Tetrahedron Letters, Vol. 33, No. 3, pp. 297-300, 1992
Printed in Great Britain

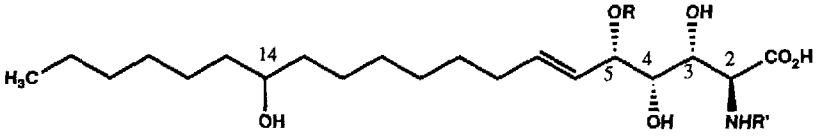
0040-4039/92 $3.00 + .00
Pergamon Press plc

DETERMINATION OF THE RELATIVE AND ABSOLUTE STEREOCHEMISTRY OF SPHINGOFUNGINS A, B, C, AND D

Frank VanMiddlesworth*, Claude Dufresne, Francine E. Wincott[1], Ralph T. Mosley, and Kenneth E. Wilson
Merck, Sharp & Dohme Research Laboratories
P.O. Box 2000, Rahway, NJ 07065, USA

Abstract: The relative and absolute stereochemistry of positions 2, 3, 4, and 5 of the recently isolated sphingofungins has been determined as 2S, 3R, 4R, 5S by spectral analysis of the rigid bicyclic derivative **5**, and enzymatic hydrolysis of **4** using a 2S specific acylase. These configurational assignments were confirmed by degradation and conversion of sphingofungin B to peracetyl deoxynojirimycin **6**.

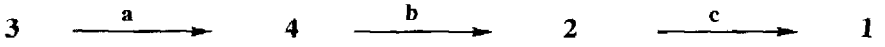
A new family of antifungal agents, the sphingofungins was recently isolated in these laboratories.[2] These compounds have been shown to be potent and specific inhibitors of serine palmitoyl transferase.[3] The structures of sphingofungins A (**1**), B (**2**), C (**3**), and D (**4**) were elucidated by NMR and MS analysis,[4] but the relative and absolute stereochemistry could not initially be determined. This communication describes the chemistry developed to interconvert the four sphingofungins and transformations to prepare the rigid bicyclic derivative **5** to define the relative stereochemistry of positions 2, 3, 4, and 5. In addition, a degradation sequence converting sphingofungin B (**2**) to the configurationally defined natural product derivative peracetyl deoxynojirimycin (**6**) is described.



sphingofungin A (**1**) R=H; R'=C(NH)NH_2
sphingofungin B (**2**) R=H; R'=H
sphingofungin C (**3**) R=Ac; R'=H
sphingofungin D (**4**) R=H; R'=Ac

Each of the four natural products could be obtained starting from the most abundant compound **3** as shown in Scheme I. Upon mild base treatment, the allylic O-acetyl of **3** undergoes an O to N acyl migration to form acetamide **4**. The acetamide of **4** is resistant to basic hydrolysis, but can be enzymatically hydrolyzed to **2** using an *Aspergillus* acylase [EC 3.5.1.14]. Since this enzyme is known to only hydrolyze 2S amino acids,[5,6,7,8] and has been used to resolve diastereomeric mixtures on this basis, the 2 configuration of the sphingofungins was assumed to be 2S. The guanidinium derivative **1**, could be prepared from amino acid **2** using a mild guanidinating agent.[9,10]

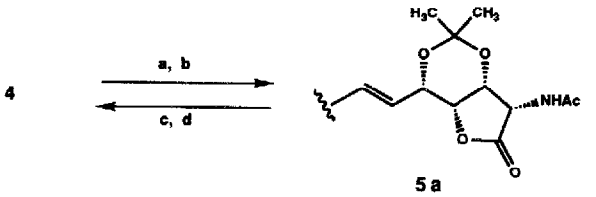
Scheme I



Reagents: (a) NEt_3, MeOH (75% yield); (b) *Aspergillus* acylase, pH 8.5 (73% yield); (c) HO_3S-C(NH)NH_2, MeOH (65% yield).

Since the sphingofungins are interconvertable through processes that retain the configurations of asymmetric centers 3, 4, and 5, the relative and absolute stereochemistry must be identical for the four compounds. In order to define the stereochemistry of positions 3, 4, and 5 of the sphingofungins, the γ-lactone-acetonide **5a** was prepared by the reactions shown in Scheme II. The five membered lactone is the major product formed upon activation with DCC, even though traces of a six membered lactone were observed. The rigid bicyclic acetonide **5a** is readily formed from the intermediate diol. To confirm that no centers had been epimerized in the lactonization-acetonide formation, **4** was regenerated from **5a** using hydrolytic conditions and was found to be unchanged by ^{1}H-NMR and HPLC.

Scheme II

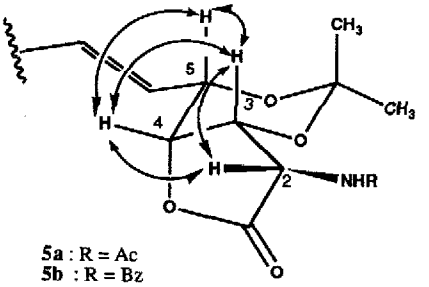


Reagents: (a) DCC, DMAP, dioxane (45% yield); (b) $MeC(OMe)_2Me$, TsOH (61% yield); (c) HOAc, THF, H_2O ; (d) LiOH, THF, H_2O (54% yield for 2 steps).

With stereocenters 3, 4, and 5 unassigned, there were 8 diastereomeric possibilities for bicyclic **5**. Computer models for each of the eight possible diastereomers were built[11] and energy minimized[12] using Merck's internally developed modeling system. Of the eight models, only the calculated ^{1}H-NMR coupling constants[13,14] of one, **5a**, were consistent with those determined experimentally. Strong evidence in support of this diastereomer was obtained from the nuclear Overhauser effects observed for **5b** (see Figure 1). These data show that each of the protons on carbons 2, 3, 4, and 5 of bicyclic **5b** are on the same side of the convex molecule which can only be accommodated by the isomer shown.

Figure 1: Observed Nuclear Overhauser effects and Observed versus Calculated Coupling Constants for **5b**.

Protons	nOe	J(calc)	J(obs)
2 → 3	5 %	3.7 Hz	3.9 Hz
2 → 4	4 %		
3 → 4	4 %	2.7 Hz	2.1 Hz
3 → 5	3 %		
4 → 5	4 %	1.6 Hz	1.6 Hz

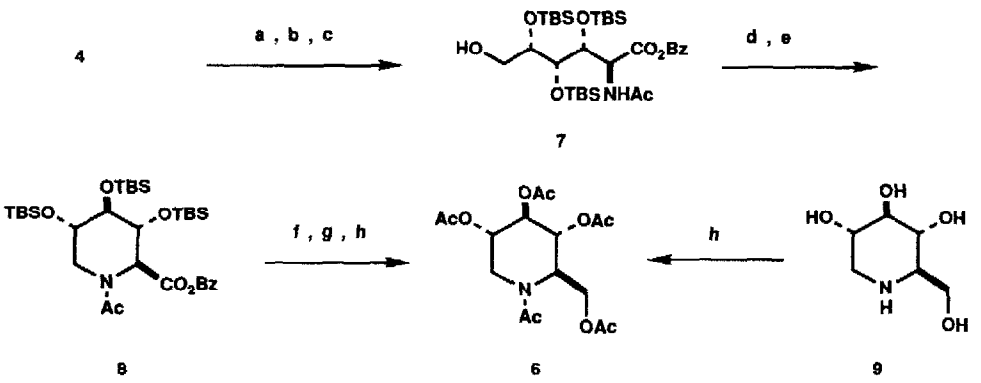


5a : R = Ac
5b : R = Bz

With the absolute configuration of position 2 determined to be S from the enzyme work, and the relative configurations determined from analysis of bicyclic **5**, stereocenters 2-5 can now be assigned as 2S, 3R, 4R, and 5S. The only ambiguity not addressed in this study is the configuration of the distal hydroxyl at C-14.

To confirm the above configurational assignments a degradation scheme was developed to convert one of the sphingofungins to a known compound. As shown in Scheme III, the functional groups of sphingofungin D (**4**) were first completely protected and the olefin cleaved by ozonolysis to yield the fully functionalized hexose amino acid **7**. Mesylation of **7** followed by deprotonation of the amide proton induced an intramolecular cyclization forming imino alditol **8**. Reduction of the ester of **8**, deprotection, and peracetylation then gave rise to **6**. Based on the earlier sphingofungin analysis, the imino-alditol exhibiting the analogous 2, 3, 4, and 5 configurations should be the commercially available deoxynojirimycin[15] (**9**). Peracetylation of **9**[15] yielded **6** which was identical by TLC, NMR, and optical rotation to the sphingofungin derived material.[16]

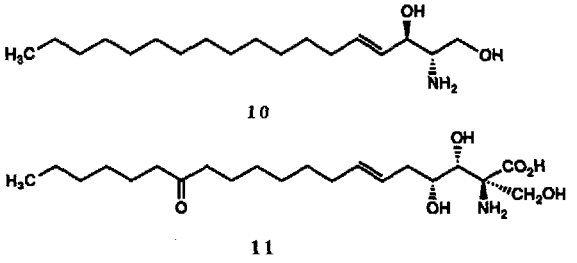
Scheme III



Reagents: (a)BzBr, $NaHCO_3$, DMF; (b)TBSOTf, 2,6-lutidine, CH_2Cl_2 (65% yield for 2 steps); (c)(i) O_3 (ii) $NaBH_4$ (91% yield); (d)MsCl, NEt_3, CH_2Cl_2 (81% yield); (e)NaH, DMF (86% yield); (f)$LiBH_4$ (65% yield); (g)HF, pyr.; (h)Ac_2O, pyr. (65% yield for 2 steps).

The chirality of the polar head group of the sphingofungins could be critical to their activity as potent inhibitors of sphingosine biosynthesis. In this regard, the spatial alignment as shown in Figure 2 seems to be opposite that of natural sphingosine, D(+)-erythro sphingosine (**10**), and similar to a related antifungal agent myriocin (**11**).[17]

Figure 2



Acknowledgement: The authors would like to thank F.A.Bouffard, O. Hensens, M. Hammond, and D. Zink for helpful discussions concerning this study.

References

1.Present address: Cortech Inc., 6840 N. Broadway, Denver, CO 80221.
2.a)VanMiddlesworth, F.; Lopez M.; Wilson, K.; Garrity, G.; Monaghan, R.; Giacobbe, R.; Zitano, L.; Fromtling, R. to be submitted to *J. Antibiotics*; b) for the isolation of a related compound, fumifungin, see Mukhopadhyay, T; Roy, K; Coutinho, L.; Rupp, R.; Ganguli, B. *J. Antibiotics* **1987**, *40*, 1050.
3. Zweerink, M.; Edison A.; Wells G.; Pinto W.; Lester R. *J. Biol. Chem.* in press.
4. Hensens, O.; Zink, D. to be submitted to *J. Antibiotics*.
5. Mori, K.; Iwasawa, H. *Tetrahedron*, **1980**, *36*, 2209.
6. Masaoka, Y.; Sakakibara, M.; Mori, K. *Agric. Biol. Chem.* **1982**, *46* , 2319.
7. Mori, K.; Funaki, Y. *Tetrahedron Lett.* **1984**, *25*, 5291.
8. Mori, K.; Otsuka, T. *Tetrahedron* **1985**, *41*, 547.
9. Miller, A.; Bischoff, J. *Synthesis* **1986**, 777-779.
10 Kim, K.; Lin Y.; Mosher, H. *Tetrahedron Lett.* **1988**, 3183-3186.
11. Thirty conformers of each diastereomer were created using a distance geometry algorithm as described in : Crippen, G. M.; Havel, T. F. *Acta. Cryst.* **1987**, *34*, 282. After energy minimization, the structures were clustered into the families shown below based on the atomic coordinates of the dioxolane ring in **5a**.
12. The energy minimizer is an MM2-X (MM2-extended) force field, developed internally at Merck. It shares many parameters with MM2 (Allinger, N. L., 1977, QCPE program 318) and differs principally in that lone pairs on heteroatoms are not used and in that electrostatic interactions take place between atom-centered charges, thus allowing proper treatment of charged systems.
13. Using the modified Karplus equation as described in: Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron*, **1980**, *35*, 2783.
14. The calculated coupling constants (Hz) for the eight possible diastereomers (and their enantiomers) are listed below:

diasteromer	family	J(2,3)	J(3,4)	J(4,5)	Avg. Energy (kcal)
2S 3S 4S 5S	twist	1.2	5.3	8.0	17.0
	chair	1.2	2.1	1.1	19.5
	boat	1.3	5.9	1.8	21.9
	chair	8.4	8.1	9.0	22.6
2S 3R 4S 5S	chair	6.4	9.6	9.3	23.1
	twist	6.7	9.3	7.8	25.6
2S 3S 4R 5S	chair	8.8	9.5	5.5	24.3
	twist	8.7	9.4	8.3	24.9
2S 3S 4S 5R	chair	1.1	2.7	1.7	18.1
	twist	2.1	6.0	7.2	20.1
	twist	5.8	8.0	3.3	22.1
	chair	7.9	8.4	7.5	28.1
2S 3R 4R 5S	chair	3.7	2.7	1.6	18.5
	twist	5.3	5.4	8.0	20.0
2S 3S 4R 5R	chair	9.0	9.5	9.3	22.7
	twist	8.7	9.3	7.7	23.8
2S 3R 4S 5R	chair	6.7	9.5	5.5	24.8
	twist	6.7	9.4	8.4	25.9
2S 3R 4R 5R	twist	4.6	4.5	7.5	17.0
	chair	7.8	7.7	9.1	17.8
	boat	3.4	2.1	1.1	18.6

15. Inouye, S.; Tsuruoka, T.; Ito, T.; Nida, T. *Tetrahedron* **1968**, *23*, 2125. Deoxynojirimycin is commercially available from Sigma Chemical Company.
16. The observed optical rotation for sphingofungin derived **6** was $[\alpha]_{366}$ = -41.8° (c=4.07), and that for deoxynojirimycin derived **6** was $[\alpha]_{366}$ = -39.3° (c=4.83).
17. Aragozzini, C; Manachini, P.; Craveri, R.; Rindone, B; Scolastico, *Tetrahedron Lett.* **1972**, 28 (21): 5493.

(Received in USA 4 October 1991)