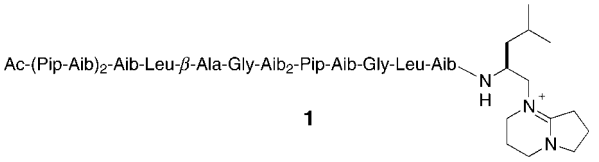
The First Total Synthesis of Efrapeptin C**

Micha Jost, Jörg-Christian Greie, Nina Stemmer, Sven David Wilking, Karlheinz Altendorf, and Norbert Sewald*

Dedicated to Professor Peter Welzel on the occasion of his 65th birthday

The efrapeptins are a class of peptide antibiotics produced by the fungus *Tolypocladium niveum* and other members of this species as a mixture of closely related sequence analogues (efrapeptins C–G).[1] They are inhibitors of F_1-ATPase and are active also against the malaria pathogen *Plasmodium falciparum*.[2] The efrapeptins are rich in α,α-dialkylated amino acids, and contain one β-alanine and several pipecolic acid residues. The C-terminus bears an unusual cationic head group derived from leucinol. Although the efrapeptins have been applied in numerous biological studies,[3] no chemical total synthesis has been reported so far.[4] This may be because the synthesis of peptides rich in α,α-dialkylated amino acids is hampered by incomplete coupling reactions caused by steric hindrance.[5] As we are interested in the structural and biological properties of efrapeptins we launched a project aimed towards the synthesis of efrapeptin C and analogues thereof.

We succeeded in assembling efrapeptin C (**1**) from three fragments: an N-terminal fragment (Pip 1–Gly 8), a central



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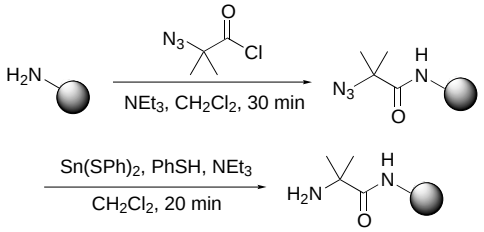
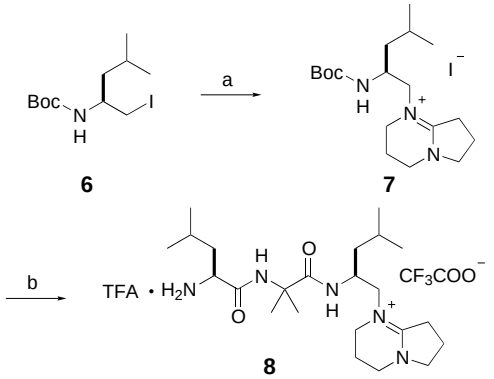
fragment (Aib 9–Gly 13), and a C-terminal fragment containing the residues Leu 14–Aib 15 as well as the head group. For the introduction of Aib residues in solid-phase syntheses we successfully modified a strategy first described by Meldal et al.,[6] in which the resin-bound amino component was acylated with the highly reactive α-azidoisobutyric acid chloride (Azib-Cl), and the azide was reduced to the primary amine. According to their original procedure reduction is achieved by treatment with 1,4-dithio-D,L-threitol (DTT) at elevated temperature and requires reaction times of up to 6 h. Use of Vilarrasa's reagent $[Et_3NH]^+[Sn(SPh)_3]^-$,[7] which is commonly applied to the reduction of alkyl and aryl azides, safeguarded the smooth conversion of the azide at room temperature within short reaction times (Scheme 1).[8] The reduction can easily be monitored by FT-IR spectroscopy and was found to be complete in all cases examined after only a few minutes.



Scheme 1. Introduction of Aib residues in solid-phase syntheses by using coupling of Azib-Cl and subsequent reduction. Aib = α-aminoisobutyric acid, Azib = α-azidoisobutyryl.

The C-terminal fragment of efrapeptin C (**1**) was synthesized starting from α-aminoisobutyric acid H-Aib-OH (**2**). Acid-catalyzed esterification of **2** with allyl alcohol to give H-Aib-OAl (**3**) and subsequent coupling of Boc-Leu-OH with **3** gave dipeptide Boc-Leu-Aib-OAl (**4**), which was deprotected at the C-terminus by allyl transfer to morpholine in the presence of $[Pd(PPh_3)_4]$ to yield Boc-Leu-Aib-OH (**5**).[9] Synthesis of the head group (Scheme 2) started from the known iodide **6**,[10] which was used to alkylate DBN in hot toluene[11] giving rise to the amidinium salt **7**. The Boc group in **7** was removed by treatment with TFA in dichloromethane and the resulting amine was subsequently coupled to **5** using N-HATU[12] as the coupling reagent. The C-terminal fragment **8** was isolated by preparative reverse-phase (RP) HPLC after cleavage of the Boc group by treatment with TFA. Compound **8** has already been described by Gupta et al.,[1] who obtained it by treatment of efrapeptin C (**1**) with HCl.

The central and N-terminal fragments of efrapeptin C (**1**) were synthesized by solid-phase methods using the highly acid-labile *o*-chlorotrityl resin.[13] Application of this type of



Scheme 2. a) DBN, toluene, reflux, 75%; b) 1. TFA/CH_2Cl_2; 2. **5** (1.2 equiv), N-HATU (1.2 equiv), DIPEA (2.2 equiv), DMF; 3. TFA/CH_2Cl_2, 27%. DBN = 1,4-diazabicyclo[4.3.0]non-5-ene, N-HATU = 1-[bis(dimethylamino)methyliumyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-3-oxide-hexafluorophosphate, DIPEA = diisopropylethylamine.

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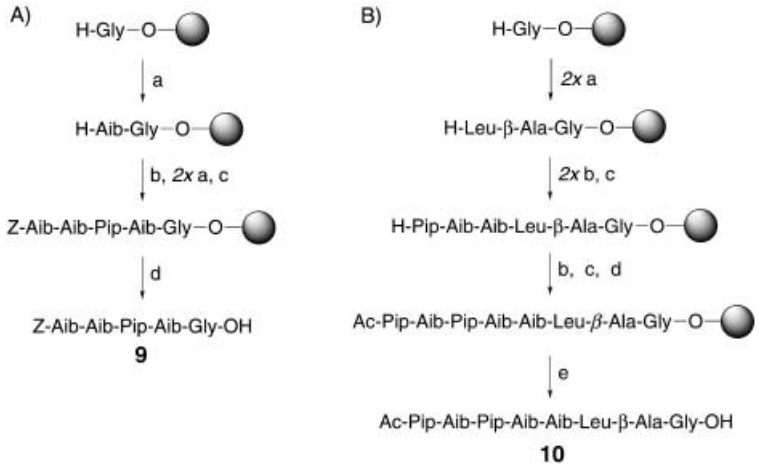
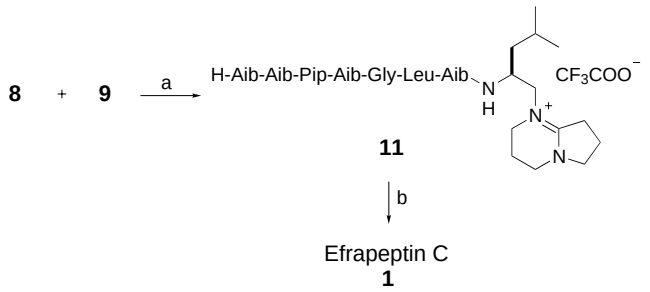
resin is crucial for the success of the synthesis because of the pronounced acid sensitivity of some -Aib-Xaa- peptide bonds.[14] Peptides **9** and **10** (Scheme 3 A and B) were synthesized according to Fmoc strategy with the exception of Aib residues, which were introduced by using Azib-Cl as described above. Proteinogenic amino acids were coupled with TBTU as the condensation reagent, and PyBOP was used for the introduction of L-pipecolic acid.



Scheme 3. A) a) 1. Azib-Cl (15 equiv), NEt_3 (22 equiv), CH_2Cl_2, 2. $Sn(SPh)_2$ (10 equiv), PhSH (30 equiv), NEt_3 (50 equiv), CH_2Cl_2; b) 1. Fmoc-Pip-OH (3 equiv), PyBOP (3 equiv), DIPEA (6 equiv), DMF; 2. DBU/piperidine, DMF; c) Z-OSu (10 equiv), NEt_3 (25 equiv), DMF/CH_2Cl_2; d) 1% TFA/CH_2Cl_2. B) a) 1. Fmoc-Xaa-OH (3 equiv), TBTU (3 equiv), DIPEA (6 equiv), DMF; 2. DBU/piperidine, DMF; b) 1. Azib-Cl (15 equiv), NEt_3 (22 equiv), CH_2Cl_2, 2. $Sn(SPh)_2$ (10 equiv), PhSH (30 equiv), NEt_3 (50 equiv), CH_2Cl_2; c) 1. Fmoc-Pip-OH (3 equiv), PyBOP (3 equiv), DIPEA (6 equiv), DMF; 2. DBU/piperidine, DMF; d) Ac_2O (30 equiv), DMAP (20 equiv), CH_2Cl_2; e) 1% TFA/CH_2Cl_2. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, Pip = L-pipecolic acid, Z-OSu = *N*-(benzyloxycarbonyloxy)succinimide, PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate, DMAP = 4-dimethylaminopyridine, TBTU = 1-[bis(dimethylamino)methyliumyl]-1*H*-benzotriazole-3-oxidetetrafluoroborate.

N-HATU was used as the coupling reagent for the final assembly of **1** from the three fragments (Scheme 4). Compound **11** was obtained upon reaction of **9** with **8** and removal of the Z group. Finally condensation of **10** with **11** gave efrapeptin C (**1**) as the trifluoroacetate.

The product was characterized by ^{1}H NMR spectroscopy and ESI-FT-ICR-MS.[15] Its homogeneity was proven by RP-HPLC. To demonstrate its bioactivity, we studied the inhibition of *E. coli* F_1-ATPase by synthetic **1** and found the K_i value to be in the range of 10 μM. This is in good agreement with a value of 21.5 μM reported by Wise et al.[16] for the mixture of efrapeptins as isolated from fermentation broths.



Scheme 4. a) 1. N-HATU, DIPEA, CH_2Cl_2/DMF, 2. H_2, Pd/C, MeOH/AcOH, 44%; b) **10** (1.3 equiv), N-HATU (1.3 equiv), DIPEA (2.3 equiv), CH_2Cl_2/DMF, 67%.

In summary we have synthesized for the first time a member of the efrapeptin class of peptide antibiotics by applying a convenient method for the introduction of Aib residues. The full sequence was assembled by a combination of solution-phase and solid-phase peptide synthesis steps with segment condensations. We are currently using this synthetic route for the synthesis of a number of efrapeptin analogues, which will be examined for bioactivity and conformational preferences to derive structure–activity relationships.

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[8] Procedure for the introduction of Aib residues in solid-phase synthesis**:** The peptidyl resin (1 equiv) was swollen in a solution of NEt_3 (22 equiv, ca. 0.7 M) in CH_2Cl_2, and Azib-Cl (15 equiv) was added. The mixture was left for 30 min and agitated occasionally. The resin was washed with CH_2Cl_2 (4×), DMF (4×), and again with CH_2Cl_2 (4×). Thiophenol (30 equiv) and NEt_3 (50 equiv) were added to a suspension of $Sn(SPh)_2$ (10 equiv; ca. 0.3 M) in CH_2Cl_2. After 5 min the resulting mixture was filtered and the yellow filtrate was added to the resin-bound azide. The mixture was left for 20 min and agitated occasionally. The resin was washed with CH_2Cl_2 (4×), DMF (4×), and again with CH_2Cl_2 (4×).

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Reactivity of Chalcogenostannate Salts: Unusual Synthesis and Structure of a Compound Containing Ternary Cluster Anions $[Co_4(\mu_4\text{-}Se)(SnSe_4)_4]^{10-}$**

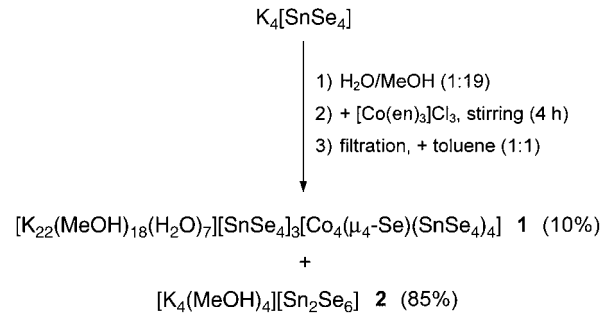
Christian Zimmermann, Maike Melullis, and Stefanie Dehnen*

Dedicated to Professor Dieter Fenske on the occasion of his 60th birthday

The stabilization of binary aggregates of main group elements $[E'_xE_y]^{q-}$ in the coordination sphere of transition-metal ions M^{n+} (E′ or E = Group 13–15 or 16 element), and thus the formation of structures that contain ternary heavy atom frameworks, is an area of increasing research activity.[1–16] Besides diverse range of molecular and crystal structures, the synthesis of some ternary M/E′/E systems that show polymeric anion substructures representing "open solid-state structures", as found in $Rb_3[AgGe_4Se_{10}]\cdot 2\,H_2O$[10] or $K_2[MnSnS_4]$,[11] have recently attracted attention. Such compounds combine both zeolite-type and semiconducting properties. If binary alloys or salts of binary anions are used for the construction of the E′/E aggregates instead of separate components, the investigations additionally serve to study the reaction behavior and stability of these systems in the presence of transition-metal compounds. However, most reports on the latter deal with binary reactants of Groups 15/16,[1,13–16] for example the synthesis of $[PPh_4]_2[Mn(CO)_3(As_3Se_3)_5]$ by using $[As_4Se_4]$.[13b] Only most recently, were some results published that considered the surfactant-templated solvothermal synthesis of mesoporous solids like $(C_{16}H_{33}NC_5H_5)_x[Pt_ySn'_4Se_{10}]$ ($x = 1.9$–2.8; $y = 0.9$–1.6).[12]

One of our current aims is to generate coordination compounds by reacting binary anions of Groups 14 and 16. However, reactions under conditions similar to those for the binary reactants from Groups 15 and 16—that is reactions with organometallic complexes in aprotic solvents—led to the reductive decomposition of the Sn–E framework under E^{2-} donation to the transition-metal ion.[17,18]

By employing protic solvents and another type of transition-metal complex, it was possible to synthesize a compound by completely transferring the binary anionic structures into the coordination sphere of the transition-metal ions. Scheme 1 depicts the reaction of $K_4[SnSe_4]$[19] and



Scheme 1. Synthesis of the compounds **1** and **2**.

$[Co(en)_3]Cl_3$ (en = 1,2-diaminoethane) in a water/methanol mixture that yielded compounds **1** and **2**.[20,21] Compounds **1** and **2** were structurally characterized by single-crystal X-ray diffraction,[22] and the formal oxidation state of the cobalt centers in **1** was additionally checked by quantum-chemical investigations. Compound **1** crystallizes as black cubes in the cubic space group $Ia\bar{3}$. It is an ionic compound that features complex, ternary anions $[Co_4(\mu_4\text{-}Se)(SnSe_4)_4]^{10-}$ that are embedded in the crystal lattice by K···Se interactions to MeOH- or H_2O-coordinated potassium cations.The composition of the C_3 symmetric anion suggests that the $[SnSe_4]^{4-}$ ions of the starting material have acted as both a ligand and—in a well-known manner—as an Se^{2-} donor. This is additionally confirmed by the formation of compound **2** which can be viewed as a dimer of $[SnSe_3]^{2-}$ ions generated by removal of Se^{2-} ions from the tetraselenostannate anions $[SnSe_4]^{4-}$. Under the assumption that the formal oxidation state of the tin atoms in **1** remains +4, the cluster anion emerges as a Co^{II} compound. Thus, in contrast to the reactions in aprotic solvents, one observed a reduction of the transition metal ions by partially released Se^{2-} ions.[23] Figure 1 gives the molecular structure of the ternary anion.

In the cluster anion, four barrelane-type $[SnCo_3Se_4]$ cages are linked by a μ_4-bridging selenium atom that centers an inner $[SeCo_4]$ fragment (barrelane = bicyclo[2,2,2]octane). In addition, the anion contains four terminal selenido ligands, which form the corners of a large tetrahedron with Se···Se edges of 1057.7(4)–1071.3(3) pm. Each of the metal atoms is almost tetrahedrally surrounded by selenido ligands. The narrow range of the observed bond lengths allows a deviation from ideal T_d symmetry by at most 1.1 pm.

Some cluster complexes are known that are topologically identical to **1**. These are chalcogenido- or pnictido-bridged clusters of the d^{10} elements, for example $[M^{II}_8(\mu_4\text{-}E)$-

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