Depsipeptides

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Siamese Depsipeptides: Constrained Bicyclic Architectures**

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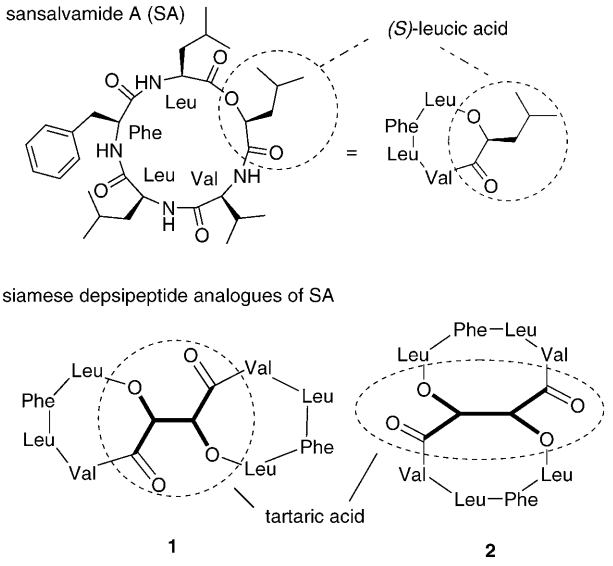
Dedicated to Professor Klaus Burger

Bioactive cyclic peptides and depsipeptides which are isolated from natural sources provide a range of key lead structures for the design of new drugs, as reflected by the notable increase in peptide- and depsipeptide-based drugs during recent years.[1] However, peptides as drugs show some drawbacks that are inherently associated with their structure. Thus, linear peptides can be degraded rapidly by proteases, and receptor affinity is low owing to the large number of conformations which they can adopt. Cyclization can overcome these problems to provide both conformational restriction and enhanced proteolytic stability.[2] Natural cyclic peptides adopt mainly a "head to tail" or "head to side chain" architecture. Bicyclic peptides are mostly formed by disulfide bridges, such in the conotoxins, or by a combination of a "head to side chain" and disulfide bridges, such in the case of thiocoralines and related natural compounds, which show a C_2 symmetry.[3] The creation of new cyclic peptide architectures is of special interest with respect to possible improvement of pharmacological properties. Consequently, several other types of cyclic peptides, such as "homodetic side chain to side chain", "backbone to backbone", as well as a combinations of these have been described.[4]

As part of one of our ongoing research programs devoted to the discovery of active depsipeptides—modeled on natural depsipeptides—a new concept of depsipeptide architecture has been designed. Cyclic depsipeptide dimers connected by a CC bond were constructed. Two kind of possible connections can be envisaged, those that share a side chain bond or those that share a backbone bond. Owing to the resulting proximity of both structurally identical cycles, they can be called Siamese depsipeptides. For Siamese depsipeptides, the hydroxy acid of the natural compound was replaced with tartaric acid and the second cycle was constructed on its additional OH and CO_2H function. Both modes of connection prevent steric interaction between the two cycles, and H-bond interactions are expected to be minimal because ester bonds are not H-bond donors and are weak H-bond acceptors.[5] The two Siamese depsipeptides proposed are shown in Scheme 1.

The cyclic depsipeptide sansalvamide A (SA), which is produced by a marine fungus and shows cancer cell cytotoxicity, was selected as a model. SA is composed of four amino acids (2 × Leu, Phe, Val) and one hydroxy acid (leucic acid).[6] More than 80 peptidic analogues, wherein the hydroxy acid and the amino acid constituents were substituted for D-amino acids and *N*-methyl amino acids with preserved or altered side chains, are already reported. Some of them exhibit greater activity and better selectivity than the natural SA.[7] However, although SA is a relatively small and structurally simple depsipeptide, an active sequence has not been disclosed to date.

The synthesis of Siamese depsipeptides is challenging because two cyclization steps are required and peptide cyclizations are often low-yielding steps. Five-membered all-



Scheme 1. Replacement of the leucic acid in sansalvamide A (SA) with tartaric acid provides Siamese depsipeptides **1** and **2**.

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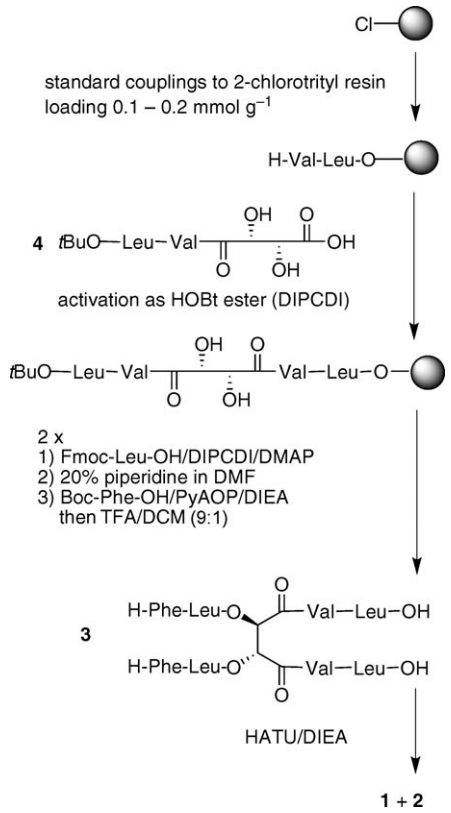
Supporting information (full experimental details, together with copies of ^{1}H NMR spectra of the depsipeptides **1**, **2**, **5**, **6**, and **7**) for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200904135.

(*S*)-peptides (as is the case for SA, where all five residues have *S* configuration) are more difficult to obtain than those bearing at least one residue with *R* configuration.[8] Therefore, we tested several cyclization strategies with D-tartaric acid (*S*,*S* configuration) as well as with L-tartaric acid (*R*,*R* configuration). Stepwise cyclizations resulted in low yields of monocycles and were therefore discarded. Finally, the symmetrically, doubly branched acyclic depsipeptide **3**, with L-tartaric acid as core unit, was found to be a key for the preparation of the Siamese depsipeptides in just one cyclization step. However, the reaction produced insufficient product when D-tartaric acid was used as the core (Scheme 2). The precursor **3** was prepared on 2-chlorotrityl resin.[9] The amino acids Val and Leu were coupled on the resin using standard protocols for Fmoc solid-phase peptide synthesis. The tartaric acid was introduced as monoamide **4** by activation as the HOBt ester. These reaction conditions tolerate unprotected secondary hydroxy groups.[10] The monoesterification of the hydroxy groups of tartaric acid in the solid phase was performed by coupling a 11-fold excess of Fmoc-Leu-OH for six hours under the conditions developed by Neises and Steglich.[11] Probably because of the steric hindrance of the Fmoc group, the bisesterification proceeded very slowly at this stage: extensive repetitions of esterification (two times more for 15 h) only resulted in moderate diester formation (50–60%). However, when the Fmoc group of the Leu ester was removed and Boc-Phe-OH was coupled, the bisesterification step (16 h) proceeded with a higher yield (80%). After coupling the second Boc-Phe-OH unit (activation with PyAOP/DIEA, 30 min), precursor **3** was cleaved from the resin and the protecting groups were completely removed (TFA/CH_2Cl_2 (9:1), 1 h). The resulting product was subjected to the double macrocyclization with HATU/DIEA in solution (2 h) without prior purification. Two products, with the expected mass for the Siamese depsipeptides, were isolated separately by semipreparative HPLC methods in 3.4% (**1**) and 5.1% (**2**) overall yield and in 96% and 91% purity by HPLC analysis, respectively.[12]



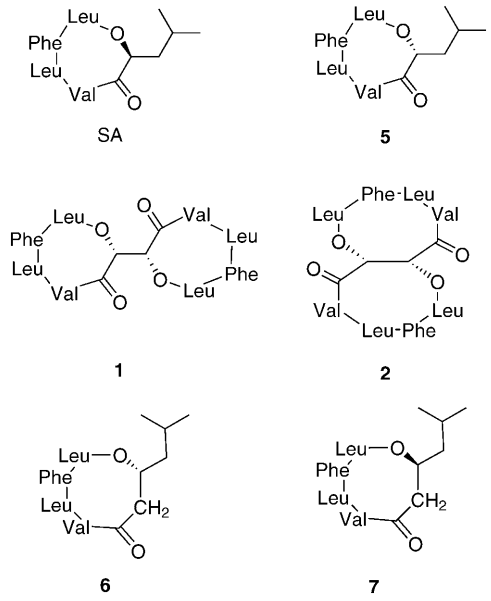
Scheme 2. Synthesis of the Siamese depsipeptides **1** and **2** through the symmetrically branched precursor **3** as the key intermediate. Boc = *tert*-butoxycarbonyl, DIEA = *N*,*N*-diisopropylethylamine, DIPCDI = diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine, DMF = *N*,*N*-dimethylformamide, Fmoc = 9-fluorenylmethyloxycarbonyl, HATU = O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole, PyAOP = (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, TFA = trifluoroacetic acid.

The proton signals of the tartaric acid residue and of the amide group in the ^{1}H NMR spectra of the two cyclization products allowed assignation of the structures. One compound showed two proton signals with the same integration and coupling constant for tartaric acid ($\delta = 5.66$ ppm, $J = 1.3$ Hz, 1H; 5.70 ppm, $J = 1.3$ Hz, 1H), and at least seven amide proton signals. We assigned this signal pattern to **1** because a rotation of the two cycles along the C–C bond was possible. The other compound showed a single proton signal for tartaric acid ($\delta = 5.82$ ppm, 2H) and four amide protons. We assigned this signal pattern to the compound with structure **2** because of its C_2-symmetry (see the NMR spectra in the Supporting Information).

With respect to SA, the configuration of the hydroxy acid residue in the Siamese depsipeptides was changed from *S* to *R*. Therefore, the SA analogue **5** with (*R*)-leucic acid was synthesized by an optimized procedure.[7a] The cycles within the Siamese depsipeptide **2** contained an additional carbon atom. The analogue **6** bearing a CH_2-homologated (*R*)-leucic acid, and **7**, bearing a CH_2-homologated (*S*)-leucic acid, were prepared to evaluate the influence of ring size on activity. The required β-hydroxy acids were introduced in the peptide chain as dipeptide building blocks prepared from the corresponding leucic acid by an Arndt–Eistert protocol developed in our laboratory (Scheme 3).[13]

The inhibitory activity of the full set of depsipeptides SA, **1**, **2**, and **5**–**7** against three human cancer cell lines (MDA-MB-231, A-549, HT-29) was tested by routine screening. Activity was observed for **1, 2,** and SA in low micromolar range and was similar for all three cell lines, however, the monocyclic analogues **5**–**7** were found to be inactive. Remarkably, the Siamese depsipeptides showed significantly higher activity than SA. In addition, we performed in-house cell-growth inhibition assays with A-549 cells, and the trend of activities was reproduced. The curves shown in Figure 1 are averaged from three independent assays and correspond to **1** as the compound with greatest activity ($IC_{50} = (1.1 \pm 0.8)$ μM), followed by **2** ($IC_{50} = (4.0 \pm 2.3)$ μM), and SA ($IC_{50} = (8.3 \pm 1.5)$ μM). No significant activity at a concentration of 100 μM was observed for the other monocycles **5**–**7**.

These results show that neither the ring expansion (an additional CH_2 group in the ring at the position of leucic acid) nor the inversion of the configuration at the chiral center of



Scheme 3. Analogues of depsipeptide SA prepared for comparison of biological activities.

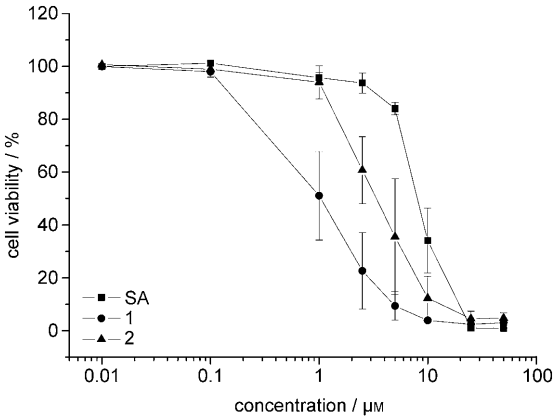


Figure 1. Effect of Siamese depsipeptides **1** (●), **2** (▲), and SA (■) on the viability of A-549 cells at 50, 25, 10, 5, 2.5, 1, 0.1, and 0.01 μM concentration.

leucic acid are favorable manipulations with respect to biological activity. Importantly, on the basis of the data of the monocyclic compounds alone, we cannot draw a conclusion as to whether the position of leucic acid or the preserved sequence is involved in receptor interaction because the loss of activity can be caused by an unfavorable shift in the conformational equilibria. However, the Siamese depsipeptides were significantly more active than the natural SA. These results strongly support the notion that our structural manipulation does not affected the "active region", but instead effects the conformational constraining part, which forces the "active region" to take the "right" conformation.[14] Furthermore, the involvement of the position of the leucic acid residue in receptor interaction can be ruled out because it is blocked by the second cycle in the Siamese depsipeptides. However, at this stage, we are unable to deduce whether the increase in activity results from a conformational constriction caused by the tartaric acid core, or whether phenomena like statistical effects (the active part is presented twice in one molecule) or multivalent binding are involved.[15]

In summary, we have described the preparation of Siamese depsipeptides, which are the first members of a new class of depsipeptide architecture with tartaric acid as the core unit. Remarkably, these compounds were synthesized in reasonable yields from a branched precursor in just one cyclization process. We have shown that the structural manipulation of the natural bioactive depsipeptide SA gives rise to analogues with greater activity, thereby providing additional information on structure–activity relationships with a small sets of compounds. This concept is currently being applied to other bioactive peptides in our laboratory and we are testing the effects of other core units.

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