(–)-Bacillamide C: the convergent approach †‡

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The newly discovered natural product bacillamide C and several derivatives were convergently synthesized for the first time and in only three steps. The key transformation constitutes a thiazole Ugi multicomponent reaction. These compounds will serve to elucidate chemical biology and SAR of this potent anti-algae natural product and shows the synthetic pathway to related natural products.

Algae bloom has recently become an important ecological and economical threat. Due to intensified farming more and more phosphate and nitrogen rich run-off is accumulating in deltas and leading to eutrophication and explosive algae bloom. Additionally, pollution from chemical factories is often adding to the bad water quality. This complicated process seems also to be supported by the local and world wide increase of temperature due to climate change. Algae species such as the harmful dinoflagellate *Cochlodinium polykrikoides* a known red tide species are associated with extensive fish kills and great economic loss *e.g.* recently in Japanese and Korean waters.[1] Another topical example is the recent algae bloom ("hu tai") in the bay of Qingdao during the Olympic games in 2008 which severely threatened the Olympic sailing events.

Thiazole-5-carboxy-2-ethylindole amide natural products have been recently described under different names including bacillamide A and C and microbiaeratin associated with diverse bioactivity including algicidal and antibacterial (Fig. 1).[2] These structurally interesting natural products have been isolated from a number of different sources, including *Bacillus* sp. SY-1 isolated during the termination of bloom by *C. polykrikoides* in the Masan Bay of Korea,[3] *Bacillus endophyticus* isolated from a Bahamian

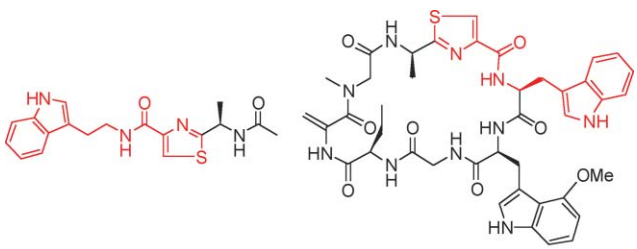

Fig. 1 Bacillamide C and argyrin B as representative bioactive natural products containing a tryptamide thiazole motif.

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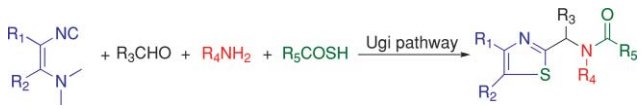
‡ Electronic supplementary information (ESI) available: Experimental details, NMR spectra and HPLC traces and a movie showing incubation of compound **10j** into HCT116 cell line. See DOI: 10.1039/b918214d

hypersaline microbial mat,[4] thermophilic *Thermoactinomyces* strain TA66-2 collected from hot spring sources of the West Anatolia in Turkey[5] and *Microbispora aerate* isolated from penguin excrement collected on the Antarctic Livingston Island.[6] In addition, the tryptamide thiazole motif observed in the bacillamides frequently occurs as a building block in potently bioactive cyclic peptides. Examples include the antibiotic zelkovamycin produced by a *Streptomyces* sp.,[7] the protein synthesis inhibitors A-21459 A and B from an *Actinoplanes* sp.,[8] and the immunosuppressive argyrins from the myxobacterium *Archangium gephyra*.[9]

Natural products have an evolutionary "built-in" biological activity, thus they often serve as valuable leads for medicinal chemistry or sometimes are useful even unmodified as drugs. Despite huge progress in synthetic methodologies, however, the multistep total synthesis of natural products for clinical trials or the market, *e.g.* epothilone derivative ZK-EPO (22 steps, 0.9% yield) or (+)-discodermolide (39 steps, 0.65% yield) is very time and resource demanding and rather the exception than the rule.[10] Especially, when PKPD (Pharmacokinetics, Pharmacodynamics) properties of the original natural product are unfortunate and many derivatives have to be synthesized to overcome these limitations, multistep sequential syntheses make these efforts often the domain of large pharma companies. Therefore a goal of future natural product chemistry must be the design of short, convergent and resource saving synthetic pathways to allow rapid access to derivatives for optimization and evaluation of their biological activities. One synthetic methodology which is very useful to reduce time and effort and widely applied in drug discovery is multicomponent reactions (MCR).[11] MCRs are the prototypes of convergent reactions and can yield large libraries of bioactive compounds in a short time based on the broad availability of their starting materials. Despite their usefulness in drug discovery, MCRs so far have been employed only sporadically in natural product total synthesis.[12]

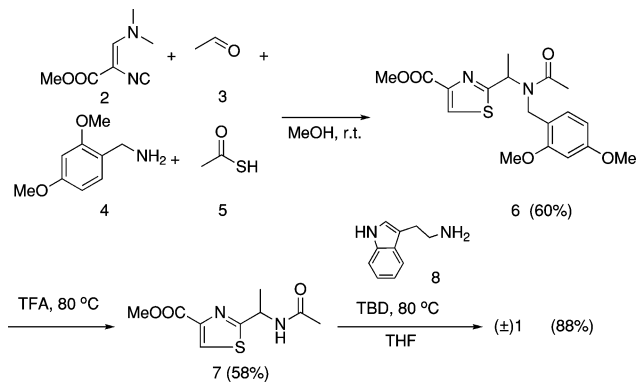
Thus in order to elucidate mode-of-action of these algicidal leads and to improve bioactivity a short, efficient and versatile synthetic access to natural products containing a thiazol-5-carboxy-2-ethylindole moiety is warranted. Additionally, the tryptamide thiazole motif caught our attention due to our recently discovered multicomponent reaction giving convergent and efficient access to peptide like thiazole back bones.[13] Here we would like to describe the first total synthesis of bacillamide C and the parallel synthesis of an array of derivatives thereof.

Retrosynthetically, using our MCR approach the central fragment of the bacillamides C is accessible using the commercially available starting materials Schöllkopf's isocyanide **2**, acetaldehyde **3**, an ammonia equivalent **4** and thioacetic acid **5** (Scheme 1).[12] We first tested our synthetic idea in preparing racemic target structure **1**. A benzylamine derivative instead of ammonia in Ugi-type reactions is required since ammonia



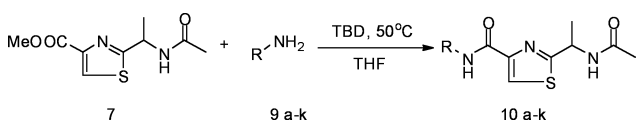
Scheme 1 Four-component thiazole component synthesis.

often displays low reactivity.[14] Thus, reaction of the four starting materials **2–5** affords the Ugi intermediate **6** in 60% yield under ambient conditions. The next step, the cleavage of the 2,4-dimethoxybenzyl protecting group is usually performed with TFA at ambient temperature.[15] However, attempts to analogously deprotect **6** under these conditions didn't show any conversion. Only prolonged treatment with TFA at 50 °C yielded the deprotected **7** in 58% yields. In order to avoid multistep saponification, activation and coupling sequences to transform the methyl ester into the amide we turned to a recently described procedure for a one-pot conversion.[16] Thus, 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) catalyzed direct amidation of **7** with tryptamine **8** yielded racemic target natural product bacillamide C **1**. Overall racemic bacillamide C is thus accessible from commercial available starting materials in three simple to perform steps in overall 30% yield (Scheme 2).



Scheme 2 Racemic four-component Bacillamide C synthesis.

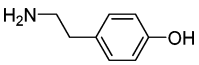
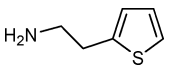
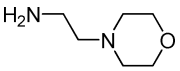
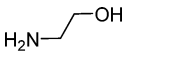
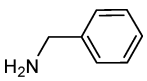
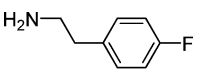
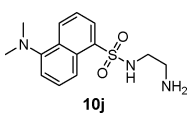
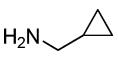
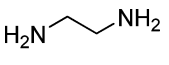
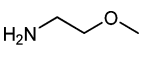
In order to explore the SAR of this new natural product we attempted to make amide derivatives based on the carboxylic acid methyl ester **7** using the same one-pot amidation (Scheme 3).[13] Thus we produced 12 amide derivatives of bacillamide C **1** shown in Table 1. Remarkably, this procedure is fast and straightforward and can yield a diversity of functionalized products. For example, we synthesized **10j**, which is a fluorescent dansyl derivative of **1**.



Scheme 3 Fast and efficient synthesis of bacillamide C derivatives.

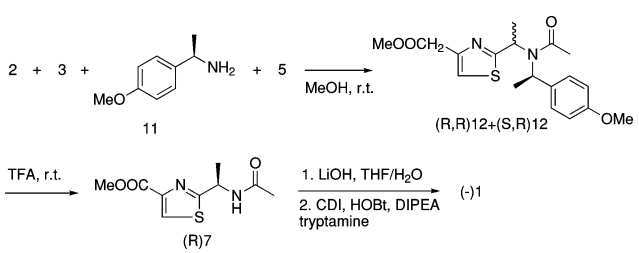
Next we considered a stereoselective approach towards **1**. It is well known, that stereoselectivity in the classical Ugi reaction can be directed by the use of chiral primary amines.[17] Different chiral amine components have been used in the past to accomplish diastereoselctivity, including phenylethylamine,[18] α-ferrocenylalkylamines, and anomeric glycosylamines.[19] When the widely used non-racemic 2-phenylethylamine was employed

Table 1 Synthesized bacillamide derivatives

Amine	Yield	Amine	Yield
 **10a**	70%	 **10g**	90%
 **10b**	90%	 **10h**	98%
 **10c**	92%	 **10i**	98%
 **10d**	85%	 **10j**	80%
 **10e**	70%	 **10l**	56%[a]
 **10f**	98%		

[a] Total yield. First treated with an excess of diamine, separated after protection with Boc_2O *in situ*, and then deprotected under TFA.

as chiral auxiliary, complete racemization (chiral HPLC analysis) was observed after deprotection using TFA (see Supporting Information). Therefore we investigated readily available chiral 4-methoxyphenylethylamine **11** as auxiliary. Thus reaction of (*R*)-1-(4-methoxyphenyl)ethylamine yielded a 1 : 1 mixture of the two diastereomers (Scheme 4) in 65% yield which can be conveniently separated by silica gel chromatography. Additionally it is known that Lewis acids can be used to enhance diastereoselectivity in the Ugi reaction.[17] In order to optimize diastereoselectivity we planned to test several Lewis acids in this new thiazole Ugi variation. The first Lewis acid we tested $ZnCl_2$ afforded no formation of the product. Surprisingly also $BF_3{\cdot}OEt_2$ yielded little of the desired product contaminated with side products. Thus we decided to separate the 1 : 1 mixture of the MCR without any additive Lewis acid with the advantage of having access to both enantiomers of the natural product. Cleavage of the chiral auxiliary lead to the amide **(R)7** without any noticeable racemisation as shown by chiral HPLC (see Supporting Information). To the best of our knowledge



Scheme 4 Stereoselective 3-step synthesis of bacillamide C **1**.

Table 2 Screening of coupling conditions for the final step to **1**

Coupling method	Yield	ee[a]
EDCI/HOBt	20%	95%
CDI/HOBt	30%	94%
IsoBuCO_2Cl/NMM	85%	<5%
TBTU	40%	0
HOAt/HATU	20%	<5%

[a] Determined by chiral HPLC (column type: DAICEL CHIRALPAK IB).

this is the first example of the use of 4-methoxyphenylethylamine as chiral auxiliary in Ugi-type reactions.[20] We propose this to be a useful and alternative method especially when labile racemisation-prone substrates are employed, *e.g.* heteroaromatic amides. Subsequent amidation under the above described TBD catalysis conditions, however, resulted in extensive racemisation. Thus we first saponified **(R)7** with 70% yield and then coupled tryptamine using CDI, HOBt and DIPEA to yield (−)-**1** in 30% yield (retention time: 74 min in chiral HPLC see supporting information). The NMR spectra obtained from our total synthetic **1** compares well with the natural product. The final peptide coupling condition was extensively investigated because of the low yield of the peptide coupling method and the observed racemization problem (Table 2).

A similar coupling problem with low yields was also reported during a coupling step of the related bacillamide A.[21] In our study 15–20% yield was accomplished without racemization using EDCI/HOBt. Iso-BuCO_2Cl/NMM in ethyl acetate yielded >80% **1** but with accompanying racemization. Other conditions such as, HOAt/HATU, TBTU, were also tried but either with low yield or racemization results. The best result was 30% yield using CDI accompanied by HOBt with slight racemization as determined by HPLC.

In order to investigate the biological properties of this thiazole natural product we are interested in derivatives able to transverse the cell membrane and exert biological effects in the cell. Therefore we prepared the fluorescent derivative **10j** and measured the cell viability into the HCT116 cell line (Fig. 2). Also we measured the dynamic cellular uptake by performing time relapsed confocal fluorescence microscopy and found that after 100 min the compound entered the cell almost quantitatively (see Supporting Information Movie).

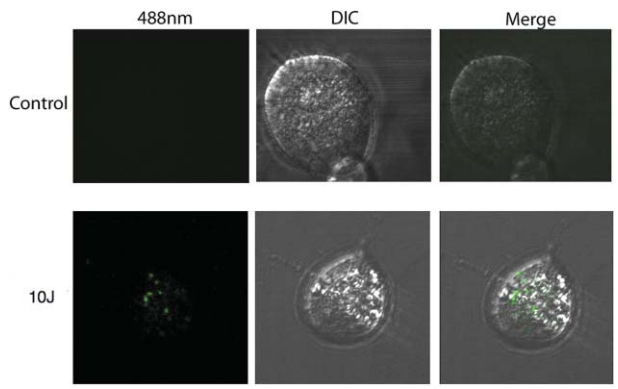


Fig. 2 Cellular uptake of **10j** in HCT116 cells observed by laser confocal microscopy. Left column: excitation with 488 nm laser; middle column: differential interface contrast (DIC); right: merge of the two images. Noteworthy, bacillamide derivative **10j** appears to localize in specific compartments of the cell.

In summary, we described the first total syntheses of the algicidal natural product bacillamide C **1**: an enantioselective and a 3-step synthesis leading to racemic **1** and derivatives. Additionally we accomplished the racemic synthesis of a fluoro derivative of the recently described neobacillamide A.[22] Our syntheses are convergent and very short and a key step is a modification of the Ugi reaction leading in one step to highly substituted thiazoles. We also describe a stereoselective synthesis of the target compound by introducing the mildly cleavable chiral 2-(4-methoxylphenyl)ethylamine for the first time as auxiliary in the thiazole-Ugi reaction. Finally we prepared several derivatives and showed that the fluorescence tagged **10j** can rapidly enter cells. The work is significant since bacillamides are potent algicidal agents and our approach can potentially access optimized derivatives by parallel chemistry.[23] Currently SAR studies are ongoing and will be reported in due course.

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