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Solid-Support Based Total Synthesis and Stereochemical Correction of Brunsvicamide A

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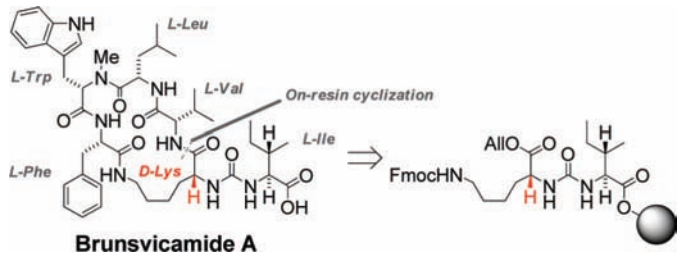
ABSTRACT



A total synthesis of the cyanobacterial metabolite brunsvicamide A and the correction of its originally assigned stereochemistry are reported. Key elements were the construction of a urea building block, peptide elongation on solid phase, and on-resin cyclization of the peptide chain, with good overall yield. Detailed structural investigations uncovered that brunsvicamide A features a previously undetected D-lysine residue in its backbone, setting the foundation for all further investigations in this compound class.

Bioactive natural products continue to be a source of inspiration for chemical biology and medicinal chemistry research.[1] The brunsvicamides (BVA) A−C are a group of cyclopeptides recently isolated from cyanobacteria (see Figure 5 for a correct structure) and were shown to inhibit the tyrosine phosphatase B of *Mycobacterium tuberculosis* (MptpB).[2] This enzyme is crucial for the host−pathogen interaction.[3] The brunsvicamides belong to a group of structurally closely related cyclic peptides, the anabaenopeptins, which have a urea moiety and N-methylation in common but differ in amino acid sequence and stereochemistry. Cyclopeptides are often characterized by increased metabolic stability, constrained conformation, and pronounced biological specificity.[4] The N-methylated amide bond is a feature which also increases conformational rigidity[5] and additionally may lead to improved pharmacological properties.[6] The urea moiety is known as a replacement for the amide bond in peptidomimetics.[7] Besides the brunsvicamides, other mem-

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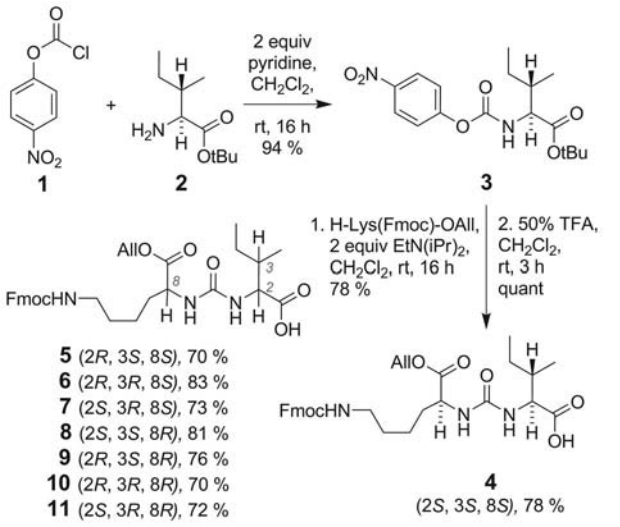
bers of this class have been reported. Many anabaenopeptins feature a D-lysine, an unusual homotyrosine, and were shown to inhibit the human carboxypeptidase A.[8] The amino acid sequence of monzamide A and B includes the very rare *allo*-isoleucine and D-valine or D-isoleucine.[9] Schizopeptin 791[10] was shown to inhibit trypsin, and the latest addition to the anabaenopeptin family was psymbamide.[11]

The sequence of brunsvicamide A features ^{1}Lys-^{2}Val-^{3}Leu-^{4}NMeTrp-^{5}Phe and forms a 19-membered cyclopeptide ring via the lysine side chain ε-NH_2. A urea function with an additional Ile residue is attached to the α-amino group of the N-terminal Lys. All amino acid residues of brunsvicamide A were initially reported to be L-configured. The inhibition of MptpB, which could be used to develop agents targeting tuberculosis, prompted us to develop a synthetic route for creating a library of BVA and related natural products for the purpose of structural confirmation and further biological evaluation.

Here, we report the first total synthesis of BVA A which led to the correction of the previously reported stereochemistry. We planned to create the urea moiety at an early synthetic stage, to elongate and then close the peptide ring on solid support. The synthesis of the urea began with the reaction of isoleucine *tert*-butylester (**2**) and 4-nitrophenyl chloroformate (**1**) to give 4-nitrophenyl carbamate **3** which can be purified by column chromatography and stored for months (Scheme 1).[12] Upon addition of Hünig's base to the

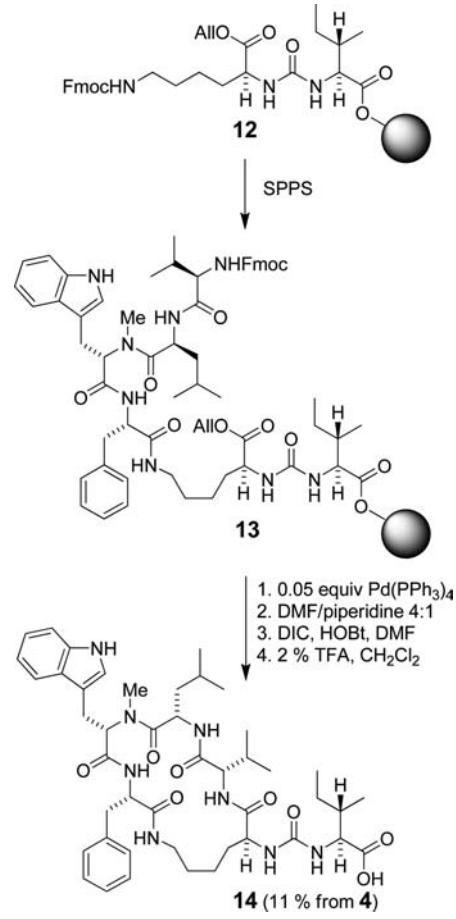
Scheme 1. Synthesis of Urea Building Blocks (**4**−**11**)



carbamate **3**, intense yellow coloration indicated the *in situ* formation of the isocyanate,[13] which was reacted with L-Lys(Fmoc)OAll to form the orthogonally protected[14] urea building block. Column chromatography removed residual 4-nitrophenol and was directly followed by *tert*-butyl ester cleavage to yield acid **4**.

Solid phase peptide synthesis (SPPS) was used to extend the peptide chain. Treatment of 2-chlorotrityl chloride resin with the purified urea building block **4**, followed by capping with methanol, gave access to the solid-support bound urea **12** (Scheme 2). To obtain sufficient loadings, it was necessary

Scheme 2. Synthesis of the Reported Brunsvicamide A Structure



to increase the reaction time to 16 h. This led to a loading of 0.6 mmol/g (UV-based Fmoc determination).[15] Alternative attempts of assembling the urea directly from solid-support attached amino acids[16] gave inferior results in our hands.

The Fmoc groups were removed with DMF/piperidine (4:1, v/v, 15 min). Peptide couplings were performed using

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either 3 equiv of the Fmoc-amino acid/DIC/HOBt (2 h, DMF, rt) or 3 equiv of Fmoc-Leu-OH/HATU/HOAt with 6 equiv of DIPEA (2 h, DMF, rt) for the coupling to the NMe-Trp. In each case, complete conversion was evident from negative Kaiser or chloranil tests.[17] Cleavage of the allylester **13** with $Pd(PPh_3)_4$ proceeded smoothly when phenylsulfinic acid sodium salt was used as a scavenger.[14,18] Deblocking of the N-terminal Fmoc group was followed by macrolactam formation on resin using DIC/HOBT in DMF. The crude product **14** was released with TFA and purified by preparative reversed phase HPLC, using MeOH/water (65:35) as eluent.

In this direct approach, **14** was obtained under pseudodilution conditions on solid support with an overall yield of 11% based on the loading of urea **12**. Using other reagent combinations for the macrocyclization (e.g., HATU/HOAt, PyBrop/DIPEA, DIC/C_6F_5OH) did not provide better results. Alternatively, the peptide precursor was released from the resin, and then macrocyclization was attempted in solution under pseudohigh dilution conditions (syringe pump). However, this strategy was more laborious and did not improve overall efficiency and was therefore abandoned.

Much to our surprise, the NMR spectra of the such obtained compound **14** were similar, but not identical, to the originally reported data for the natural product.[2] An HPLC coinjection of synthetic **14** and natural BVA revealed that they were not identical (Figure 1). However, the MS−MS fragmentation patterns were identical with the patterns of the natural product. This finding indicated that the structural differences should reside in the stereochemistry of the residues. We compared the recorded NMR spectra with the reported data and found major differences for the signals belonging to the L-lysine and L-isoleucine (Figure 2 and Supporting Information).

Since many of the anabaenopeptins contain a D-lysine, and the monzamides feature the very uncommon *allo*-isoleucine, we decided to synthesize a collection of all eight possible stereochemical combinations of lysine and isoleucine in brunsvicamide A (Figure 3). The required *tert*-butyl esters of D-*allo*-Ile-OH, D-Ile-OH, and L-*allo*-Ile-OH, respectively, were readily obtained with *tert*-butyl isourea.[19] The 4-nitrophenyl carbamates **3b**−**d** were prepared and each reacted with L- and D-Lys(Fmoc)OAll to give the ureas **5**−**11** (Scheme 1). Eight portions of 2-chlorotrityl resin were loaded with the individual stereoisomers. Peptide chain elongation, allyl ester cleavage, and ring closure were performed as before and simplified by the successful application of IRORI MacroKans and RFID technology.[20] The compounds **15**−**21** were obtained smoothly in good and reproducible overall yield (13−18%, Figure 3).

HPLC coinjection experiments narrowed the selection of possible candidates to compounds **18** and **21**. We then found

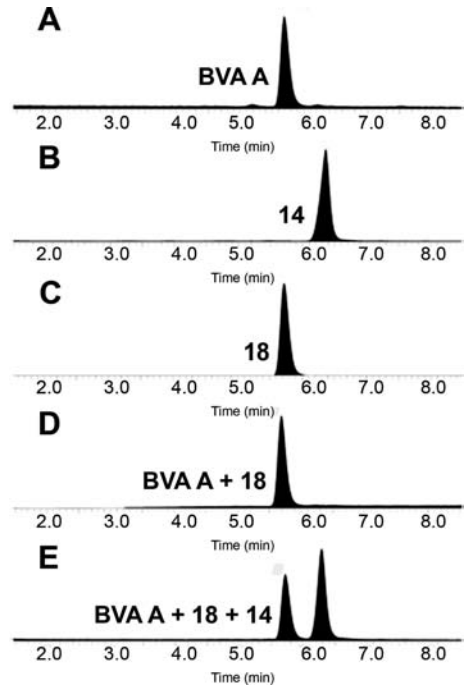

Figure 1. HPLC chromatograms showing coinjections of synthetic and natural brunsvicamides. (A) Authentic sample of BVA A. (B) **14** with L-Lys as initially reported. (C) **18** with D-Lys. (D) Coinjection of authentic sample and **18**. (E) Coinjection of authentic sample, **18**, and **14**.

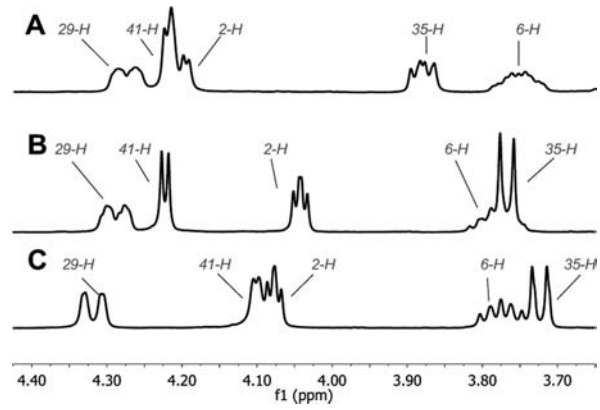

Figure 2. ^1H spectra showing characteristic signals of BVA (500 MHz, MeOH-d_4). (A) **14** with L-Lys as initially reported.[2] (B) **18** with D-Lys (pH = 4). (C) **18** (pH = 10).

that the compound **18** had NMR spectra with only minor differences from those reported earlier, whereas **21** showed major differences to the reported data. After consideration of the original data in conjunction with our synthetic efforts, we decided to remeasure the samples for compound **18** at different concentrations and pH values, both of which were not clear from the isolation procedure.

It was found that at pH = 10 all the signals now matched those initially observed for the isolated material (Figures 2 and 4). In the HPLC coinjection, compound **18** and the

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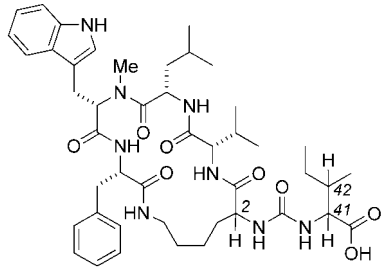


14 (41-*S*, 42-*S*, 2-*S*), 11 %; **18** (41-*R*, 42-*S*, 2-*R*), 16 %
15 (41-*R*, 42-*S*, 2-*S*), 14 %; **19** (41-*R*, 42-*S*, 2-*R*), 18 %
16 (41-*R*, 42-*R*, 2-*S*), 15 %; **20** (41-*R*, 42-*R*, 2-*R*), 13 %
17 (41-*S*, 42-*R*, 2-*S*), 16 %; **21** (41-*S*, 42-*R*, 2-*R*), 15 %

Figure 3. Structures of synthesized stereochemical variants of brunsvicamide A.

natural product were indistinguishable as well (Figure 1). This led us to the conclusion that the Lys residue in brunsvicamide A must be D-configured and by inference should also be assumed D for the other brunsvicamides. This finding is consistent with the occurrence of D-Lys in all other

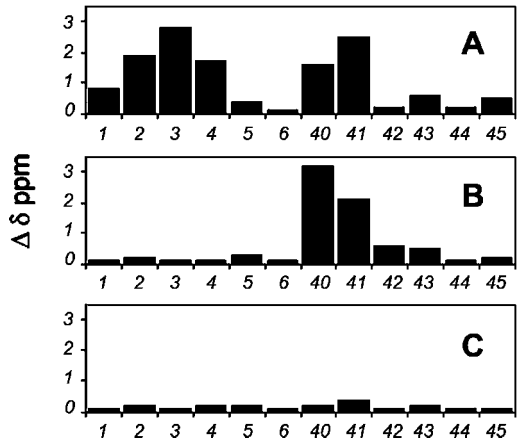
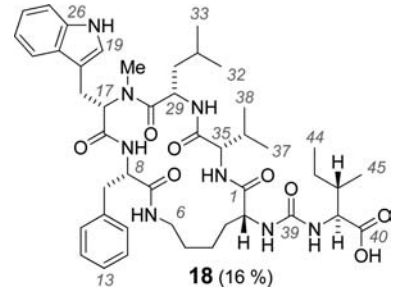


Figure 4. Chemical shift differences of ^{13}C signals reported for isolated BVA A and synthetic material (125 MHz, MeOH-d_4). (A) **14** with L-Lys as initially reported. (B) **18** with D-Lys (pH = 4). (C) **18** (pH = 10).

anabaenopeptins reported to date which are produced from cyanobacteria.[21]

In summary, a total synthesis of brunsvicamide A (**18**, Figure 5) has been accomplished on solid support from a urea building block which is easily varied. The solid phase synthesis entailed peptide chain elongation, allyl ester cleavage, and a practical on-resin cyclization.



18 (16 %)

Figure 5. Brunsvicamide A with its correct stereochemistry.

The very efficient 12-step sequence gave access to a collection of eight brunsvicamide A stereoisomers in 11−18% overall yield and allowed the identification of the correct stereochemistry of the brunsvicamides which feature a previously undetected D-lysine residue.

These studies highlight the vital role that chemical synthesis continues to play in confirming the structures of promising natural products of low abundance.[22,23] Efforts directed toward the synthesis of other members of the anabaenopeptin family of natural products and studies of their biological activity are underway.

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Note Added after ASAP Publication. This paper was published ASAP on July 1, 2008. A change was made to Scheme 1. The revised paper was reposted on July 3, 2008.

Supporting Information Available: Experimental details, characterization of the compounds discussed, detailed NMR characterization of compounds **14** and **18**, HPLC traces, and ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) A referee pointed out that amino acid stereochemistry determination by chiral GC (as reported in ref 2) is typically reliable. We agree, but note that depending on the amount and quality of the isolated materials misassignments can occur, especially with noncanonical linkages (e.g., urea) or less common stereoisomers (e.g., *allo*). For a related case see: (a) Zampella, A.; Randazzo, A.; Borbone, N.; Luciani, S.; Trevisis, L.; Debitus, C.; D'Auria, M. V. *Tetrahedron Lett.* **2002**, *43*, 6163–6166. For a general account on the technology, see: (b) Fountoulakis, M.; Lahm, H. W. *J. Chromatogr. A.* **1998**, *826*, 109–134.