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Natural Products Synthesis

Total Synthesis of Thiamyxins A–C and Thiamyxin E, a Potent Class of RNA-Virus-Inhibiting (Cyclo)depsipeptides

*Kevin Bauer and Uli Kazmaier**

Dedicated to Professor Manfred T. Reetz on the occasion of his 80th birthday

Abstract: We present the first total synthesis of the thiamyxins A–C and the now fully characterized thiamyxin E, an interesting class of thiazole- and thiazoline-rich depsipeptides with diverse antiviral activity. The synthesis features a parallel closing of two methyl thiazoline units, with low epimerization of the very labile adjacent stereocenter. It also includes the three-step synthesis of an uncommon hydroxy acid and the oxidation-free elimination of a phenylselenide to form a dehydroalanine moiety. The exploitation of the acid-labile stereocenter at the isoleucine moiety and the reopening of the macrolactones gave access to the four thiamyxins with good yields and diastereomeric purities from a single precursor. The modular total synthesis allows further testing of the biological activity and gives opportunities to explore the pharmacophore and antiviral target through derivatization.

There is high biological and synthetic interest in many thiazole- and thiazoline-containing natural products, such as thiangazole,[1] didehydromirabazole,[2] baringolin,[3] and apratoxin,[4] due to their striking tumor-selective cytotoxicity, antibiotic and/or antiviral activities. The recently highlighted[5] thiamyxins represent a new group of thiazole/thiazoline-rich natural products, isolated from a myxobacterial strain of the *Myxococcaceae* family (MCy9487) by Müller and co-workers.[6]

The depsipeptides appear in their cyclic form, thiamyxin A and B, as well as the open-chain hydroxy acid thiamyxin C and as the corresponding glycerol ester thiamyxin D (Figure 1). The latter two are presumably shunt products of the cyclization process and were isolated as inseparable mixtures of diastereomers, differentiating only at the isoleucine α-center (*).

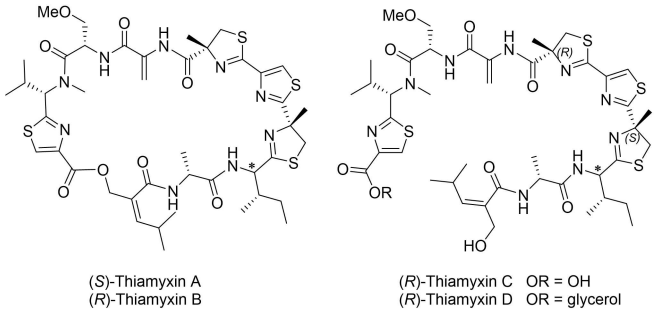


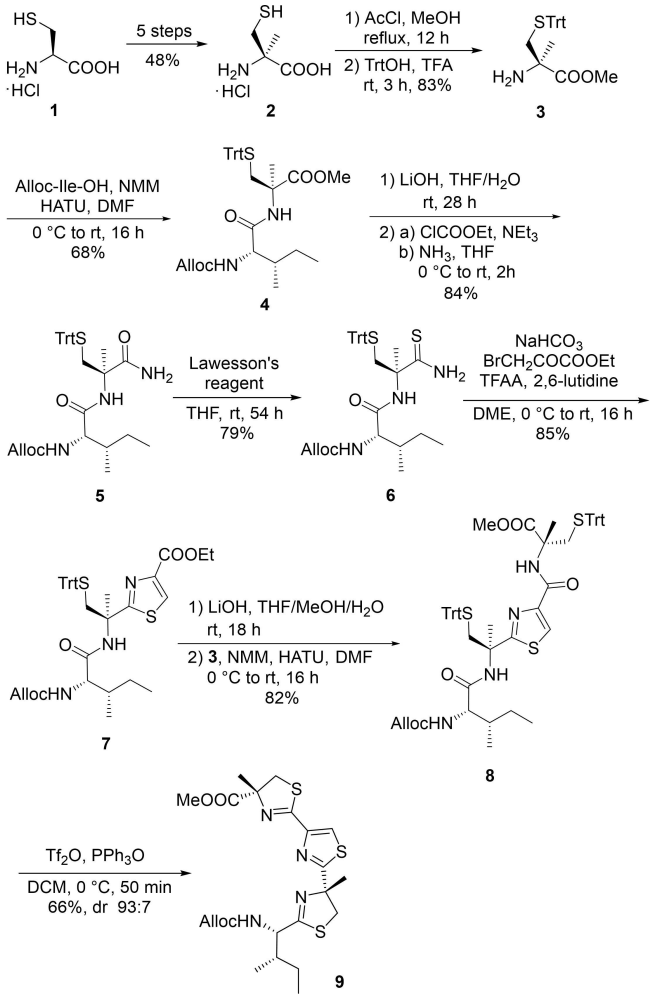
Figure 1. Thiamyxins from MCy9487.

The responsible polyketide synthase (PKS)/non-ribosomal peptide synthase (NRPS) hybrid gene cluster contains two epimerization domains, one in the Ala-incorporating module and one in the Ile-incorporating module. The higher production rate and the D-*allo*-isoleucine moiety depict thiamyxin B as the main product of the biosynthetic gene cluster assembly line. Thiamyxins show interesting activity against RNA viruses (human pathogenic corona, Dengue, and Zika virus) with high nanomolar or low micromolar IC_{50} values. More importantly, a fivefold difference in their cytotoxic effects indicates a distinguished mode of action for their antiviral activity. A total synthesis of this series of natural products could enable derivatization options for investigating biosynthetic pathways, modes of action, and pharmacophores, and could ultimately deliver substantial amounts of material for bioactivity testing, overcoming the low production rates of MCy9487. The most challenging part of the synthesis will be the thiazoline–thiazole–thiazoline fragment with a labile adjacent stereocenter at the isoleucine (*). Epimerization at this position has to be avoided, because the diastereomers of thiamyxin C can probably not be separated.[6]

The interesting biological activities of this compound class and our interest in the total synthesis of peptidic natural products[7] motivated us to develop a synthetic protocol which gives access to the whole thiamyxin family taking advantage of the configurational lability of the isoleucine α-center (*).

Starting with the thiazoline–thiazole–thiazoline fragment (Scheme 1), α-methyl cysteine **2** was synthesized from **1** using the protocol reported by Pattenden et al.[8] Subsequent methyl ester formation followed by tritylation under acidic conditions[9] gave the protected α-methyl cysteine **3** in good

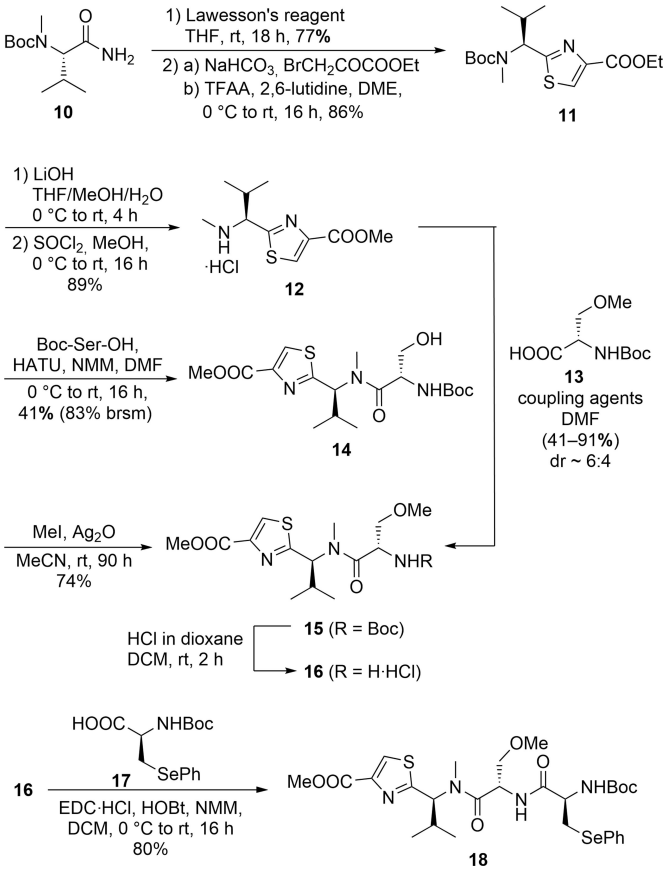
[*] K. Bauer, Prof. Dr. U. Kazmaier
Organic Chemistry I, Saarland University
Campus C4.2, 66123 Saarbrücken (Germany)
E-mail: u.kazmaier@mx.uni-saarland.de



Scheme 1. Synthesis of bisthiazoline building block **9**. DMF = *N*,*N*-dimethylformamide, NMM = *N*-methyl morpholine, TFAA = trifluoroacetic anhydride.

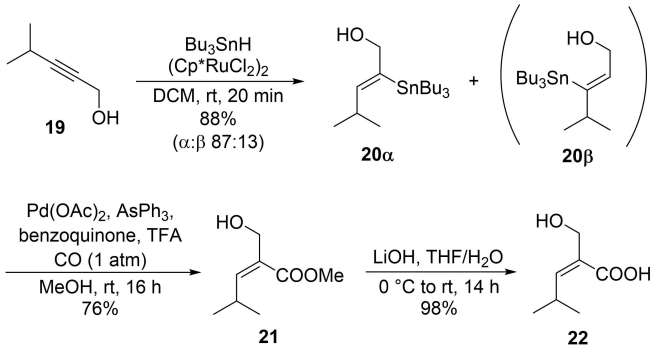
yields. Standard coupling of *N*-allyloxycarbonyl (Alloc) protected isoleucine using HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) in DMF provided dipeptide **4**. Instead of the very expensive D-*allo*-isoleucine, we used low-priced L-isoleucine, anticipating a racemization of the usually fragile stereocenter at the C2 exomethine position next to the thiazolines.[10] A primary amide was installed by saponification of the methyl ester, activation of the acid and quenching with an aqueous ammonia solution. The amide **5** was transformed with the Lawesson reagent into thioamide **6**,[11] which was then used to generate a thiazole by a modified Hantzsch procedure, as described by Aguilar and Meyers.[12] Such a sequence has been used previously[13] and provided thiazole **7** with 84% yield. Using methanol as a cosolvent in the following saponification greatly improved the reaction time and was crucial in achieving full conversion. A further uronium-type peptide coupling with protected α-methyl cysteine **3** gave peptide **8** in high yield. For thiazoline formation, a "low-epimerization protocol" by dehydrogenation with a phosphorous(V) species, as reported by Kelly and co-workers,[14a] gave the best result with 66% yield and a diastereomeric ratio (dr) of 93:7. Another promising approach by molybdenum catalysis, following the procedure developed by Ishihara and co-workers,[14b] gave the bisthiazoline **9** in only 40–48% yield with dr 1:1, independent on the molybdenum catalyst used. Although the epimerization of the C2 exomethine position was an intended process, it should be suppressed as long as possible for a cleaner synthesis with manageable analytics. Thus, although our first attempt was to undertake thiazoline formation at the end of the total synthesis, unfortunately, none of the above-mentioned protocols[14] was successful. Thomas and co-workers reported similar problems with a phosphorous(V) approach on a highly functionalized intermediate during their total synthesis of precursors of vioprolide.[15] As a result, we discontinued this late-stage approach and bis-thiazoline **9** was used as a building block in the later assembly.

The next building block **18** (Scheme 2) started with the known primary amide of *N*-methyl valine **10**.[16] A further sequence of the Lawesson reagent and a modified Hantzsch reaction gave thiazole **11**, which has been synthesized before by Pattenden and co-workers using a similiar protocol, and performing the *N*-methylation as the final step.[13d] Saponification of the ethyl ester and subsequent esterification with parallel Boc-deprotection produced amine **12** as the



Scheme 2. Synthesis of the north-eastern part of the thiamyxins. DME = 1,2-dimethoxyethane.

hydrochloride salt. Different attempts to couple **12** with *O*-methylated serine **13**,[17] EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide)/HOBt (*N*-hydroxy benzotriazole), HATU, PyAOP ((7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate), BOP-Cl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride)), including base-free couplings with the free amine of **12** and EDC/HOBt, resulted in epimerization at the serine α-stereocenter.

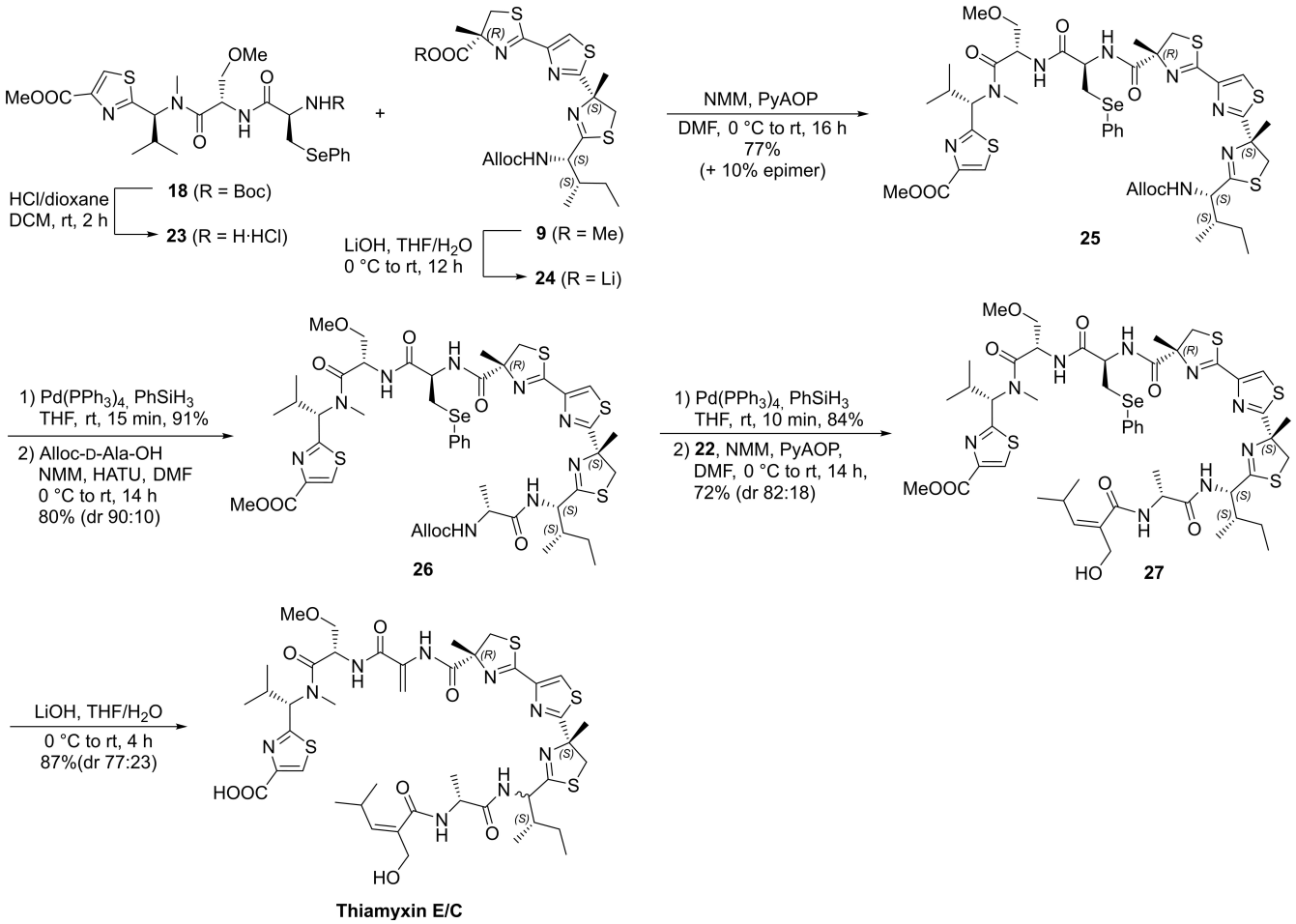


Scheme 3. Synthesis of the unusual *Z*-alkenoic acid **22**.

Finally, direct coupling with Boc-protected serine and reisolation of rather unreactive **12** as the free amine proved to be the most satisfying approach with 41 % or 83 % yield based on recovered starting material. The resulting peptide **14** was *O*-methylated with silver(I) oxide and iodomethane to give **15**.[17] After Boc-deprotection, the hydrochloride salt **16** and *N*-Boc phenyl selenocysteine **17**[18] gave building block **18** in high yield, which serves as a precursor for the north-eastern fragment of the thiamyxins.

The unusual *Z*-alkenoic acid (Scheme 3) in the southern part of the thiamyxins was synthesized in a short three-step procedure, starting with propargylic alcohol **19**.[19] *Trans* hydrostannation by the protocol of Rummelt and Fürstner[20] led to the *Z*-stannyl alkene **20α** and the β-addition side product **20β** in a ratio of 87:13. The side product **20β** could either be chromatographically separated or, much more easily, as the five-membered-ring lactone after the subsequent palladium-catalyzed methoxycarbonylation.[21] Following this protocol, we were able to access the *Z*-alkenoic acid **22** after saponification in an overall yield of 58 % over three steps.

After Boc-deprotection of **18**, peptide **23** was coupled with lithium carboxylate **24** (Scheme 4) to avoid acid-catalyzed epimerization at the labile isoleucine center. The



Scheme 4. Coupling of the building blocks and final deprotection.

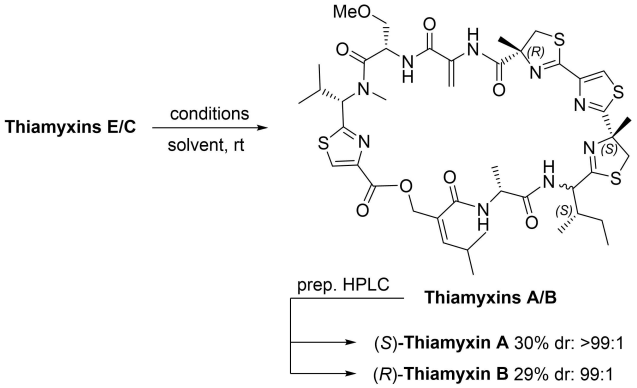
coupling with PyAOP provided compound **25** in 77 % yield, contaminated with 10 % of the epimerization product, which could be separated by column chromatography. Subsequent palladium-catalyzed Alloc-deprotection under neutral conditions using phenylsilane as an allyl scavenger, followed by a HATU coupling with Alloc-D-Ala-OH, yielded compound **26** in 73 % yield over two steps. This coupling resulted in another 10 % epimerization. At this point, we used the mixture of diastereomers in the next Alloc-deprotection and reaction with *Z*-alkenoic acid **22**. This resulted in the thiamyxin precursor **27** with a diastereomeric ratio of 82:18. The remaining steps were planned as saponification of the methyl ester, followed by oxidative elimination to generate the dehydroalanine moiety. To our surprise, using more than two equivalents of lithium hydroxide during saponification led to complete elimination of the phenylselenide, resulting in a mixture of the *S*-configured so far undescribed thiamyxin E as the main diastereomer, and *R*-configured thiamyxin C as the minor diastereomer in a ratio of 77:23. In attempts to purify the compounds by preparative HPLC with 0.1 % $HCOOH_{aq}$/MeCN, the diastereomers remained inseparable but further isomerized to a 1:1 mixture, showcasing again the lability of the α-stereocenter at the isoleucine.

To get access to the thiamyxins A and B, the next step was the macrolactonization, which was not a trivial issue. A selection of the screened conditions is shown in Table 1. A Steglich-type esterification[22] with DIC (*N*,*N*′-diisopropylcarbodiimide)/DMAP (4-dimethylaminopyridine) resulted in full conversion into the unreactive *N*-acyl urea,[23] while no product formation was observed (entry 1).

A standard peptide coupling reagent, PyAOP, resulted in only 10 % product formation together with several unknown side products (entry 2). In the third entry, a recently published protocol by Zhao and co-workers[24] using a ynamide as a coupling reagent was tested. In the first step, full conversion into the α-acyloxy enamide was achieved, but the acid-catalyzed second step did not promote any cyclization. Under Yamaguchi conditions,[25] with TCBC (2,4,6-trichlorobenzoyl chloride) in toluene (with MeCN as cosolvent due to solubility issues) 31 % yield could be achieved (entry 4). Other Yamaguchi-type macrolactonization reactions using MNBA (2-methyl-6-nitrobenzoic anhydride)[26] showed good progress in toluene/MeCN and proceeded even better in MeCN (entries 5 and 6). At this stage, the best results were obtained with TCBC in MeCN, providing a mixture of thiamyxin A and B in 63 % yield after only 1 h at room temperature, despite the high dilution of 2 mM (entry 7). Purification by preparative HPLC gave diastereomerically pure thiamyxin A and B in 30 % and 29 % yield, respectively. To our surprise, using 0.1 % aqueous formic acid as the eluent to improve separation did not cause any epimerization. Clearly, the cyclic derivatives are less sensitive to acid-catalyzed epimerization as compared to the open-chain variants thiamyxin C and E.

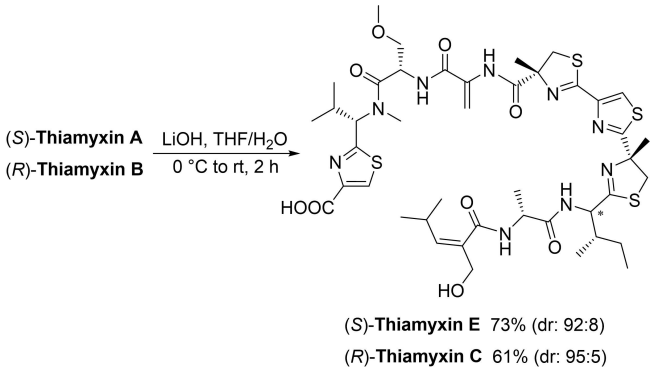
Finally, to get access to stereoisomerically pure open-chain thiamyxins, we saponified the pure thiamyxins A and B using lithium hydroxide (Scheme 5). This resulted in the formation of thiamyxins E and C with dr 92:8 and 95:5, respectively. The NMR spectroscopic data of the synthesized thiamyxins A–C are in accordance with the published data of Müller and co-workers.[6] Comparison with the NMR

Table 1: Macrolactonization of thiamyxins E/C.



Entry	Conditions	Solvent	Yield [%]
1	2 equiv DIC, 5 equiv DMAP, 48 h	DMF	–
2	5 equiv PyAOP, 5 equiv Et_3N, 10 equiv DMAP, 72 h	DMF	10[a]
3	1) 1.0 equiv MyTSA 2) 0.05 equiv pTsOH*H_2O, 5 days	DCM	–
4	1.05 equiv TCBC, 5 equiv Et_3N, 10 equiv DMAP, 30 h	Tol/MeCN	31[b]
5	1.1 equiv MNBA, 5 equiv Et_3N, 10 equiv DMAP, 24 h	Tol/MeCN	48[a]
6	1.1 equiv MNBA, 5 equiv Et_3N, 10 equiv DMAP, 18 h	MeCN	60[a]
7	1.05 equiv TCBC, 5 equiv Et_3N, 10 equiv DMAP, 1 h	MeCN	63[b]

[a] Yield determined by LC–MS. [b] Yield of the isolated product. DIC = *N*,*N*′-diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine, MyTSA = *N*-methyl ynetoluenesulfonamide, pTsOH*H_2O = *p*-toluenesulfonic acid monohydrate, TCBC = 2,4,6-trichlorobenzoyl chloride, MNBA = 2-methyl-6-nitrobenzoic anhydride.



Scheme 5. Accessing thiamyxin E and thiamyxin C by saponification.

raw data of the Rolf Müller group showed that synthetic thiamyxin E matches the minor diastereomer in their reported thiamyxin C sample (see the Supporting Information).

In conclusion, we have reported the first total synthesis of the cyclic depsipeptides thiamyxin A and B as well as the open-chain derivatives C and E. The route features the parallel ring closure of two methyl thiazolines with low epimerization, a short three-step procedure to the uncommon *Z*-alkenoic acid **22**, and macrolactonization under Yamaguchi conditions. By saponification of the macrolactones, we synthesized thiamyxin C and, additionally, the fully characterized diastereomer thiamyxin E with good diastereomeric purity, confirming the structure and configuration of the thiamyxins reported by Müller and coworkers.[6] We accessed four natural products by the same route by taking advantage of the very acid labile isoleucine α-stereocenter. This modular total synthesis of the thiamyxins could enable a broad range of derivatization for studying the pharmacophore of thiamyxins or elucidating the antiviral target. Further testing of the antiviral activity of the synthesized thiamyxins is currently ongoing.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: Macrolactonization · Natural Products · Peptides · Thiazolines · Total Synthesis

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