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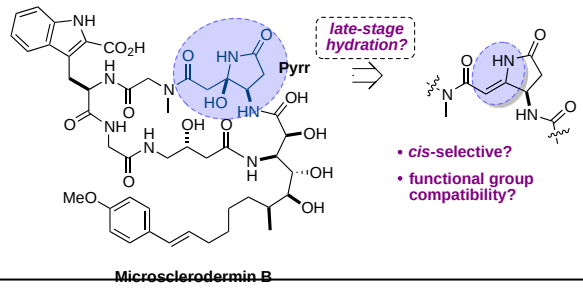

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Diastereoselective synthesis of the 5-hydroxy-pyrrolidinone amino acid of the microsclerodermins and model studies for an end-game strategy for microsclerodermin B

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ABSTRACT

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.

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 *Microscleroderma herdmani*.[2] Notably Müller recently reported the isolation of microsclerodermins L and M from terrestrial myxobacteria *Chondromyces*, *Jahnella* and *Sorangium*;[3] furthermore with a proposal of their biosynthetic origin which showed that the related natural products pedein A and B also derive from the same pathway.[4] The microsclereodermin 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 (**Figure 1**).

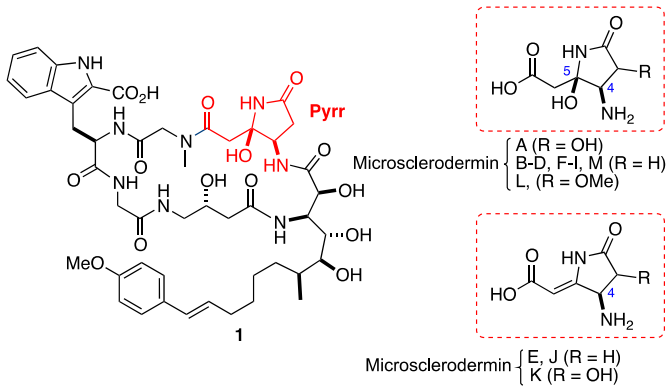

Figure 1. Microsclerodermin B (**1**) and the constituent pyrrolidinone amino acids of the family. The relative stereochemistry of the *cis*-pyrrolidinone amino acid in microsclerodermin A has not been determined.

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 residues;[6] 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-

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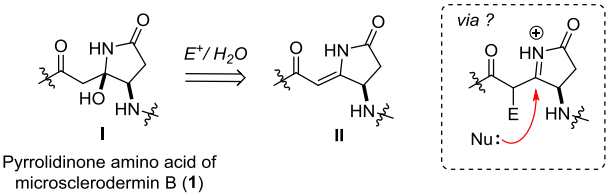
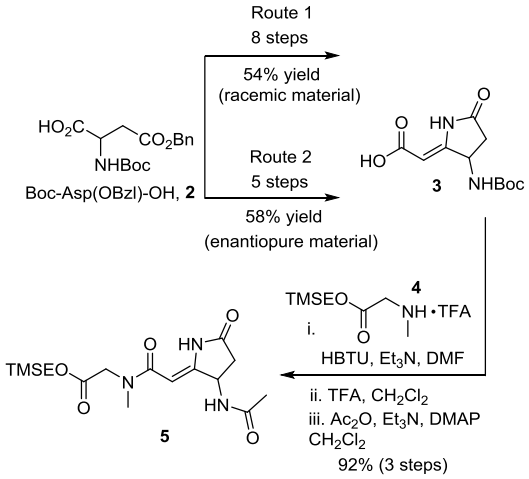
hydroxypyrrolidinone amino acid,[9] they found that it easily dehydrated, in line with previous observations made by Faulkner.[1] As part of our efforts we recently disclosed the total synthesis of microsclerodermin J and dehydromicrosclerodermin B, along with the structural reassignment of the C4 pyrrolidinone stereocentre in both natural products and concurrently the adjacent C5 stereocentre in microsclerodermin B.[10] Whilst we 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.



Scheme 1. Proposed hydration strategy to prepare the 5-hydroxypyrrolidinone of microsclerodermin B

Results and discussion

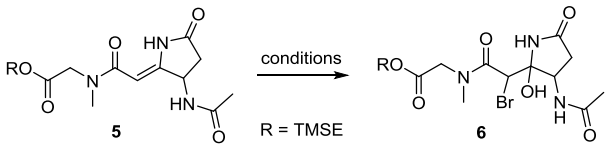
Because of the reported ease with which the 5-hydroxypyrrolidinone dehydrated we reasoned that in order to incorporate this residue into a viable total synthesis, hydration of the unsaturated pyrrolidinone should be conducted as late as possible in any synthetic sequence. As a result we felt it prudent 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 β-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 Ma[8] and Shioiri (route 1)[9] but later *via* a new route involving a Blaise reaction (route 2),[11] which we then utilised in the synthesis of microsclerodermin J and dehydromicrosclerodermin 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 sarscosine (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**).



Scheme 2. Synthesis of dipeptide **5**. TMSE = (trimethylsilyl)ethyl

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/H_2O 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 CH_2Cl_2/H_2O could also be employed to deliver the bromohydrin in similar yield (76%) (entry 3). Interestingly only the mono-brominated hydroxypyrrolidinone 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.

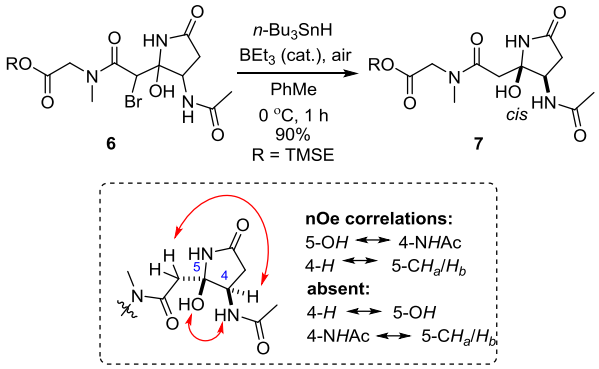
Table 1. Hydroxybromination of dipeptide **5**



Entry	Bromine source[a]	Solvent	Yield of **6** /%[b]
1	NBS	THF/H_2O	57
2	TBCD	THF/H_2O	81
3	Br_2	CH_2Cl_2/H_2O	76

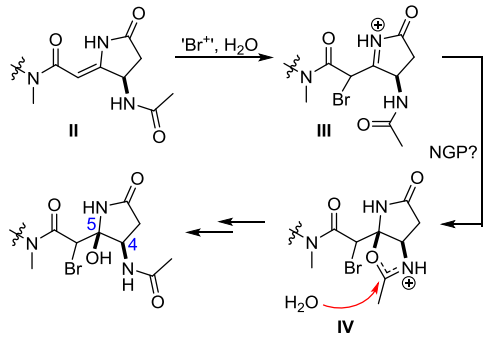
[a] 2.5 equivalents used. [b] Isolated yields after purification by flash column chromatography; NBS = *N*-bromosuccinimide, TBCD = 2,4,4,6-tetrabomo-2,5- cyclohexadien-1-one.

With bromohydrin **6** in hand our attention then turned to debromination and subsequent establishment of the stereochemistry of the pyrrolidinone product (**Scheme 3**). After some optimisation we found that treatment of the bromohydrin **6** with tributyltin hydride and catalytic amounts of triethylborane as an initiator,[12] could cleanly abstract the bromine atom at low temperature to furnish the desired γ-hydroxypyrrolidinone **7** in excellent yield (90%).[13] Elevated temperature ^{1}H NMR studies revealed the hydroxypyrrolidinone **7** to be a single diastereoisomer.[14] 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-hydroxypyrrolidinone amino acid of the microsclerodermins,[9] with dipeptide **7** being represented in eight family members.



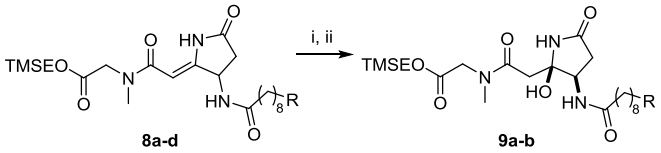
Scheme 3. Radical-mediated debromination of bromohydrin **6** and nOe spectroscopy correlations to determine the pyrrolidinone 4,5-*cis*-diastereoselectivity

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 hemi-aminal ring. As evidence against this route, both Shiori[9] and ourselves[15] have observed diastereoisomeric hemi-aminals from simpler model systems; these clearly had not equilibrated to the *cis* diastereisomer. 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.[16] 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.



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

Since the β-amino acid of microsclerodermins A and B, AMMTD, contains an electron rich *p*-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 alkyne or an olefin would be able to serve this purpose. Accordingly we set out to test this hypothesis and prepared a series of four dipeptides functionalised with long-chain acids (to mimic AMMTD), with either a 4-methoxystyrene, alkyne, or terminal olefin, along with an unsubstituted variant for comparison (**8a-d**) (see ESI for details). Treatment of these derivatives with NBS in THF/H_2O (TBCD also gave comparable yields with these derivatives) revealed, as suspected, that when styrene derivative **8a** was exposed to these conditions none of the desired bromohydrin was observed, and competing bromination could be detected on the styrene moiety itself (**Table 2**, entry 1). Pleasingly both alkyne **8b** (entry 2) and olefin **8c** (entry 3) underwent clean and regioselective hydroxybromination in good yield (65% and 73% respectively), albeit with elongated reaction times (~10 h). For 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-hydroxypyrrolidinones **9b-d**.



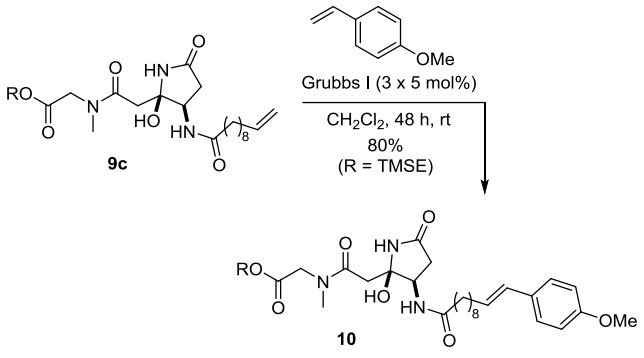
Reagents and conditions:i. NBS, THF/H_2O, 10 h;
ii. *n*-Bu_3SnH, BEt_3 (cat.), air, PhMe, 0 °C, 1 h

Entry	R	Step i. /%[a]	Step ii. /%[a]
1	**a** (*E*)-CH=CH(4-MeOC_6H_4)	– [b]	NA
2	**b** C≡CH	65	90
3	**c** CH=CH_2	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.

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

Our final task as part of our model studies was to derivatise either alkyne **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.[17] After some experimentation we found that olefin **9c** underwent efficient CM with 4-methoxystyrene at room temperature *via* treatment with three 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 (^3J = 15.6 Hz) with no signs of any dehydration of the sensitive γ-hydroxypyrrolidinone functionality (**Scheme 5**). For completeness an elevated temperature ROESY experiment was conducted which revealed **10** to be a single 4,5-*cis*-diasteoisomer (as expected, in line with previous observations, see ESI for details).



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

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-hydroxypyrrolidinone 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;[18] further experimentation will be required to achieve this aim.

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.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at

References and notes

1. (a) Bewley, C. A.; Debitus, C.; Faulkner, D. J. *J. Am. Chem. Soc*. **1994**, *116*, 7631-7636. (b) Schmidt, E. W.; Faulkner, D. J. *Tetrahedron* **1998**, *54*, 3043-3056. (c) Qureshi, A.; Colin, P. L.; Faulkner, D. J. *Tetrahedron* **2000**, *56*, 3679-3685.
2. (a) Zhang, X.; Jacob, M. R.; Rao, R. R.; Wang, Y. H.; Agarwal, A. K; Newman, D. J.; Khan, I. A; Clark, A. M.; Li, X. C. *Res. Rep. Med. Chem.* **2012**, *2*, 7-14. (b) Zhang, X.; Jacob, M. R.; Rao, R. R.; Wang, Y. H.; Agarwal, A. K; Newman, D. J.; Khan, I. A; Clark, A. M.; Li, X. C. *Res. Rep. Med. Chem.* **2013**, *3*, 9-10.
3. Hoffmann, T.; Müller, S.; Nadmid, S.; Garcia, R; Müller, R. *J. Am. Chem. Soc.* **2013**, *135*, 16904-16911.
4. Kunze, B.; Bohlendorf, B.; Reichenbach, H.; Hofle, G. *J. Antibiot.* **2008**, *61*, 18-26.
5. (a) Wright, A. E.; Pomponi, S. A.; Longley, R. E.; Isbrucker, R. A. US6384187B1, **2002** (b) Guzmán, E. A.; Maers, K.; Roberts, J.; Kemami-Wangun, H. V.; Harmody, D.; Wright, A. E. *Invest. New Drugs* **2015**, *33*, 86-94.
6. (a) Sasaki, S.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett*. **1997**, *38*, 3013-3016. (b) Sasaki, S.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1999**, *40*, 3187-3190. (e) Chandrasekhar, S.; Sultana, S. S. *Tetrahedron Lett*. **2006**, *47*, 7255-7258. (f) Shuter, E. C.; Duong, H.; Hutton, C. A.; McLeod, M. D. *Org. Biomol. Chem.* **2007**, *5*, 3183-3189. (g) Hjelmgaard, T.; Faure, S.; Lemoine, P.; Viossat, B.; Aitken, D. J. *Org. Lett*. **2008**, *10*, 841-844. (h) Tarrade-Matha, A.; Valle, M. S.; Tercinier, P.; Dauban, P.; Dodd, R. H. *Eur. J. Org. Chem.* **2009**, *5*, 673-686. (i) Burnett, C. M.; Williams, R. M. *Tetrahedron Lett*. **2009**, *50*, 5449-5451. (j) Davies, S. G.; Fletcher, A. M.; Foster, E. M.; Lee, J. A.; Roberts, P. M.; Thomson, J. E. *J. Org. Chem*. **2013**, *78*, 2500-2510.
7. Pullin, R. D. C.; Rathi, A. H.; Melikhova, E. Y.; Winter, C.; Thompson, A. L.; Donohoe, T. J. *Org. Lett*. **2013**, *15*, 5492-9545.
8. Zhu, J.; Ma, D. *Angew. Chem. Int. Ed*. **2003**, *42*, 5348-5351.
9. (a) S. Sasaki, Y. Hamada, T. Shioiri, *Synlett* **1999**, *4*, 453-455. (b) T. Shioiri, S. Sasaki, Y. Hamada, *Arkivoc* **2003**, 103-122; (c) Hamada and Shioiri prepared an isolated γ-hydroxypyrrolidinone amino acid with a 3:1 diastereomeric ratio.
10. Melikova, E. Y.; Pullin, R. D. C.; Winter, C.; Donohoe, T. J. *Angew. Chem. Int. Ed*. **2016**, *55*, 9753-9757.
11. Hoang, C. T.; Bouillere, F.; Johannesen, S.; Zulauf, A.; Panel, C.; Pouilhes, A.; Gori, D.; Alezra, V.; Kouklovsky, C. *J. Org. Chem.* **2009**, *74*, 4177-4187.
12. Renaud, P.; Beauseigneur, A.; Brecht-Forster, A.; Becattini, B.; Darmency, V.; Kandhasamy, S.; Montermini, F.; Ollivier, C.; Panchaud, P.; Pozzi, D.; Scanlan, E. M.; Schaffner, A.-P.; Weber, V. *Pure Appl. Chem.* **2007**, *79*, 223-233.
13. Using AIBN as an initiator required elevated temperatures, which led to dehydration; using Pd/C under hydrogen also led to dehydration.
14. Amide rotamers are present at room temperature.
15. T. J. Donohoe, *unpublished results.*
16. For an example see: Jha, R.; Davis, J. T. *Carbohydrate Res*. **1995**, *227*, 125-134.
17. Chatterjee, A. K.; Choi, T.-L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.*, **2003**, *125*, 11360-11370.
18. See reference 10 for details; the unsaturated pyrrolidinone starting material was consumed but only undefined and inseparable side products were obtained.