ORGANIC LETTERS

2006
Vol. 8, No. 4
649−652

Synthesis of 1-Deoxysphingosine Derivatives with Conformationally Restricted Pyrrolidinediol Head Groups

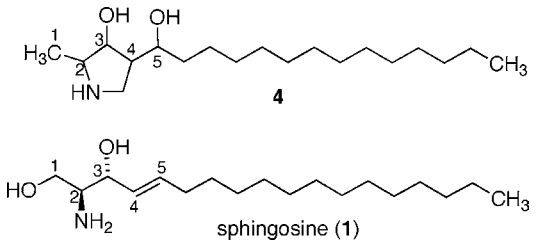
Ann M. Dougherty,[†] Frank E. McDonald,*[,†] Dennis C. Liotta,*[,†] Steven J. Moody,[‡] David C. Pallas,[‡] Carrie D. Pack,[§] and Alfred H. Merrill[§]

Departments of Chemistry and Biochemistry and the Winship Cancer Institute, Emory University, Atlanta, Georgia 30322, and School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332

fmcdona@emory.edu; dliotta@emory.edu

Received November 23, 2005

ABSTRACT



A family of cyclic 1-deoxysphingolipid derivatives of structure 4 has been designed and synthesized, which may serve as tumorigenesis suppressors for various cancers. Compound 4 is a second-generation analogue developed from sphingosine (1), in which a hydroxyl substituent is moved from C1 to C5 and a methylene is added for conformational rigidity between the C2-nitrogen substituent and C4. The synthetic chemistry for pyrrolidine ring closure at C3–C4 features ring-closing metathesis followed by hydroboration-oxidation.

Sphinoglipids are natural products found in most cell membranes and are structurally characterized by a long carbon chain "sphingoid" base that is derivatized with amide-linked fatty acids and various polar headgroups.[1] Sphingoid bases have additional functions as cellular mediators and protein kinase C (PKC) inhibitors,[2] affecting the growth, differentiation, migration, and apoptosis of cells. Extensive research efforts have resulted in several synthetic approaches to sphingoid bases[3] and structural analogues, encouraged in part by recent discoveries regarding the anticancer activity of sphingolipids. In 2003 Menaldino et al. reported that 1-deoxysphingoid bases of general structure **3** (Figure 1) were growth inhibitory and cytotoxic at concentrations up to 10-fold lower than for sphingosine (**1**) and up to 50-fold more potent than the corresponding *N*-acylated ceramide **2** in HT29 and DU145 cell lines.[4] As the primary alcohol of sphingosine is phosphorylated in vivo (resulting in undesired mitogenic/anti-apoptotic activity), the design of 1-deoxy-analogues **3** prevents phosphorylation, and by moving the hydroxyl group to the 5-position, lipophilicity of **3** is similar to that of sphingosine (**1**). To minimize *N*-acylation activity, we have further modified the 1-deoxysphingoid lead structure **3** to cyclic pyrrolidinediol **4**, which also provides confor-

[†] Department of Chemistry.
[‡] Department of Biochemistry and Winship Cancer Institute.
[§] Georgia Institute of Technology.

(1) *Biochemistry*, 2nd ed.; Voet, D., Voet, J. G., Eds.; Wiley: New York, 1995.

(2) (a) Hannun, Y. A.; Loomis, C. R.; Merrill, A. H., Jr.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 12604. (b) Merrill, A. H., Jr.; Sereni, A. M.; Stevens, V. L.; Hannun, Y. A.; Bell, R. M.; Kinkade, J. M., Jr. *J. Biol. Chem.* **1986**, *261*, 12610.

(3) (a) Garner, P.; Park, J. M.; Malecki, E. *J. Org. Chem.* **1988**, *53*, 11061. (b) Herold, P. *Helv. Chim. Acta* **1988**, *71*, 354. (c) Nimkar, S.; Menaldino, D.; Merrill, A. H.; Liotta, D. *Tetrahedron Lett.* **1997**, *38*, 7687.

(4) (a) Menaldino, D. S.; Bushnev, A.; Sun, A.-M.; Liotta, D. C.; Symolon, H.; Desai, K.; Dillehay, D. L.; Peng, Q.; Wang, E.; Allegood, J.; Trotman-Pruett, S.; Sullards, M. C.; Merrill, A. H. *Pharmacol. Res.* **2003**, *47*, 373. (b) For second-generation synthesis of aminodiol compounds **3**: Wiseman, J. M.; McDonald, F. E.; Liotta, D. C. *Org. Lett.* **2005**, *7*, 3155.

10.1021/ol052839v CCC: $33.50
Published on Web 01/21/2006

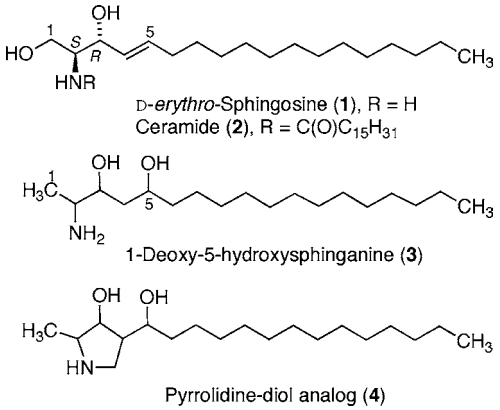


Figure 1. Structures of sphingosine (**1**), ceramide (**2**), and 1-deoxyanalogues **3** and **4**.

mational restriction of the polar groups. This communication describes the synthesis and biological evaluation of several stereoisomers of **4**, prepared in highly convergent fashion.

We envisioned that the cyclic pyrrolidinediol analogue **4** could be prepared from the functionalized dihydropyrrole **5**, which in turn would arise from ring-closing metathesis[5] of the diallylamine **6** (Figure 2).

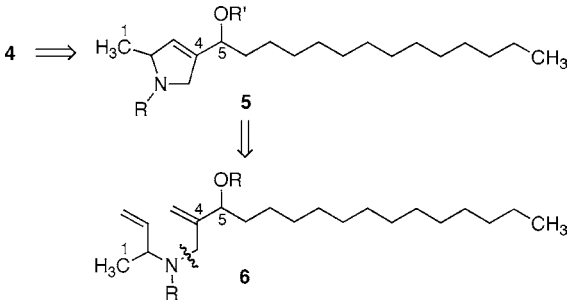
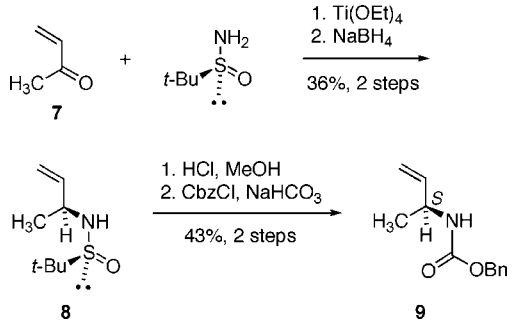


Figure 2. Retrosynthesis for cyclic 1-deoxysphinganine.

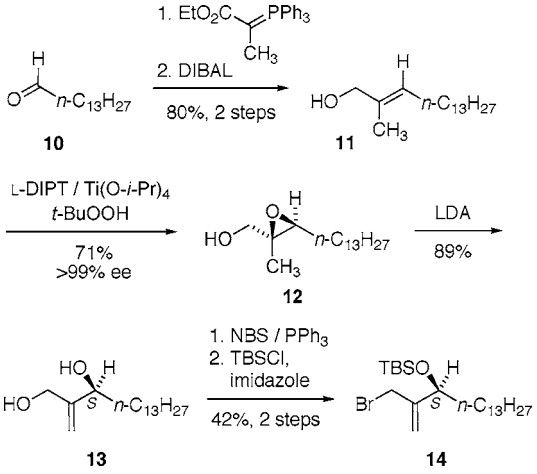
We initially planned to prepare 2-amino-3-butene from the amino acid L-alanine, but racemization occurred under all conditions attempted via α-aminoaldehyde intermediates.[6] Therefore, enantioselective synthesis of 2-amino-3-butene was accomplished by sodium borohydride reduction of the chiral sulfinimide[7] derived from methyl vinyl ketone (**7**), providing sulfinamine **8** as the major product of a 7:1 mixture of diastereomers (Scheme 1). The minor diastereomer was separated from **8** by careful silica gel chromatography.[8] Acidic cleavage[9] of the chiral auxiliary and Cbz-protection of nitrogen provided compound **9**.

Scheme 1. Enantioselective Synthesis of *N*-Cbz-2-amino-3-butene (**9**)



Several approaches were explored for preparation of the fragment bearing carbons 4 and 5. The best route involved asymmetric epoxidation of **11** to **12** (Scheme 2), followed by LDA elimination[10] to give the allylic diol **13**. Differentiation of the primary alcohol as the bromide and protection of secondary alcohol as the silyl ether afforded chiral nonracemic synthon **14** for carbon−nitrogen coupling. The enantiomer of **14** was likewise prepared beginning with epoxidation of **11** with D-DIPT.

Scheme 2. Stereoselective Synthesis of Allylic Bromide (**14**)



(5) Kirkland, T. A.; Grubbs, R. H. *J. Org. Chem.* **1997**, *62*, 7310.

(6) (a) Albeck, A.; Persky, R. *J. Org. Chem.* **1994**, *59*, 653. (b) McKillop, A.; Taylor, R. J. K.; Watson, R. J.; Lewis, N. *Synthesis* **1994**, 31. (c) Takai, K.; Hotta, Y.; Oshima, K.; Nozaki, H. *Tetrahedron Lett.* **1978**, *27*, 2417. (d) Nishizawa, R.; Saino, T.; Takita, T.; Suda, H.; Aoyagi, T.; Umezawa, H. *J. Med. Chem.* **1977**, *20*, 510.

(7) (a) Borg, G.; Cogan, D. A.; Ellman, J. A. *Tetrahedron Lett.* **1999**, *40*, 6709. (b) Cogan, D. A.; Liu, G.; Ellman, J. *Tetrahedron* **1999**, *55*, 8883. (c) Ellman, J. A.; Owens, T. D.; Tang, T. P. *Acc. Chem. Res.* **2002**, *35*, 984. (d) Weix, D. L.; Ellman, J. A. *Org. Lett.* **2003**, *5*, 1317. (e) Zhou, P.; Chen, B.; Davis, F. A. *Tetrahedron* **2004**, *60*, 8003.

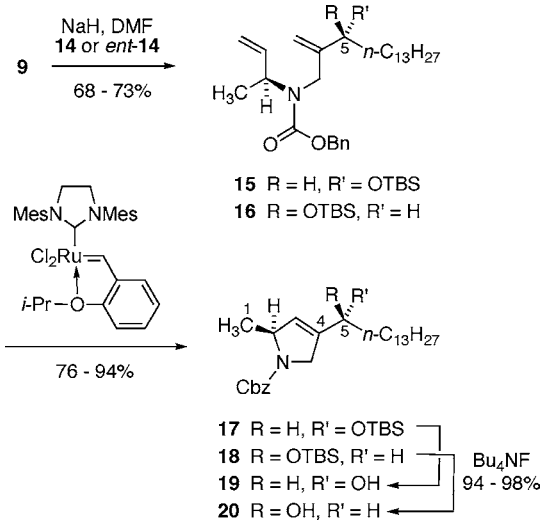
(8) We also explored addition of vinylmagnesium bromide to the sulfinimide derived from acetaldehyde, but this reaction proceeded with poor diastereoselectivity, reaching a maximum of 3:1 dr favoring compound **8** when 8 equiv of CH_2=CHMgBr was used.

(9) Liu, G.; Cogan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913.

(10) (a) Kang, S. H.; Jun, H. S. *Chem. Commun.* **1998**, 1929. (b) Hao, J. L.; Aiguade, J.; Forsyth, C. J. *Tetrahedron Lett.* **2001**, *42*, 821. (c) Gao, Y.; Klunder, J. M.; Hanson, R. M.; Masamune, H.; Ko, S. Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

Reaction of carbamate **9** with sodium hydride[11] and *N*-alkylation of each enantiomer of allylic bromide **14** provided the dienes **15** and **16** (Scheme 3), which each

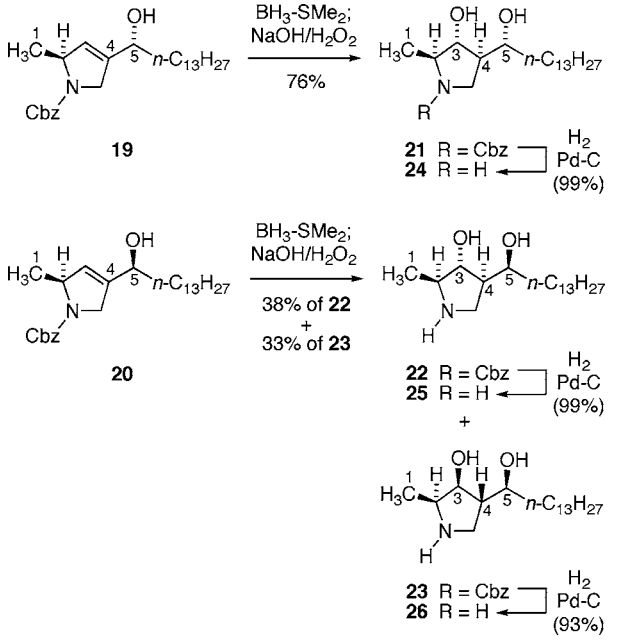
Scheme 3. Coupling of **9** and **14** and Ring-closing Metathesis to Diastereomeric Dihydropyrrolidines **17** and **18**



underwent ring-closing metathesis to dihydropyrroles **17** and **18** in excellent yield, using the Hoveyda metathesis catalyst.[12]

A variety of conditions were explored for the introduction of the C3 alcohol via anti-Markovnikov hydration, with the best results obtained with hydroboration[13] of the alcohols **19** and **20** with either borane-dimethyl sulfide or thexylborane followed by alkaline hydrogen peroxide oxidation and hydrogenolytic removal of the *N*-Cbz protective group.[14] As

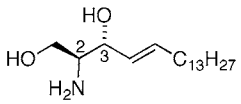
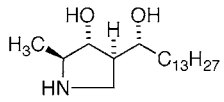
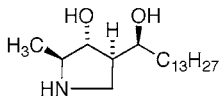
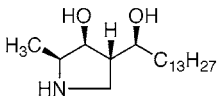
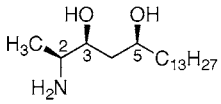
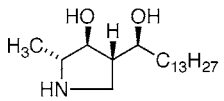
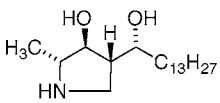
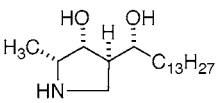
Scheme 4. Stereoselectivity of Hydroboration of **19** vs **20** and Completion of Target Compound Syntheses



expected, diastereomer **19** produced a single pyrrolidinediol diastereomer **21**, arising from stereoinduction from the C5-hydroxyl (Still−Barrish model),[15] reinforcing the steric effect from the methyl substituent attached at C2. In contrast, diastereomer **20** gave a separable mixture of diastereomers **22** and **23**, consistent with opposing effects of stereoinduction from C2 and C5 chiral allylic postions.[16]

After hydrogenolysis of the *N*-Cbz protective group, pyrrolidinediol stereoisomers **24**−**26** and their enantiomers

Table 1. Biological Evaluation of Pyrrolidinediol Analogs **24**−**26** and *ent*-**24**−**26**

	controls (ref. 4)	cytotoxicity, EC_{50} (Hill slope)		
				
	(2*S*,3*R*)-sphingosine **1**	**24**	**25**	**26**
DU-145 [a]	13.9 μM (-2.7)	ND [c]	13.0 μM (-2.3)	31.4 μM (-2.7)
DU-145 [b]	46.0 μM (-2.4)	13.2 μM (-2.4)	9.9 μM (-1.8)	17.5 μM (-2.1)
HT-29 [a]	30.0 μM (-2.0)	ND [c]	10.0 μM (< -4) [d]	13.0 μM (-0.28)
				
	(2*S*,3*S*,5*S*)-**3**	*ent*-**24**	*ent*-**25**	*ent*-**26**
DU-145 [a]	7.0 μM (-3.8)	49.9 μM (-3.3)	14.9 μM (< -4) [d]	16.0 μM (< -4) [d]
DU-145 [b]	10.2 μM (-3.2)	15.4 μM (-2.4)	5.2 μM (-1.5)	16.7 μM (-2.2)
HT-29 [a]	7.9 μM (-3.7)	29.4 μM (-3.5)	10.4 μM (-2.2)	14.4 μM (-3.0)

[a] In ethanol solution. [b] In 1:1 ethanol/ethyl acetate solution, with serum-starved cells. [c] ND = not determined, due to insufficient sample. [d] For these analyses, the Hill slope of the toxicity curve was too steep to provide an exact value.

(prepared from *ent*-**8** following the same synthetic route) were evaluated for cytotoxicity in DU-145 (human prostate carcinoma) and HT-29 (human colon carcinoma) cell lines (Table 1).[17,18] We were pleased to observe that pyrrolidinediol compounds **25** and *ent*-**25** exhibited cytotoxicity against DU-145 cells in the same range as acyclic (2*S*,3*S*,5*S*)-**3**, and the compounds **25**, *ent*-**25**, **26**, and *ent*-**26** were uniformly more cytotoxic against HT-29 cells in comparison with D-*erythro*-sphingosine (**1**).[19] The range of biological activities among the various stereoisomers of cyclic structures **24**−**26** is relatively small, and thus further structure−activity studies on other cyclic aminodiols **24**−**26** as well as first-generation aminodiols **3** are warranted.

Acknowledgment. This research was supported by the National Institutes of Health, through the National Cooperative Drug Discovery Grant program U19 CA 87525 and R01 CA 57327 to D.C.P.

Supporting Information Available: Experimental procedures and characterization data for compounds **8**−**26**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL052839V

(11) For example, see: Briot, A.; Bujard, M.; Gouverneur, V.; Mioskowski, C. *Eur. J. Org. Chem.* **2002**, 139.

(12) (a) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H *J. Am. Chem. Soc.* **2000**, *122*, 8168. (b) Kinderman, S. S.; Doodeman, R.; van Beijma, J. W.; Russcher, J. C.; Tjen, K. C. M. F.; Kooistra, T. M.; Mohaselzadeh, H.; van Maarseveen, J. H.; Hiemstra, H.; Schoemaker, H. E.; Rutjes, F. P. J. T *Adv. Synth. Catal.* **2002**, *344*, 736. (c) The Grubbs second-generation catalyst $Cl_2(PCy_3)$(IMes)Ru═CHPh also provided metathesis products **17** and **18**, but a longer reaction time was required and it proved difficult to remove all traces of the Ru catalyst from the products.

(13) (a) Brown, H. C.; Vara Prasad, J. V. N.; Gupta, A. K. *J. Org. Chem.* **1986**, *51*, 4296. (b) Lutjens, H.; Knochel, P. *Tetrahedron: Asymmetry* **1994**, *5*, 1161.

(14) Other hydroboration reagents, such as 9-BBN, or hydrosilylation directed by the C5-alcohol gave no reaction and recovery of substrate. The silyl ether compounds **17** and **18** were either unreactive or gave unsatisfactory yields under all hydroboration conditions attempted.

(15) Still, W. C.; Barrish, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 2487.

(16) After our synthetic work was completed, we learned of another study involving hydroboration of dihydropyrrole-substituted with oxygenated substituents: Cren, S.; Wilson, C.; Thomas, N. R. *Org. Lett.* **2005**, *7*, 3521.

(17) DU-145 cell cytotoxicity assays were also conducted under serum-starved conditions (protocol *b*, Table 1) to minimize artifacts of protein binding, but results are also reported without serum deprivation (protocol *a*), since serum removal can affect sphingolipid metabolism: Colombaioni, L.; Frago, L. M.; Varela-Nieto, I.; Pesi, R.; Garcia-Gil, M. *Neurochem. Int.* **2002**, *40*, 327.

(18) Compounds **24**−**26** exhibited poor solubility in ethanol solvent vehicles, therefore experiments were conducted in a 1:1 ethanol/ethyl acetate mixture as well as in ethanol. Control experiments indicated that addition of ethyl acetate was neither toxic to nor inhibited the growth of DU-145 cells.

(19) *N*-Cbz compounds **21**−**23** and *ent*-**21**−**23** were also evaluated, as well as *N*-acetyl derivatives of **24**−**26** and *ent*-**24**−**26**, and were generally less potently cytotoxic than the cyclic amines **24**−**26** and enantiomers.