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A concise, total synthesis and antibacterial evaluation of 2-hydroxy-1-(1*H*-indol-3-yl)-4-methylpentan-3-one

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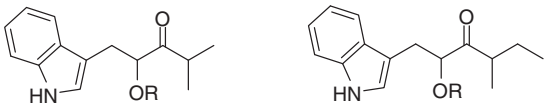
ABSTRACT

Treatment of racemic 2-hydroxy-3-(1*H*-indol-3yl)propionic acid methyl ester (**5**) with isopropyl magnesium chloride provided the title compound **1** and its isomer, 3-hydroxy-1-(indol-3-yl)-4-methylpentan-2-one (**9**). Both enantiomers (>96% ee) of each component were obtained via semi-preparative chiral supercritical fluid chromatography (SFC). In contrast to previous reports, these compounds, as well as their acetate derivatives, were not active or very weakly active against 16 bacterial strains, including *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*.

From our literature survey on indole-containing antibiotics, we became interested in the small group of natural products **1**–**4** (Fig. 1). These compounds were first isolated by Nealson and coworkers[1] in 1981 from the cultured broth of *Xenorhabdus nematophilus*. They were believed to be among the active ingredients of the antibiotic cocktail that the symbiotic bacteria in the gut of entomopathogenic nematodes produce to prevent other bacteria and fungi in the environment from invading and putrefying the infected insect, thus helping the nematodes to complete their life cycle.[2] Importantly, subsequent work by Sundar and Chang[3] showed that the indole-containing fraction isolated by thin layer chromatography from the *X. nematophilus* extract caused severe inhibition of RNA synthesis in *Bacillus cereus* and *Escherichia coli* K12. These data indicated a novel mechanism of action, which could be useful in the development of new antibacterial drugs.[4] Curiously, there have been no reports on the total synthesis of these compounds. While the previous findings were promising, there were shortcomings: no minimum inhibitory concentration (MIC) values had been reported for any of these four compounds in the pure form. Furthermore, assay data on the clinically relevant bacterial strains are limited.[1,3,5] To address these issues, we decided to synthesize compound **1** to: (i) confirm the structure assignment of the natural product; (ii) determine MIC values against a number of the prevalent pathogens using **1** and related compounds; and (iii) evaluate their potential application in drug development programs.

Initially, the synthetic plan was analogous to the syntheses of kurasoin B[6] and soraphinol A.[7] Thus, the commercially available (racemic) 2-hydroxy-3-(1*H*-indol-3yl)propionic acid was converted to the Weinreb amide **6** (Scheme 1). Treatment of this amide with either 2-propenyl magnesium bromide or isopropyl magnesium chloride did not provide any detectable product. The corresponding *tert*-butyldimethylsilyl ether derivative **7** also did not react with the Grignard reagents.

These failures could be due to the bulkiness of the nucleophiles and the amides. Indeed, when the less cumbersome methyl ester **8** was prepared and treated with isopropyl magnesium chloride, reaction occurred readily to provide the desired product **1** and its tauto-isomer **9** (Scheme 2). The ratio of these two products varied based on the quenching conditions. When the reaction was quenched with methanol, followed by aqueous HCl, the ratio of **1**:**9** was 1.2:1, and when quenched with aqueous $NaHCO_3$ or TBAF solutions the ratio varied from batch to batch, in the range of 1.2:1–7:1. Although the isomeric ratio does not change in neutral solutions, these isomers were not separable by silica gel or C18 reversed-phase chromatography.



1, R = H
2, R = Ac

3, R = H
4, R = Ac

Figure 1. Indole natural products from *Xenorhabdus nematophilus*.

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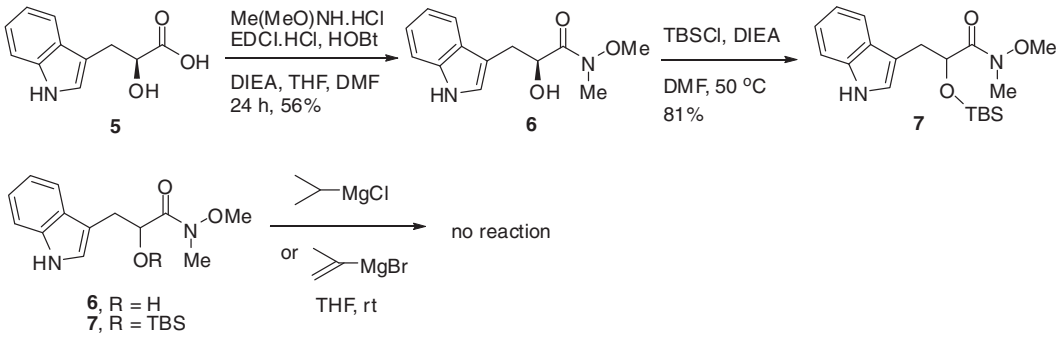
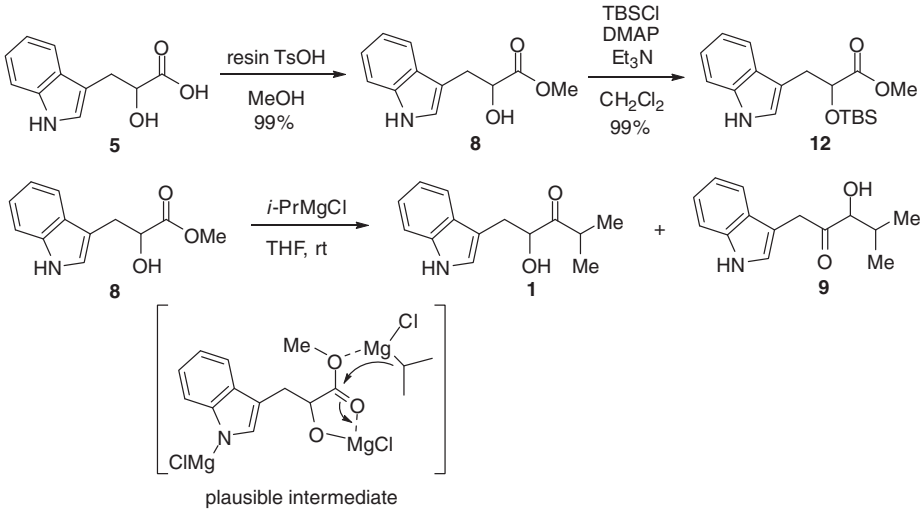
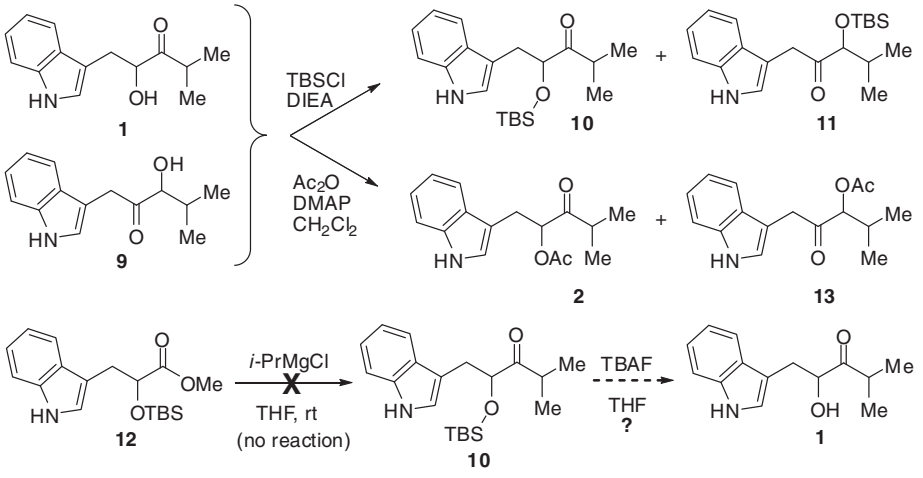


Scheme 1. Synthesis and reactions of Weinreb amides.



Scheme 2. Synthesis of **1** and **9**.



Scheme 3. Synthesis of the derivatives of **1** and **9**.

The corresponding *tert*-butyldimethylsilyl ether (**10**, **11**) or acetate ester (**2**, **13**) derivatives were prepared but they were also inseparable by silica gel chromatography (Scheme 3). An attempt to prepare **10** by treatment of methyl 2-(*tert*-butyldimethylsilyloxy)-3-(1*H*-indol-3-yl)propanoate (**12**) with isopropyl magnesium chloride was unsuccessful.[8] This failure indicated that the free hydroxyl group played an important role in the addition reaction. The hydroxyl group provides a chelating site for magnesium and thus activates the carbonyl center towards nucleophilic attack by the Grignard reagent.

Interestingly, when a 2:1 mixture of **1** and **9** was submitted to semi-preparative chiral SFC column chromatography, four enantiomerically enriched compounds (ee >96%) were isolated (Fig. 2). ^{1}H, ^{13}C NMR, mass spectra and optical rotation values of the four isomeric compounds were measured to confirm their identities. Among these, (−)-**1**, (+)-**9** and (−)-**9** are new compounds. The ^{1}H

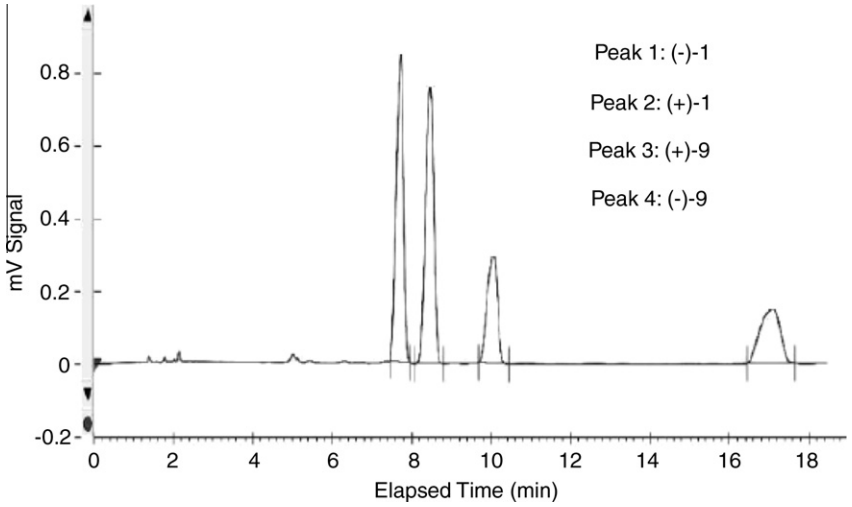


Figure 2. Chiral supercritical fluid chromatography (SFC) of compounds **1** and **9**.

Table 1
^{1}H and ^{13}C NMR comparisons for compounds **1** and **3**

^{1}H NMR of **1**			^{13}C NMR of **3**		^{13}C NMR of **1**	
Ref. 9[a]	Ref. 1[a]	Ours[a]	Ref. 9[a]	Ref.1[b]	Ref.1[b]	Ours[b,c]
8.05	8.08	8.10	215.8	190.2	190.2	215.4
1H, bs	1H, bs	1H, bs				(215.9)
7.63	7.63	7.62	136.1	136.1	136.1	136.4
1H, d	1H, d	1H, d				(136.1)
7.36	7.36	7.34	127.4	128.0	128.0	128.6[d]
1H, d	1H, d	1H, d				(127.4)
7.20	7.16	7.20	122.8	121.0	121.2	123.2
1H, t	2H, m	1H, t				(122.8)
7.14		7.14	122.2	120.2	120.1	122.3
1H, t		1H, t				(122.2)
7.11	7.10	7.06	119.5	117.9	117.7	119.8
1H, d	1H, s	1H, d				(119.5)
4.67	4.62	4.67	118.6	117.7	117.0	119.1
1H, m	1H, dd	1H, dd				(118.7)
3.45		3.47	111.2	109.5	109.5	111.5
1H, d		1H, bs				(111.2)
3.31	3.33	3.31	110.9	109.0	109.2	110.9
1H, dd	1H, dd	1H, dd				(110.7)
3.06	3.05	3.05	76.2	73.7	73.4	75.4
1H, dd	1H, dd	1H, dd				(75.0)
2.88	2.90	2.88	43.1	42.2	34.4	36.4
1H, hep	1H, m	1H, m				(36.5)
1.13	1.10	1.13	29.8	29.0	28.2	30.2
3H, d	3H, d	3H, d				(30.0)
1.02	0.98	1.02	27.1	26.8	17.2	19.2
3H, d	3H, d	3H, d				(19.5)
			14.8	15.2	15.4	17.4
						(17.3)
			11.4	11.4		

[a] Spectrum recorded in $CDCl_3$.
[b] Spectrum recorded in C_6D_6.
[c] Values in brackets were recorded in $CDCl_3$.
[d] Signal overlapped with the solvent peak.

Table 2
Optical rotation comparisons for compound **1**

Ref. 8	Ref. 1	Ours
86 (*c* 1.1, $CHCl_3$)	65.4 (*c* 1.2, $CHCl_3$)	75 (*c* 0.76, $CHCl_3$)

NMR spectrum and optical rotation value of the compound eluted as peak two matched those reported in the literature for **1** (Tables 1 and 2).[1,9] Curiously, the ^{13}C NMR spectrum of this compound was not identical to that reported by the Nealson group.[1] On examination of their data, we found that some of the chemical shifts did not seem to correlate with the structure. Particularly, the carbonyl carbon signal recorded at 190.2 ppm was too low for such a ketone. Our value was 215.4 ppm.

We noticed that Webster and co-workers[9] also isolated compounds **1**–**4** from *Xenorhabdus bovienii* A2. Their ^{1}H NMR spectra of all four compounds matched the Nealson data and our data. However, only the ^{13}C NMR data for compound **3** was recorded.[10] In comparing these data with the Nealson report we found similar discrepancies: the carbonyl signal was recorded at 215.8 ppm by Webster and at 190.2 ppm by Nealson. Because the spectral data and the optical rotation value recorded by Webster were consistent with ours, it was concluded that the structural assignment of the natural product **1** was correct.

The only remaining issue was the absolute configuration of compound **1**. Although there have been no experimental reports on the determination of the absolute configuration of compounds **1**–**4**, in the supporting information part of a recent publication, Balskus and Walsh[7] depicted the configuration of the hydroxyl-bearing stereogenic center in compounds **1**–**4** as *S*, based on a hypothetical general biosynthetic pathway for the acyloin natural products.[11] Considering that other compounds in this family, such as kurasoin A and B,[12] soraphinol A[13] and B,[14] sattabacin and 4-hydroxysattabacin[15] all have the *S* configuration with positive values of optical rotation, it is probable that the configuration of (+)-**1** also is *S*.

The MIC assays against 16 prevalent bacterial strains[16] were performed on the amide **6**, the racemic mixtures of **1** and **9**, of **2** and **13**,[17] and the enantiomerically enriched samples of (+)-**1**, (−)-**1**, (+)-**9** and (−)-**9**. To our disappointment, these samples showed either no or only insignificant antibacterial activities (MIC values at 80 μg/mL or higher). Notably, our assays included bacteria such as *E. coli*, *B. subtilis* and *S. aureus*, which had been shown to be susceptible to the cultured broth[5] or the indole-containing fraction of the broth[3] from the *Xenorhabdus* spp. It is possible, therefore, that there were other components in the mixtures that caused the observed antibiotic activities in the early assays.[18]

In conclusion, we have established a concise synthesis of 2-hydroxy-1-(1*H*-indol-3-yl)-4-methylpentan-3-one (**1**) and confirmed the structure assignment of this natural product. Our *in vitro* assays revealed that the title compound and its tauto-isomer **9** in their pure forms, as well as their acetate derivatives in racemic form, are not active against a broad range of Gram-negative and Gram-

positive bacteria. Additional studies will be necessary to deconvolute the active antibiotic cocktail produced by the *Xenorhabdus* spp.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.003.

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8. It was expected that **10** could be converted to **1** without isomerization by treatment with TBAF because the same reagent had been used for a similar transformation in the synthesis of soraphinol A in Ref. 7.
9. Li, J.; Chen, G.; Webster, J. M. *J. Nat. Prod.* **1995**, *58*, 1081.
10. We contacted Professor Webster to request the ^{13}C NMR spectrum of **1**, but this record was not available.
11. The hypothesis is in good agreement with the previous proposal by Chang and co-workers based on their radiolabeling and culturing experiments that tryptophan, isoleucine, leucine and valine could be involved in the biosynthesis of **1**–**4** in Ref. 3.
12. Kurasoin A: $[\alpha]_D$ 7.0 (*c* 0.1, MeOH), kurasoin B: $[\alpha]_D$ 22.0 (*c* 0.1, $CHCl_3$); Sunazuka, T.; Hirose, T.; Zhi-Ming, T.; Uchida, R.; Shiomi, K.; Harigaya, Y.; Omura, S. *J. Antibiot.* **1997**, *50*, 453.
13. Soraphinol A: $[\alpha]_D$ 28.1 (*c* 0.3, MeOH); Li, X.; Zee, O. P.; Shin, H. J.; Seo, Y.; Ahn, J.-W. *Bull. Korean Chem. Soc.* **2007**, *28*, 835.
14. Soraphinol B: $[\alpha]_D$ 1.3 (*c* 0.07, MeOH); Ahn, J. W.; Li, X.; Zee, O. P. *Bull. Korean Chem. Soc.* **2007**, *28*, 1215.
15. Sattabacin: $[\alpha]_D$ 35.0 (*c* 0.7 $CHCl_3$), 4-hydroxysattabacin: $[\alpha]_D$ 14.0 (*c* 0.3, $CHCl_3$); Lampis, G.; Deidda, D.; Maullu, C.; Madeddu, M. A.; Pompei, R. *J. Antibiot.* **1995**, *48*, 967.
16. Tested bacteria: *E. coli* (2), *P. aeruginosa* (2), *A. baumanii*, *S. maltophilia*, *K. pneumonia* (2), *B. subtilis*, *B. anthracis*, *S. aureus*, *MRSA*, *E. faecalis*, *VRE faecalis* and *B. cepacia.*
17. This mixture might also be separable by chiral SFC.
18. One such component could be nematophin; Li, J.; Chen, G.; Webster, J. M. *Can. J. Microbiol.* **1997**, *43*, 770.