Diastereoselective synthesis of the 5-hydroxy-pyrrolidinone amino acid of the microsclerodermins and model studies for an end-game strategy for microsclerodermin B

Christian Winter, Robert D.C. Pullin, Timothy J. Donohoe*
Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford OX1 3TA, UK

A R T I C L E   I N F O
Article history:
Received 6 December 2016
Accepted 19 December 2016
Available online 21 December 2016

Keywords:
Amino acids
Cyclic peptides
Stereoselective synthesis
Peptide coupling
Metathesis

A B S T R A C T
The first diastereoselective synthesis of the 5-hydroxy-pyrrolidinone amino acid common to eight members of the microsclerodermin family is presented. Our strategy involves formal hydration of an unsaturated precursor via the use of a two-step hydroxybromination-debromination protocol; this procedure provides exclusively the requisite 4,5-cis-pyrrolidinone. Furthermore model studies are presented that indicated the potential viability of this hydration strategy in the context of a synthesis of microsclerodermin B.

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Introduction
The microsclerodermins (assigned as variants A-M) represent an intriguing class of cyclic peptide natural product. First reported in 1994, microsclerodermins A-I were isolated by Faulkner from the marine sponges Microsclerodema sp. and Theonella sp.,1 with Li later isolating J and K from the sponge Microsclerodema herdmani.2 Notably Müller recently reported the isolation of microsclerodermins L and M from terrestrial myxobacteria Chondromyces, Jahnella and Sorangium3; furthermore with a proposal of their biosynthetic origin which showed that the related natural products peedin A and B also derive from the same pathway.4 The microsclerodermin family displays both antifungal activity and cytotoxicity against various cell lines.1–5 Structurally, the microsclerodermins share a common 23-membered cyclic hexapeptide motif. Of the six aminoacid residues, three are conserved; glycine (Gly), sarcosine (Sar) and γ-amino-β-hydroxybutanoic acid (GABOB), with differences arising in the nature of a substituted tryptophan (Trp), a complex polyhydroxylated β-amino acid and a β-amino-γ-lactam (pyrrolidinone, Pyrr) residue, which is found either in an unsaturated ‘dehydrated’ form (E, J and K) or a ‘hydrated’ 5-hydroxy form (ten remaining members) with a 4,5-cis relative configuration in all cases (Fig. 1).

The microsclerodermins have been the focus of continued synthetic work since their first isolation. Most reports have focused on the preparation of the polyhydroxylated β-amino acid residues6; indeed we have reported the synthesis of the stereopentad β-amino acid AMMTD (present in A, B, J and K).7 However, fewer studies have focused around the pyrrolidinone amino acid. In 2003 Ma reported the total synthesis of microsclerodermin E,8 which possesses an unsaturated pyrrolidinone residue and whilst early studies by Shioiri and Hamada demonstrated the synthesis of an isolated 5-hydroxypyrrolidinone amino acid,9 they found that it easily dehydrated, in line with previous observations made by Faulkner.1 As part of our efforts we recently disclosed the total synthesis of microsclerodermin J and dehydromicrosclerodermin B, along with the structural reassignment of the C4 pyrrolidinone stereocentre in both natural products and concurrently the adjacent C5 stereocentre in microsclerodermin B.10 Whilst we have so far been unsuccessful in our attempts to prepare microsclerodermin B (1) itself, our synthetic strategy was designed to incorporate a late-stage hydration of the unsaturated pyrrolidinone amino acid II of dehydromicrosclerodermin B in order to reveal the sensitive γ-hydroxypyrrolidinone I (Scheme 1) at a late stage. Details of the synthesis of the 5-hydroxypyrrolidinone amino acid and supporting model studies for a synthesis of microsclerodermin B are now reported.

* Corresponding author.
E-mail address: timothy.donohoe@chem.ox.ac.uk (T.J. Donohoe).

http://dx.doi.org/10.1016/j.tetlet.2016.12.045
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Results and discussion

Because of the reported ease with which the 5-hydroxypyrrolidinone dehydrated we reasoned that in order to incorporate this residue into a viable total synthesis, hydration of the unsaturated pyrrolidinone should be conducted as late as possible in any synthetic sequence. As a result we felt it prudent to first prepare a suitable model system in which to test the validity of this strategy. We selected the unsaturated sarcosine-pyrrolidinone dipeptide 5, where the N-acetamide would provide a simple model of the amino acid present in the microsclerodermins. Preparation of the unsaturated pyrrolidinone amino acid 3 commenced from aspartic acid derivative 2, originally accomplished with a modification of chemistry reported by Zhu and Ma8 and Shioiri (route 1)9 but later via a new route involving a Blaise reaction (route 2),11 which we then utilized in the synthesis of microsclerodermín J and dehydromicrosclerodermín B.10 While the second route had the advantage of allowing the preparation of acid 3 in enantiopure form, the first route (giving racemic material in our hands) was used in this model study. Protecting group manipulation then allowed preparation of the sarcosine-pyrrolidinone dipeptide 5 through coupling of acid 3 with sarcosine (trimethylsilyl)ethyl ester (4) using HBTU. Removal of the Boc group and acylation with acetic anhydride delivered dipeptide 5 in high overall yield (Scheme 2).

All our attempts to hydrate the dipeptide 5 with either Brønsted or Lewis acids failed. Therefore, we sought to react the constituent enamide with a separate electrophile, which could later be removed, in aqueous solution to effect a formal hydration. Halogenation, in particular bromination, was an attractive option. We were delighted to find that direct treatment of 5 with 2.5 equivalents of either NBS (Table 1, entry 1) or TBCD (entry 2) in THF/H2O cleanly gave a mono-brominated bromohydrin, 6 in good yield (57% and 81% respectively) and in short reaction times (<1 h). Further experimentation revealed that bromine in CH2Cl2/H2O could also be employed to deliver the bromohydrin in similar yield (76%) (entry 3). Interestingly only the mono-brominated hydroxy-pyrrolidinone was observed in these reactions, despite the fact that 2.5 equivalents of brominating agent was used. In all cases a single diastereoisomer (unassigned at this stage) was observed. With bromohydrin 6 in hand our attention then turned to debromination and subsequent establishment of the stereocchemistry of the pyrrolidinone product (Scheme 3). After some optimisation we found that treatment of the bromohydrin 6 with

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**Table 1**

| Entry | Bromine source | Solvent | Yield of 6[^a] |
|-------|----------------|---------|--------------|
| 1     | NBS           | THF/H2O | 57           |
| 2     | TBCD          | THF/H2O | 81           |
| 3     | Br2           | CH2Cl2/H2O | 76          |

[^a]: 2.5 equivalents used.

[^b]: Isolated yields after purification by flash column chromatography; NBS = N-bromosuccinimide, TBCD = 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one.
tributyltin hydride and catalytic amounts of triethylborane as an initiator, could cleanly abstract the bromine atom at low temperature to furnish the desired γ-hydroxypropylidinone 7 in excellent yield (90%). Elevated temperature 1H NMR studies revealed the hydroxypropylidinone 7 to be a single diastereoisomer. Furthermore, NOE studies then revealed the desired 4,5-cis configuration of the substituents on the pyrrolidinone ring. The removal of the bromide constitutes the first diastereoselective synthesis of this γ-hydroxypropylidinone amino acid of the microsclerodermins, with dipeptide 7 being represented in eight family members.

The 4,5-cis-diastereoselectivity that was observed in the two-step hydration could be rationalised by two different pathways. Firstly, thermodynamic control could be operative, forming the more stable (OH-NH cis) diastereoisomer by reversible opening of the semi-aminal ring. As evidence against this route, both Shiori and ourselves have observed diastereoisomeric hemi-aminals from simpler model systems; these clearly had not equilibrated to the cis diastereoisomer. Secondly, the cis configuration could be reached by considering the role of the 4-NHac functional group (Scheme 4). Treatment of the unsaturated pyrrolidinone with TBCD or NBS could form an N-acyliminium, III. This can subsequently be trapped intramolecularly by the adjacent N-Hac group to afford a dihydrooxazolium ion IV that may be subsequently hydrolysed by attack at the acyl carbonyl to yield the 4,5-cis-pyrrolidinone. Clearly, more experimental data is required to distinguish between the two pathways, and it would then be interesting to consider the consequences of such reactivity on the biosynthesis of this amino acid.

Since the β-amino acid of microsclerodermins A and B, AMMTD, contains an electron rich β-methoxy styrene moiety we were concerned that this moiety would not be compatible with our two-step hydroxybromination-debromination strategy. If the hydration proved incompatible, we required a functional group in a late-stage intermediate that could subsequently be manipulated to the styrene as a final step in any total synthesis. We felt that either an olefin or an alkylene would be a more suitable way to achieve this goal when this methodology was applied to a late stage intermediate on route to dehydromicrosclerodermin B; further experimentation will be required to achieve this aim.

Comparison, unsubstituted derivative 8d gave a comparable yield (61%) (entry 4). We were delighted to find that all three compounds then underwent efficient (~90%) radical-mediated debromination under our optimised conditions (as long as the amount of tributyltin hydride was kept at ≤1.1 equivalents in the case of the olefin) to reveal the desired functionalised γ-hydroxypropylidinones 9b-d.

Our final task as part of our model studies was to derivatise either alkylene 9b or olefin 9c to the corresponding p-methoxystyrene, as present in AMMTD. We believed that terminal olefin 9c appeared the best candidate, through the use of a cross metathesis (CM) reaction. After some experimentation we found that olefin 9c underwent efficient CM with 4-methoxystyrene at room temperature via treatment with five portions of Grubbs I catalyst and an excess of the styrene. Pleasingly these conditions provided a high yield of the desired coupled styrene 10 (80%), exclusively as the (E)-isomer (J = 15.6 Hz) with no signs of any dehydration of the sensitive γ-hydroxypropylidinone functionality (Scheme 5). For completeness an elevated temperature ROESY experiment was conducted which revealed 10 to be a single 4,5-cis-diastereoisomer (as expected, in line with previous observations, see ESI for details).

At this stage we believed this end-game model could pave a way, not only for the first total synthesis of microsclerodermin B, but the first synthesis of any microsclerodermin that possesses the 5-hydroxypropylidinone residue. At this stage, however, we have been unable to accomplish this goal when this methodology was applied to a late stage intermediate on route to dehydromicrosclerodermin B; further experimentation will be required to achieve this aim.

**Scheme 4.** Proposed mechanism to account for the observed 4,5-cis-diastereoselectivity of the two-step formal hydration.

**Scheme 5.** (E)-Selective cross metathesis of olefin 9c with 4-methoxystyrene in the presence of the 5-hydroxypropylidinone amino acid.

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**Table 2.** Hydroxybromination and debromination of microsclerodermin dipeptide mimics 8a-d.

| Entry | R          | Step I/%a | Step ii/%a |
|-------|------------|-----------|------------|
| 1     | a (E)-CH=CH(4-MeO-C6H4) |           | NA         |
| 2     | b CH=CH   | 65        | 90         |
| 3     | c CH=CH2  | 73        | 90         |
| 4     | d H       | 61        | 91         |

a Isolated yields after purification by flash column chromatography.
b Undesired bromination of the aromatic ring was observed.
Conclusion

In summary, we have reported the first diastereoselective synthesis of the sensitive 5-hydroxypyrrolidinone amino acid common to eight microsclerodermin family members. Through a two-step hydroxybromination-debromination procedure we were able to prepare the amino acid as part of a dipeptide and as the requisite 4,5-cis-disastereoisomer. Further experimentation revealed this pyrrolidinone to be stable to cross metathesis conditions and this factor allowed the introduction of a 4-methoxystyrene unit as found in the β-amino acid of microsclerodermin B.

Acknowledgments

We thank the German Academic Exchange Service (DAAD) (CW) and the EPSRC, EP/I010343/1 (RDCP) for funding. Choon Boon Cheong is thanked for assistance with NMR spectroscopy.

A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.12.045.

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18. See Ref. 10 for details; the unsaturated pyrrolidinone starting material was consumed but only undefined and inseparable side products were obtained.