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Asymmetric Synthesis of (2*S*,3*R*)-3-Amino-2-Hydroxydecanoic Acid: The Unknown Amino Acid Component of Microginin

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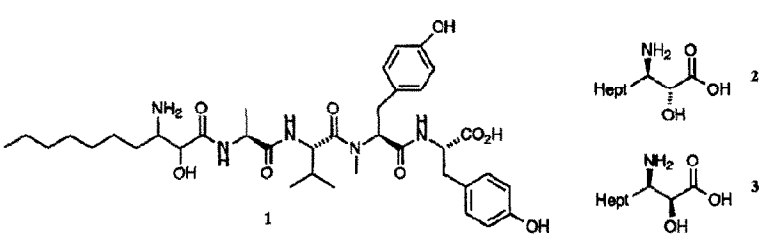
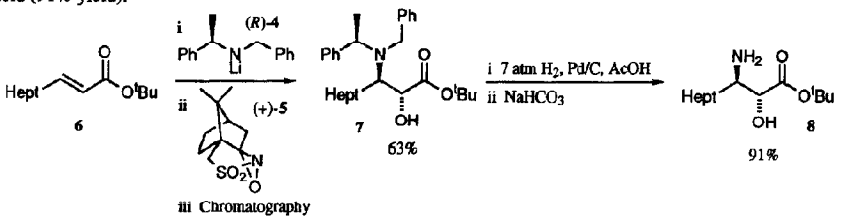
Abstract: 3-Amino-2-hydroxydecanoic acid (AHDA) is an unusual amino acid purported to occur in the recently isolated angiotensin-converting enzyme inhibitor microginin. In order to elucidate the stereochemistry of the naturally occurring material, and thus complete the structural assignment of microginin, both the (2*R*,3*R*)-*anti*-diastereoisomer and the (2*S*,3*R*)-*syn*-diastereoisomer of AHDA have been prepared. Comparison of the ^1H and ^{13}C nmr spectroscopic data of the synthetic amino acids with that reported for the naturally occurring material indicates that the *relative* stereochemistry of the AHDA found in microginin is *syn*. The *absolute* stereochemistry of the natural amino acid is shown to be (2*S*,3*R*) by comparison of its reported CD spectrum with that recorded for the synthetic material prepared herein.

Microginin **1** is a pseudopentapeptidic angiotensin-converting enzyme (ACE) inhibitor which has recently been isolated from the freshwater blue-green alga *Microcystis aeruginosa*.[1] A number of techniques, including 2D nmr and chemical degradation, were employed to elucidate the partial structure of **1** with 3-amino-2-hydroxydecanoic acid (AHDA) being implicated as the *N*-terminal component. The structure of this novel β-amino-α-hydroxy amino acid was suggested by the ^1H and ^{13}C nmr spectra, and the FAB mass spectrum, of a sample isolated by hydrolysis of microginin.[1] On the basis of a Cotton effect in the CD spectrum, it was further suggested that the α-stereogenic centre in this acid was of the *R* configuration, however this must be regarded as tentative, especially since the configuration of the β-stereogenic centre remained undefined.[1] In order to complete the structural and stereochemical assignment of microginin, this communication describes the asymmetric synthesis of authentic samples of the homochiral *anti* and *syn* diastereoisomers (2*R*,3*R*)-AHDA **2** and (2*S*,3*R*)-AHDA **3** for spectroscopic comparison with the naturally occurring acid.



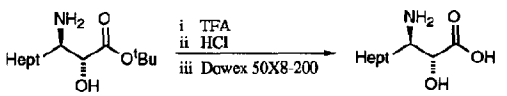
We have previously shown that the conjugate addition of lithium (*R*)-(α-methylbenzyl)benzylamide (*R*)-**4** to *tert*-butyl cinnamate, followed by *in situ* hydroxylation with (+)-(camphorsulfonyl)oxaziridine (+)-**5**, provides the corresponding *anti* -β-amino-α-hydroxy amino acid derivative with excellent diastereoselectivity (92% d.e.).[2] We anticipated therefore that the corresponding reaction using the readily available[3] enoate **6** would provide the basis for an efficient synthesis of **2**. Indeed, the tandem addition-hydroxylation of **6** using (*R*)-**4** and (+)-**5** provided the desired adduct **7** with good diastereoselectivity (88% d.e.) and this material was

isolated as a single diastereoisomer in 63% yield after flash chromatography. Subsequent catalytic debenzylation of **7** occurred without complication to afford the free β-amino-α-hydroxy ester **8** in excellent yield (91% yield).



The *anti* relative stereochemistry within **8** was confirmed by conversion to the oxazolidinone **9** using carbonyl diimidazole (CDI). Analysis of the ring proton coupling constant in **9** ($J_{4,5}$ = 8.6 Hz) confirmed the *cis* stereochemistry[4,5] which follows directly from the *anti* arrangement in **8**, and hence **7**. The *absolute* stereochemistry of **7** follows from addition of (*R*)-**4** to the *Re*-face of **6** in an analogous manner to that previously elucidated for a number of related enoates.[2,5,6]

With the stereochemistry within **8** established, a convenient synthesis of the *anti* amino acid **2** was available. Thus, treatment of **8** with trifluoroacetic acid (TFA) to remove the *tert*-butyl group, conversion of the resultant TFA salt to the corresponding hydrochloride, and ion-exchange chromatography afforded the free amino acid **2** in 59% isolated yield: $[\alpha]_D^{25}$ +3.4 (*c* 0.70, 1N HCl).



Analysis of the ^{1}H nmr and ^{13}C nmr spectra of synthetic **2** was completely consistent with the designated structure but a comparison with the spectroscopic data reported[1,7] for the naturally occurring acid (see tables) revealed sufficient inconsistency to suggest that **2** was not the component of microginin. Consequently, it appeared that the naturally occurring amino acid was of the *syn* relative stereochemistry and, in order to unequivocally confirm this assignment, a synthesis of (2*S*,3*R*)-AHDA **3** was undertaken.

We anticipated that **3** could be readily prepared from the *anti*-β-amino-α-hydroxy ester **8** using our previously reported oxazoline inversion protocol.[2] Thus, the *N*-benzoyl derivative **10** was prepared by treatment of **8** with one equivalent of benzoyl chloride and readily isolated in 83% yield. Intramolecular cyclisation of **10** under Mitsunobu conditions, with attendant stereochemical inversion at the α-centre, subsequently furnished the *trans*-oxazoline **11** ($J_{4,5}$ = 6.3 Hz) in good yield (84%). Finally, exhaustive hydrolysis of **11** with 6N HCl, and ion exchange chromatography of the resultant hydrochloride salt, afforded

the desired free amino acid **3** as a single diastereoisomer in excellent yield (92%): $[\alpha]_D^{25}$ +5.4 (*c* 0.59, 1N HCl). As anticipated, the ^{1}H nmr and ^{13}C nmr spectra of synthetic homochiral **3** correlated with the data recorded[1] for the natural acid (see tables) and confirmed that the unusual amino acid component found in microginin was indeed 3-amino-2-hydroxy-decanoic acid with *syn* relative stereochemistry.

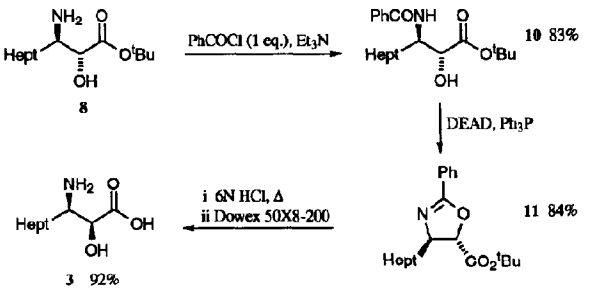


Table 1: ^{1}H nmr data for *anti* and *syn* 3-amino-2-hydroxydecanoic acid **2** and **3**

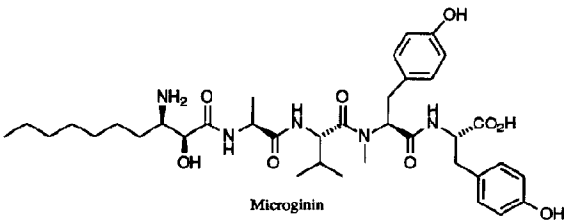
^{1}H nmr (d^6-DMSO)	**2**	**3**	Natural Acid[1]
C<u>H</u>(OH)CHN	δ3.35, d, *J* 8.1 Hz	δ3.55, br	δ3.54, m
CH(OH)C<u>H</u>N	δ2.82, m	δ3.10, br	δ3.08, br
$CH_3(C\underline{H}_2)_5$	δ1.71–1.26, m	δ1.60–1.20, m	δ1.3–1.2, m
$C\underline{H}_3(CH_2)_5$	δ0.86, t, *J* 6.9 Hz	δ0.86, t, *J* 6.8 Hz	δ0.85, t

Table 2: ^{13}C nmr data for *anti* and *syn* 3-amino-2-hydroxydecanoic acid **2** and **3**

^{13}C nmr (d^6-DMSO)	**2**	**3**	Natural Acid[1]
<u>C</u>O$_2$	-	δ173.4	-
<u>C</u>H(OH)CHN	δ70.3	δ69.5	δ69.60
CH(OH)<u>C</u>HN	δ53.9	δ53.0	δ52.30
$CH_3(\underline{C}H_2)_5$	δ31.3	δ31.4	δ31.17
	δ30.2	δ29.1	δ28.90
	δ29.0	δ28.8	δ28.90
	δ28.6	δ28.7	δ28.48
	δ24.8	δ25.3	δ25.20
	δ22.1	δ22.3	δ22.07
$\underline{C}H_3(CH_2)_5$	δ14.0	δ14.2	δ13.95

It has been reported[1] that the CD spectrum of the AHDA isolated from microginin exhibited a negative Cotton effect at 215nm and, presumably on the assumption that the β-configuration was irrelevant, this was suggested to implicate the *R* stereochemistry at the α-stereogenic centre. However, the CD spectrum for synthetic **3** *also* indicated a negative Cotton effect in the same region[8] (216nm) indicating that the previous stereochemical assumption was erroneous and that, in fact, **3**, with the (2*S*,3*R*) configuration, is the unknown amino acid component of microginin.[9]

In conclusion, the unknown amino acid component of microginin has been established as (2*S*,3*R*)-3-amino-2-hydroxydecanoic acid **3** by an independent asymmetric synthesis and spectroscopic comparison with data reported for a sample secured by hydrolysis of the natural product. These studies complete the structural and stereochemical assignment of microginin, for which the revised structure is depicted below.



Microginin

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1. T. Okino, H. Matsuda, M. Murakami and K. Yamaguchi, *Tetrahedron Lett.*, 1993, **34**, 501.
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7. We thank Dr. Murakami for advising us of the solvent (d^6-DMSO) and instruments (600MHz ^1H nmr; 75MHz ^{13}C nmr) employed in the characterisation of the naturally occurring acid. The nmr spectra of the synthetic amino acids **2** and **3** reported herein were recorded using a Bruker AM500 spectrometer (500MHZ ^1H nmr; 125MHz ^{13}C nmr).
8. The *anti* diastereoisomer **2** also exhibited a negative Cotton effect but at significantly lower wavelength (204 nm).
9. Dr. Murakami has recently informed us that he has independently reassessed the configuration of the 3-amino-2-hydroxydecanoic acid found in microginin also as (2*S*,3*R*).

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