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Synthesis of 1-deoxysphingosine derivatives with conformationally restricted pyrrolidinediol head groups

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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 analog 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.

Sphingolipids 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 bases³ and structural analogs, 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 ten-fold lower than for sphingosine (1), and up to fifty-fold more potent than the corresponding *N*-acylated ceramide 2 in HT29 and

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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>.

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-deoxyanalogs **3** prevents phosphorylation, and by moving the hydroxyl group to the 5-position, lipophilicity of **3** is similar to sphingosine (**1**). In order to minimize *N*-acylation activity, we have further modified the 1-deoxysphingoid lead structure **3** to a cyclic pyrrolidine-diol **4**, which also provides conformational 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 pyrrolidine-diol analog **4** could be prepared from the functionalized dihydropyrrole **5**, which in turn would arise from ring-closing metathesis⁵ of the diallylamine **6** (Figure 2).

We initially planned to prepare 2-amino-3-butene from the amino acid L-alanine, but racemization occurred under all conditions attempted via *alpha*-aminoaldehyde intermediates.⁶ Therefore, enantioselective synthesis of 2-amino-3-butene was accomplished by borohydride reduction of the chiral sulfinamide⁷ 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 cleavage⁹ of the chiral auxiliary and Cbz-protection of nitrogen provided compound **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 elimination¹⁰ 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 non-racemic synthon **14** for carbon-nitrogen coupling. The enantiomer of **14** was likewise prepared beginning with epoxidation of **11** with d-DIPT.

Reaction of carbamate **9** with sodium hydride¹¹ and *N*-alkylation of each enantiomer of allylic bromide **14** provided the dienes **15** and **16** (Scheme 3), which each 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 hydroboration¹³ 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 expected, diastereomer **19** produced a single pyrrolidine-diol diastereomer **21**, arising from stereinduction 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 stereinduction from C2 and C5 chiral allylic positions.¹⁶

After hydrogenolysis of the *N*-Cbz protective group, pyrrolidine-diol stereoisomers **24** – **26** and their enantiomers (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 pyrrolidine-diol 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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 potent cytotoxic than the cyclic amines **24** - **26** and enantiomers.

Figure 1. Structures of Sphingosine (1), Ceramide (2), and 1-Deoxyanalogs 3 and 4

Figure 2. Retrosynthesis for cyclic 1-deoxysphinganine

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

Scheme 2. Stereoselective synthesis of allylic bromide (14)

Scheme 3. Coupling of **9** and **14**, and ring-closing metathesis to diastereomeric dihydropyrrolidines **17** - **18**

Scheme 4. Stereoselectivity of hydroboration of **19** vs. **20**, and completion of target compound syntheses

Table 1
Biological evaluation of pyrrolidine-diol analogs 24 - 26 and *ent*-24 - 26

controls (ref. 4)		cytotoxicity, EC ₅₀ (Hill slope)
(2 <i>S</i> ,3 <i>R</i>)-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 <i>S</i> ,3 <i>S</i> ,5 <i>S</i>)- 3		<i>ent</i> - 24	<i>ent</i> - 25	<i>ent</i> - 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.