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STUDIES ON MORPHOLINOSPHINGOLIPIDS: POTENT INHIBITORS OF GLUCOSYLCERAMIDE SYNTHASE

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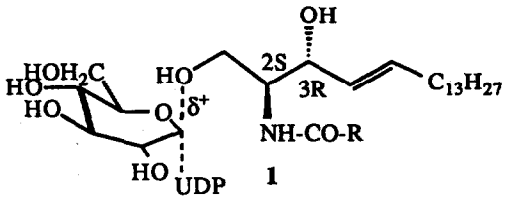
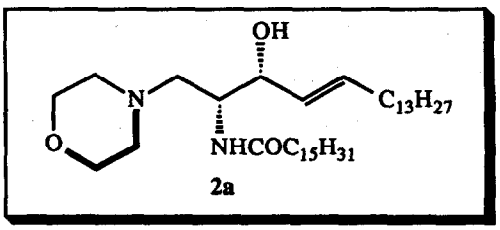
Abstract: Synthetic 1-morpholino-1-deoxyceramides were designed to inhibit glucosylceramide synthase. The most potent inhibitor **2a** possesses the unnatural R,R-configuration of D-threo-sphingosine.

Eucaryotic glycosphingolipids (GSLs) affect membrane physical properties, cell-cell and cell-matrix interactions, adhesiveness, cellular immune responses, and differentiation.[1] GSLs have also been implicated in cancer cell metabolism.[2] For example, malignant cells display marked abnormalities in their relative proportions of glycolipids,[3] and produce GSLs with novel linkage and sugar specificities.[4] Tumor cells also synthesize and shed excessive levels of GSLs which suppress lymphocyte responses in the host's immune system.



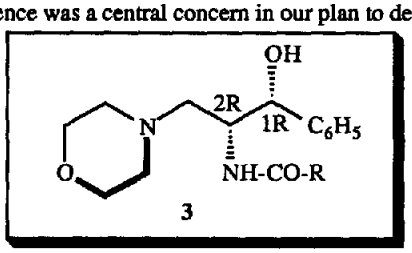
1



2a

GSL biosynthesis begins with the coupling of UDP-glucose to C1 of an N-acylsphingosine (ceramide). The reaction is catalyzed by glucosylceramide synthase (GlcCer synthase; ceramide:UDP-glucose glucosyltransferase; EC 2.4.1.80), probably via a transition structure like **1**.[5] The enzyme plays a pivotal role in GSL biosynthesis and represents a promising cancer chemotherapy target, since inhibitors can retard or arrest tumor growth.[6] Here we report studies defining 1-morpholino-1-deoxyceramides such as **2a** as potent GlcCer synthase inhibitors.

Inhibitor design was based on the observation that D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) **3** is an active competitive inhibitor of GlcCer synthase.[7] Presuming that the morpholine ring mimics the cationic charge of the GlcCer synthase transition state **1**, the *syn*-stereochemistry in **3** suggests that the bioactive (1R,2R)-PDMP stereoisomer is at variance with the D-*erythro*-or *anti*-configuration of naturally-occurring GSLs shown in (2S,3R)-1. This stereochemical difference was a central concern in our plan to design more potent, sphingosine-based GlcCer synthase inhibitors containing glucopyranose analogs having ring conformations related to **1**.[8] To probe this stereochemical issue, we developed syntheses of all four isomeric 1-morpholino-1-deoxyceramides **2a-d**, as shown in the Scheme.



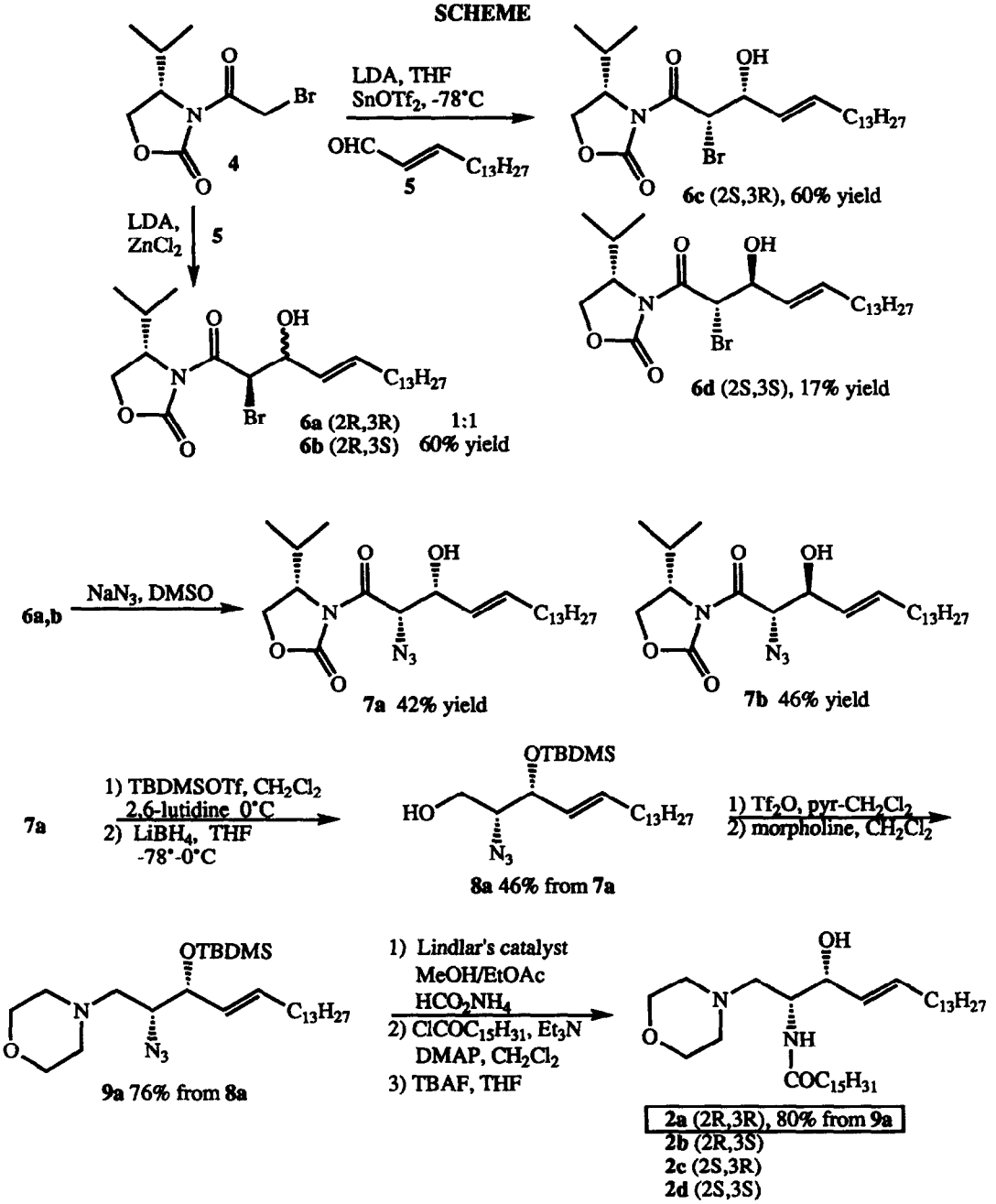
3

Our synthetic approach was based on the method of Evans *et al.* for the enantioselective aldol condensation of oxazolidinone **4** with unsaturated aldehyde **5**.[9] According to Abdel-Magid *et al.*,[10] the observed stereochemistry of condensation is influenced by chelation to the oxazolidinone oxygen in the transition state. In fact, condensation of **5** with the tin(II) enolate of **4** (prepared as shown in the Scheme) gave mostly **6c**, whereas the Zn(II) enolate of **4** gave an inseparable mixture of **6a** and **6b**. Both reactions gave minor amounts of the (2S,3S) isomer **6d**, which could be separated by flash column chromatography.

The mixture of bromohydrins **6a** and **6b** could not be resolved, but was separable after conversion to the corresponding azides **7a** and **7b**, following the procedure of Nicolaou *et al.*[11] Alcohol **7a** was then protected as its *t*-butyldimethylsilyl ether, and the chiral auxiliary was reductively removed to afford sphingol ether **8a**. The primary alcohol in **8a** was next activated as its triflate, and displacement with morpholine afforded aminoether **9a**. Reduction of the azide group in **9a** was effected by transfer hydrogenation using ammonium formate as the hydrogen source. Subsequent N-acylation and desilylation led to the final product **2a** in good overall yield.[12] This consitutes the first use of metal-dependent aldol condensations in sphingosine synthesis, and the first synthesis of 1-azaceramides. Diastereomers **2b-d** were synthesized in like fashion from aldol adducts **6b-d**.

Morpholinoceramides **2a-d** were evaluated as inhibitors of GlcCer synthase from Madin-Darby canine kidney cell homogenates, using octanoyl sphingosine as glucose acceptor (thermostatted ultrasonic bath, triplicate assays).[13] Diastereomer **2a** was clearly the most powerful inhibitor of GlcCer synthase (73% inhibition at 5μM), and was significantly more potent than PDMP **3** (16-20% inhibition at 5μM) or isomers **2b-d** (5-20% at 5μM).

SCHEME


LDA, THF
SnOTf2, -78°C
4
5
6c (2S,3R), 60% yield
LDA,
ZnCl2
5
6d (2S,3S), 17% yield
6a (2R,3R) 1:1
6b (2R,3S) 60% yield
6a,b
NaN3, DMSO
7a 42% yield
7b 46% yield
7a
1) TBDMSOTf, CH2Cl2
2,6-lutidine 0°C
2) LiBH4, THF
-78°-0°C
8a 46% from 7a
1) Tf2O, pyr-CH2Cl2
2) morpholine, CH2Cl2
9a 76% from 8a
1) Lindlar's catalyst
MeOH/EtOAc
HCO2NH4
2) ClCOC15H31, Et3N
DMAP, CH2Cl2
3) TBAF, THF
2a (2R,3R), 80% from 9a
2b (2R,3S)
2c (2S,3R)
2d (2S,3S)

These findings set the stage for the refinement of GlcCer synthase transition structure mimics, including bi-substrate analogs and other active inhibitors based on the corresponding glucoamidrazone and glucoamidoxime derivatives[8] of sphingosine.

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