, A. Riera, *J. Org. Chem.* **1997**, *62*, 8425–8431.
[45] M. J. O'Donnell, J. T. Cooper, M. M. Mader, *J. Am. Chem. Soc.* **2003**, *125*, 2370–2371.
[46] A. Enright, F.-R. Alexandre, G. Roff, I. G. Fotheringham, M. J. Dawson, N. J. Turner, *Chem. Commun.* **2003**, 2636–2637.
[47] G. J. Roff, R. C. Lloyd, N. J. Turner, *J. Am. Chem. Soc.* **2004**, *126*, 4098–4099.
[48] T. Ooi, D. Kato, K. Inamura, K. Ohmatsu, K. Maruoka, *Org. Lett.* **2007**, *9*, 3945–3948.
[49] J. M. Waisvisz, M. G. Vanderhoeven, B. T. Nijenhuis, *J. Am. Chem. Soc.* **1957**, *79*, 4524–4527.
[50] a) F. A. Davis, R. E. Reddy, J. M. Szewczyk, *J. Org. Chem.* **1995**, *60*, 7037–7039; b) T. P. Tang, J. A. Ellman, *J. Org. Chem.* **1999**, *64*, 12–13.
[51] T. Yamada, M. Yagita, Y. Kobayashi, G. Sennari, H. Shimamura, H. Matsui, Y. Horimatsu, H. Hanaki, T. Hirose, S. Omura, T. Sunazuka, *J. Org. Chem.* **2018**, *83*, 7135–7149.
[52] T. Yamada, K. Suegane, S. Kuwata, H. Watanabe, *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1088–1093.
[53] T. Yamada, T. Miyazawa, S. Kuwata, H. Watanabe, *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1827–1830.
[54] S. Ackermann, H.-G. Lerchen, D. Haebich, A. Ullrich, U. Kazmaier, *Beilstein J. Org. Chem.* **2012**, *8*, 1652–1656, No. 1189.
[55] a) U. Kazmaier, C. Hebach, *Synlett* **2003**, 1591–1594; b) R. Pick, M. Bauer, U. Kazmaier, C. Hebach, *Synlett* **2005**, 757–760.
[56] a) M. Bauer, F. Maurer, S. M. Hoffmann, U. Kazmaier, *Synlett* **2008**, 3203–3207; b) U. Kazmaier, S. Ackermann, *Org. Biomol. Chem.* **2005**, *3*, 3184–3187; c) U. Kazmaier, A. Persch, *Org. Biomol. Chem.* **2010**, *8*, 5442–5447.
[57] F. Hata, A. Matsumae, K. Abe, Y. Sano, M. Otani, S. Omura, Kitasato Institute for Infectious Diseases, Japan, **1972**, Fermentative preparation of bottromycin. JP47010036B, **1972**.
[58] Y. Kobayashi, M. Ichioka, T. Hirose, K. Nagai, A. Matsumoto, H. Matsui, H. Hanaki, R. Masuma, Y. Takahashi, S. Omura, T. Sunazuka, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6116–6120.
[59] W. J. Miller, L. Chaiet, G. Rasmussen, B. Christensen, J. Hannah, A. K. Miller, F. J. Wolf, F. J. Wolf, *J. Med. Chem.* **1968**, *11*, 746–749.
[60] H.-G. Lerchen, G. Schiffer, H. Broetz-Oesterhelt, A. Mayer-Bartschmid, S. Eckermann, C. Freiberg, R. Endermann, J. Schuhmacher, H. Meier, N. Svenstrup, S. Seip, M. Gehling, D. Haebich, AiCuris GmbH & Co. KG, Germany. **2006**, Fermentative and chemical synthesis of bottromycin derivative analogs for use in treatment or prevention of bacterial infections in humans or animals. WO2006103010A1, **2006**.
[61] N. Tanaka, T. Nishimura, S. Nakamura, H. Umezawa, *J. Antibiot. Ser. A* **1966**, *19*, 149–154.

Manuscript received: August 11, 2020
Revised manuscript received: October 19, 2020
Version of record online: November 6, 2020

REVIEW