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Bioorganic & Medicinal Chemistry Letters 17 (2007) 911–915
Bioorganic & Medicinal Chemistry Letters

Total synthesis of (*R*,*R*,*R*)- and (*S*,*S*,*S*)-schweinfurthin F: Differences of bioactivity in the enantiomeric series

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Received 30 September 2006; revised 14 November 2006; accepted 20 November 2006
Available online 12 December 2006

Abstract—Total synthesis of the (*R*,*R*,*R*)- and (*S*,*S*,*S*)-enantiomers of the natural product schweinfurthin F has been completed. Comparisons of spectral data and optical rotations with those reported for the natural product, as well as a variety of bioassay data, allow assignment of the natural material as the (*R*,*R*,*R*)-isomer.

Schweinfurthins F and G (**1a** and **2**, Fig. 1) recently were isolated from an extract of *Macaranga alnifolia* fruits as part of a search for cytotoxic natural products conducted by the Kingston group.[1] These compounds have the same carbon skeleton but a simpler oxidation pattern than vedelianin (**3**), a prenylated stilbene originally isolated from the leaves of *Macaranga vedeliana* by Thoison et al. as part of an ethnobotanical study of plants from New Caldonia[2] and also recovered from the *M. alnifolia* extract. All three compounds incorporate a hexahydroxanthene skeleton identical to that of three closely related terpenoids, schweinfurthins A, B, and D (**4**–**6**), isolated from the African species *Macaranga schweinfurthii* by Beutler et al.[3,4] In all three studies, the gross structures and relative stereochemistry were established by extensive analyses of spectroscopic data, but the absolute stereochemistry was not assigned.

Vedelianin (**3**) and schweinfurthins A and B (**4** and **5**) have potent and selective cytotoxicity in the 60 cell line anticancer screen of the National Cancer Institute (NCI) with mean GI_{50} values of 0.08, 0.36, 0.81 μM, respectively.[3,4] Even more intriguing than their potency is their pattern of sensitive and resistant cell lines. The NCI has developed a bioinformatics algorithm called COMPARE that can cluster data from the 60 cell line assay by pattern of sensitivity, and these patterns have been found to correlate with mechanism of action.[5] The schweinfurthins appear to be novel in this respect, because these natural products show no significant correlation to any other compound in the NCI standard agent database of clinically used anticancer agents.[3,4]

As part of an ongoing program aimed at the total synthesis of the schweinfurthins[6,7] and illumination of the mechanism of their activity, we have disclosed a stereoselective route to the 3-deoxyhexahydroxanthene substructure of stilbenes **1a** and **2**.[8] We now describe the stereoselective total synthesis of the (*R*,*R*,*R*)- and (*S*,*S*,*S*)-enantiomers of schweinfurthin F (**1a**) in optically pure form, along with the preparation of several analogues of this natural product. The synthesis establishes the absolute stereochemistry of natural schweinfurthin F, and allows consideration of biological activity in this light. Subsequent measurements of their impact on DNA synthesis via ^{3}H-thymidine incorporation assays have revealed significant differences in the activity of the two enantiomers, and different biological activity also was observed in two enantiomeric pairs of analogues.

Keywords: Schweinfurthin; Synthesis; Enantiomer; Activity.
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doi:10.1016/j.bmcl.2006.11.096

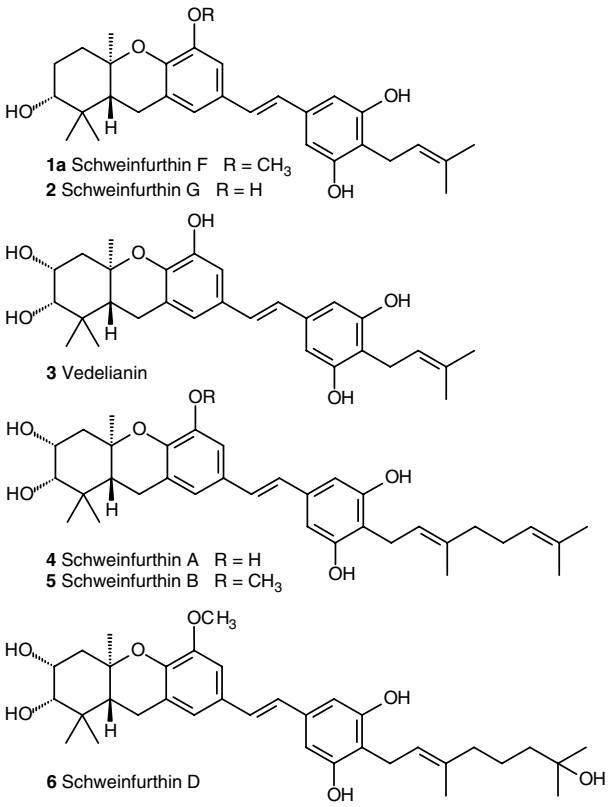


Figure 1. Representative schweinfurthins shown as the (2*R*,4a*R*,9a*R*) or (2*R*,3*R*,4a*R*,9a*R*) enantiomers.

We envisioned a highly convergent synthesis of compound **1a** (Fig. 2) in which construction of the stilbene olefin would be conducted near the end of the sequence, allowing access to either enantiomeric series from a common benzylic phosphonate and the enantiomeric aldehydes **7a** and **7b**. Both aldehydes are available via a previously published route,[8] which leaves synthesis of the required phosphonates (e.g., compound **8**) as the necessary starting point.

Synthesis of phosphonate **8** (Scheme 1), and the monomethylated analogue **13** which we sought for its potentially greater stability, began with the known benzylic

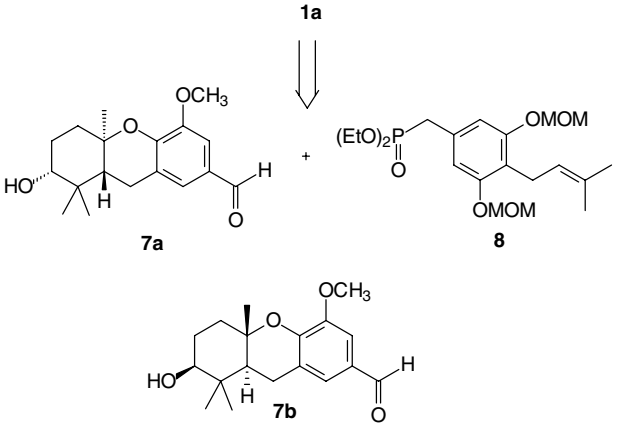
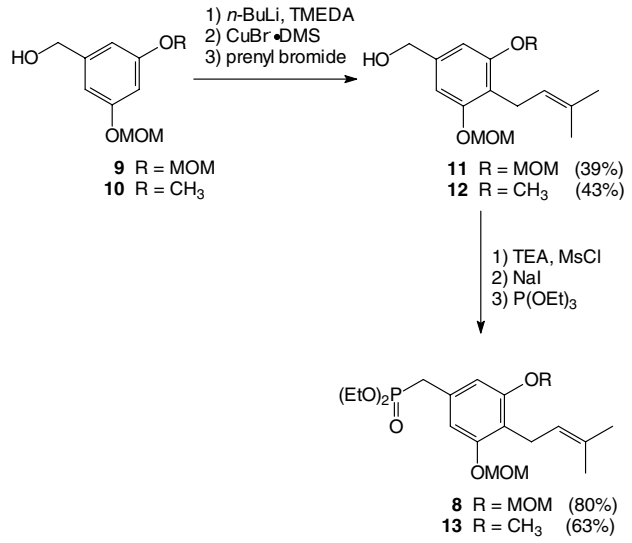
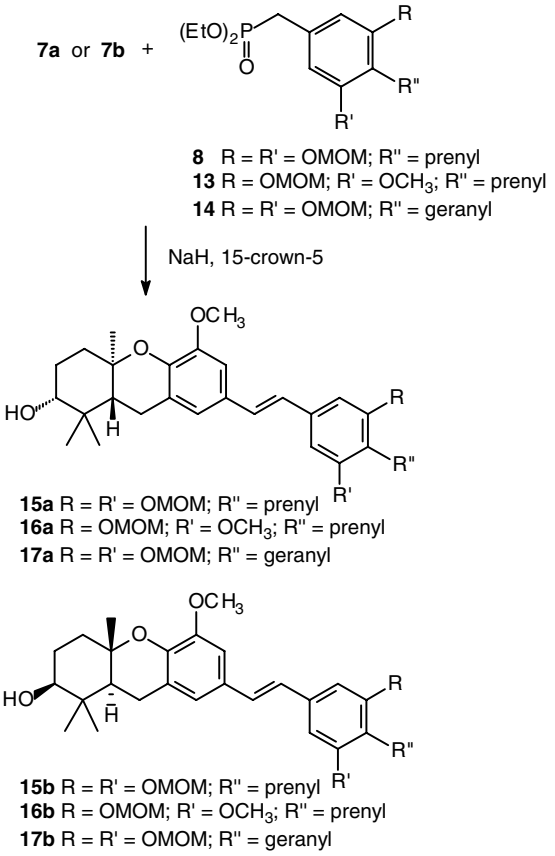


Figure 2. Retrosynthetic analysis.



Scheme 1. Synthesis of phosphonates **8** and **13**.

alcohols **9** and **10**.[9] Directed *ortho* metalation followed by transmetalation of the resulting aryl lithium to the cuprate and alkylation with prenyl bromide afforded the C-alkylated products **11** and **12**, respectively, without the need for protection of the benzylic alcohol.[7] Treatment of these benzylic alcohols with methanesulfonyl chloride followed by transformation into the iodide



Scheme 2. Stilbene synthesis.

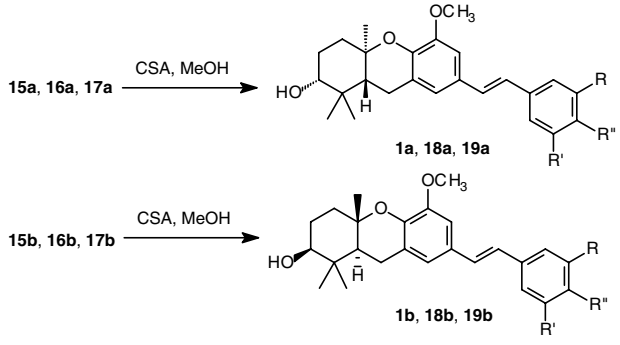
Table 1. Synthesis of the protected stilbenes **15a**–**17a** and **15b**–**17b**

Aldehyde	Phosphonate	R	R′	R″	Stilbene	Yield (%)
7a	**8**	OMOM	OMOM	C_5H_9	**15a**	71
7b	**8**	OMOM	OMOM	C_5H_9	**15b**	72
7a	**13**	OMOM	OCH_3	C_5H_9	**16a**	44
7b	**13**	OMOM	OCH_3	C_5H_9	**16b**	74
7a	**14**	OMOM	OMOM	$C_{10}H_{17}$	**17a**	82
7b	**14**	OMOM	OMOM	$C_{10}H_{17}$	**17b**	57

and final Arbuzov reaction with triethyl phosphite gave the desired phosphonates **8** and **13** in good yields.

Phosphonates **8** and **13**, and the known phosphonate **14**[7,10] were allowed to react (Scheme 2) with enantiopure tricyclic aldehydes **7a** and **7b** in the presence of sodium hydride and a crown ether,[11] to give the protected stilbenes **15a**, **16a**, and **17a**, and then **15b**, **16b**, and **17b**, respectively, in good yields (Table 1). These protected phenols were then subjected to acidic hydrolysis through treatment with camphorsulfonic acid[12] to afford the desired compounds **1a**, **18a**, and **19a**, and **1b**, **18b**, and **19b** (Scheme 3 and Table 2). The ^1H and ^{13}C NMR spectra for compound **1a** were virtually identical to those reported for natural schweinfurthin F, and the optical rotation was closely parallel (natural, $[\alpha]_D^{22}$ +50.8 (*c* 0.06, CH_3OH); synthetic $[\alpha]_D^{22}$ +53.4 (*c* 0.007, CH_3OH)). While the spectral data for the (*S*,*S*,*S*)-enantiomer **1b** also were very similar, the rotation was negative (−55.8 (*c* 0.005, CH_3OH)). On this basis natural schweinfurthin F was assigned as the (*R*,*R*,*R*)-enantiomer. Further support for this assignment can be found in the bioassay data.

An earlier synthetic study[8] afforded 3-deoxy-schweinfurthin B enriched in the (*R*,*R*,*R*)-isomer **19a** (68% ee). This



Scheme 3.

Table 2. Synthesis of the target schweinfurthins

Protected stilbene	R	R′	R″	Product	Yield (%)
15a	OH	OH	C_5H_9	**1a**	69
15b	OH	OH	C_5H_9	**1b**	53
16a	OH	OCH_3	C_5H_9	**18a**	66
16b	OH	OCH_3	C_5H_9	**18b**	72
17a	OH	OH	$C_{10}H_{17}$	**19a**	40
17b	OH	OH	$C_{10}H_{17}$	**19b**	53

enantioenriched material was found to have a pattern of activity across the cell lines that was highly correlated with natural schweinfurthin B (**5**, Pearson correlation coefficient = 0.75), and a comparable mean GI_{50}.[8] We recently reported assays on a series of analogues of similar enantiopurity, and have shown that the 'right-half' resorcinol substructure is required for correlated differential activity in the 60 cell line screen.[13]

Because the absolute stereochemistry of the natural products was not known at the outset of these studies, the initial choice of enantiomeric series was arbitrary and the correlated activity seen in the enriched material could be due to the primary enantiomer, the minor component, or a combination. While bioactivity often is associated with one enantiomer of a pair, there are numerous examples where more complex relationships exist.[14–16] Based on the initial screens, a working hypothesis was formed that the desired bioactivity would result from the (*R*,*R*,*R*)-isomers. To test this idea more fully, we studied the set of three enantiomeric pairs described herein.

We initially tested two pairs of enantiomers for growth inhibition using a ^3H-thymidine incorporation assay of DNA synthesis in RPMI-8226 human-derived myeloma cells (Fig. 3). This cell line was chosen for these studies because it is highly susceptible to natural schweinfurthins.[3,4] The graphs for the two (*R*,*R*,*R*)-enantiomers **1a** and **19a** were very similar, as were those for the two (*S*,*S*,*S*)-isomers **1b** and **19b**, but the profiles differed

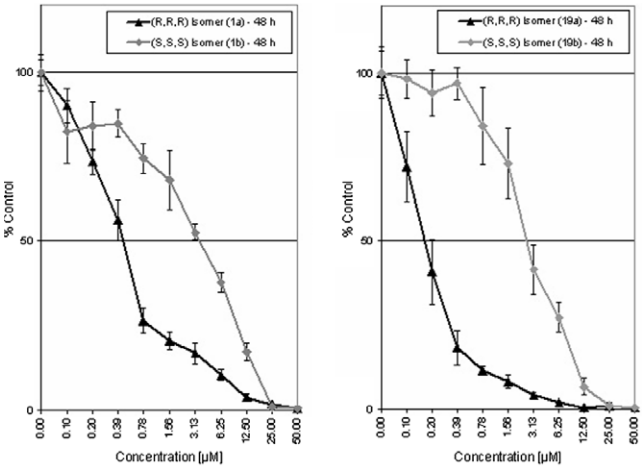


Figure 3. DNA synthesis assays in RPMI-8226 human-derived myeloma cells.

Table 3. Activity in the NCI 60-cell line screen

Compound	Stereochemistry	Mean GI_{50} (μM)	GI_{50} Range (log units)
1a	(*R*,*R*,*R*)	0.41	4.00
1b	(*S*,*S*,*S*)	0.13	3.10
3		0.08	3.41
4		0.36	3.11
5		0.81	3.08
18a	(*R*,*R*,*R*)	0.87	3.05
18b	(*S*,*S*,*S*)	4.6	0.97
19a	(*R*,*R*,*R*)	0.87	3.25
19b	(*S*,*S*,*S*)	2.2	2.32

substantially between the two enantiomeric series. Both enantiomers of schweinfurthin F (**1a** and **1b**) inhibited growth in this assay but the (*R*,*R*,*R*)-isomer **1a** consistently showed the higher activity. For example, at the 48 h time point the (*R*,*R*,*R*)-isomer **1a** displayed an IC_{50} = 0.58 μM versus 3.40 μM for the (*S*,*S*,*S*)-isomer **1b**. Similarly both enantiomers of 3-deoxyschweinfurthin B (**19a**, **19b**) showed activity, with the (*R*,*R*,*R*)-compound **19a** displaying an IC_{50} = 0.21 μM and the (*S*,*S*,*S*)-isomer **19b** an IC_{50} = 4.5 μM at 48 h. Thus, both (*R*,*R*,*R*)-compounds inhibited growth more potently than their corresponding (*S*,*S*,*S*)-enantiomers.

The results in RPMI-8226 cells appeared to indicate that the original choice of the (*R*,*R*,*R*)-enantiomer was a fortuitous one. Nevertheless, compounds **1a**, **1b**, **18a**, **18b**, **19a**, and **19b** all were tested at NCI in the 48 h, 60 cell line screen. As indicated in Table 3, these compounds showed significant anti-proliferative activity at the GI_{50} level, most often with a 1000-fold range of response across the cell lines. The activity of the (*R*,*R*,*R*)-compounds also was highly correlated with that of the natural products **3**–**5** (e.g., a Pearson correlation of **1a** with schweinfurthin A (**4**) = 0.78).

The (*S*,*S*,*S*)-compounds also inhibited growth considerably, but they did not show significant Pearson correlations with the natural products **3**–**5** or amongst the other members of the (*S*,*S*,*S*) enantiomeric series (e.g., **19b** vs. **4** and **19b** versus **18b**). (*S*,*S*,*S*)-Schweinfurthin F (**1b**), which we assign as the unnatural enantiomer, showed the most potent anti-proliferative activity of all the compounds tested, and is only slightly less active than vedelianin, the most potent compound in the natural family to date. The two methylated stilbenes **18a** and **18b** also showed significant activity, although the (*R*,*R*,*R*)-enantiomer **18a** was the more active isomer.

A comparison of the mean graphs of the enantiomers **1a** and **1b** (see Supplemental material) shows some notable differences. One example of the large divergence of bioactivity can be seen in the response of the glioma-derived SNB 75 cell line where stilbene **1a** displayed a GI_{50} = 10 nM and its enantiomer **1b** had a GI_{50} = 1.7 μM. The activity pattern is reversed in the U251 cell line of the CNS panel, where the (*S*,*S*,*S*)-enantiomer **1b** displays very high activity with a GI_{50} < 10 nM, while the (*R*,*R*,*R*)-enantiomer **1a** shows a GI_{50} = 13 μM. In an extreme example, the breast cancer cell line HS 578T shows a 10,000-fold difference in activity between the two enantiomers, being essentially resistant to compound **1a** and displaying a GI_{50} of <10 nM with compound **1b**. These and other differences lead to a low Pearson correlation between the two enantiomers (0.40) and may indicate an underlying difference in mechanism of action. Similar differences were observed between enantiomers **18a** and **18b**, and between the enantiomers **19a** and **19b**. However, the three (*R*,*R*,*R*) compounds **1a**, **18a**, and **19a** gave relatively close values for their mean GI_{50}'s and ranges between 3 and 4 log units, while the three (*S*,*S*,*S*) compounds were less consistent (cf. Table 3).

In conclusion, we report here the total synthesis of both enantiomers of schweinfurthin F (**1a** and **1b**), and assignment of the natural product as the (*R*,*R*,*R*)-enantiomer **1a**. Both the optical rotations and the bioassay results support assignment of the (*R*,*R*,*R*)-enantiomer as the natural product in the specific case of schweinfurthin F, and it is likely that this stereochemistry is found throughout the family of natural schweinfurthins. In the NCI 60 cell line panel, the (*R*,*R*,*R*)-isomer **1a** shows both high activity and a high degree of correlation with the other natural products **3**–**5**. The (*S*,*S*,*S*)-isomers also can display very high activity, but the members of the (*S*,*S*,*S*)-series show low Pearson correlations with the other natural products and with each other. This divergence of activity between the two enantiomeric series suggests a potential difference in mechanism of action. The potent activity of the (*S*,*S*,*S*)-enantiomer **1b** also indicates that continued exploration of this series may be rewarding as well. Further synthetic studies and efforts aimed at determining the biological targets of these agents will be disclosed in due course.

Acknowledgments

We thank Prof. David G. I. Kingston for providing spectral data on schweinfurthin F prior to publication, and Domenic Scudiero and the Developmental Therapeutics Program, NCI, for 60 cell line testing. Financial support from the Roy J. Carver Charitable Trust, the Breast Cancer Research Program (DAMD17-01-1-0276 and DAMD17-02-1-0423), the University of Iowa Graduate College, an Oncology Research Training Award from the Holden Comprehensive Cancer Center's Institutional National Research Service Award (2 T32 CA79445), and the Predoctoral Training Program in the Pharmacological Sciences (2 T32 GM067795) is gratefully acknowledged. This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.096.

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