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Synthesis and characterization of some atypical sphingoid bases

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ABSTRACT

Sphingolipids are ubiquitous and abundant components of all eukaryotic and some prokaryotic organisms. Sphingolipids show a large structural variety not only between the different species, but also within an individual cell. This variety is not limited to alterations in the polar headgroups of e.g. glycosphingolipids, but also affects the lipophilic anchors comprised of different fatty acids on the one hand and different sphingoid bases on the other hand. The structural variations within different sphingoid bases e.g. in pathogens can be used to identify novel biomarkers and drug targets and the specific change in the profile of common and uncommon sphingolipids are associated with pathological conditions like diabetes or cancer. Therefore, the emerging field of sphingolipidomics is dedicated to collect data on the sphingolipidome of a cell and hence to assign changes therein to certain states of a cell or to pathological conditions. This powerful tool however is still limited by the availability of structural information about the individual lipid species as well as by the availability of appropriate internal standards for quantification. Herein we describe the synthesis of a variety of 1-deoxy-sphingoid bases. 1-DeoxySphingolipids have recently acquired significant attention due to its pathological role in the rare inherited neuropathy, HSAN1 but also as predictive biomarkers in diabetes type II. Some of the compounds synthesized and characterized herein, have been used and will be used to elucidate the correct structure of these disease-related lipids and their metabolites.

1. Introduction

Sphingolipids are important components of mammalian cells. They are defined by a sphingoid base, usually a C-18 2-amino-1,3-diol, that is mostly *N*-acylated to form ceramide, which in turn serves as a lipid anchor for a variety of polar head groups.[1] Such polar head groups include phosphoryl choline (forming sphingomyelins) or a variety of several hundred mono- and oligosaccharides.[2,3] The latter species are grouped as glycosphingolipids (GSL) and contribute to the cell's glycocalyx and thus cell-cell and cell-ligand interactions.[1] For example, GSL determine the blood groups of the A, B, 0 system.[4] The acidic GSL, the ganglioside GM1, is used by cholera toxin to enter target cells[5] and the ganglioside GM3 modulates the activity of the insulin receptor.[6] While the variety of both, the sphingolipid head groups and the acyl chains have been subject to intense research in the past, the existence of structural variations in the sphingoid base has been much less appreciated so far.[7] Indeed, most organisms contain a predominant sphingoid base, like the "canonical" sphingosine (2*S*,3*R*,4*E*-2-aminooctadec-4-ene-1,3-diol) in mammals or phytosphingosine in plants and fungi.[8] Typically, the first and rate limiting step of sphingolipid biosynthesis is the condensation of serine and palmitoyl-CoA to form 3-Ketosphinganine, which is catalyzed by the enzyme serine-palmitoyl-CoA transferase (SPT), which is conserved in all eukaryotes examined so far.[8] 3-Ketosphinganine is then reduced to sphinganine (also termed "dihydrosphingosine") followed by *N*-acylation and desaturation at the Δ4 position. In mammalian cells, this desaturation occurs always after *N*-acylation, meaning that sphingosine (SO) is not an intermediate of *de novo* biosynthesis, but rather of the catabolism of complex sphingolipids.[9] SO itself can be again re-acylated to ceramide or terminally degraded. Variations to the sphingoid base are mostly introduced downstream of the SPT reaction. Interestingly, studies in pathogenic fungi revealed the abundance of unusual sphingoid bases, which derive from individual biosynthetic pathways, formed by specialized enzymes that might provide interesting pharmacological targets for antifungal therapies.[10,11] Additional examples for atypical sphingoid bases are 1-deoxy-sphingoid bases spisolusine or the xestoaminols, which are formed by the use of alanine over serine and which have been originally isolated from a variety of marine animals. 1-Deoxy-sphingoid bases showed pronounced cytotoxic properties to certain cancer cell lines and were tested as an experimental cancer therapy.[12] Later it was

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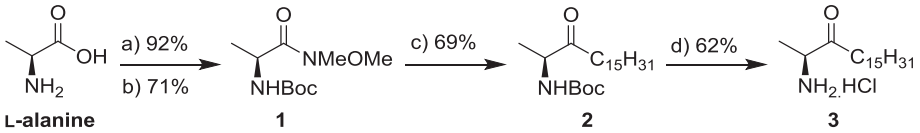


Scheme 1. Synthesis of 3-keto-1-deoxysphinganine analogue. Reagents and conditions: (a) Boc_2O, 1 M NaOH, dioxane, −10 °C to r.t., 12 h. (b) *N,O*-dimethylhydroxylamine HCl, EDCI·HCl, DCM, −15 °C, 4 h. (c) (i) Mg, 1,2-DBE (drops), 1-bromopentadecane, Et_2O, reflux, 2 h, and then (ii) **1**, DCM:Et_2O; 0 °C-rt, 3 h. (d) AcCl, MeOH, 0 °C, 2 h.

demonstrated that pathologically elevated 1-deoxysphingolipids cause the rare inherited neuropathy HSAN1 which has been attributed to several mutations in the SPT genes. These mutations induce a permanent shift in the substrate affinity of SPT leading to an increased utilization of alanine over its canonical substrate serine.[13] In a recent study, a branched 1-deoxysphinganine was synthesized from 2-carboxylaziridine and it was shown that this analogue of *C. elegans* sphingolipids did not rescue a genetic sphingoid base depletion.[14] In addition, 1-deoxysphingolipids are also elevated in type 2 diabetes mellitus and might thereby also involved in the frequent diabetic polyneuropathy.[15,16] Although the exact molecular mechanism for 1-deoxysphingolipid formation in diabetes is still unknown. 1-Deoxysphingolipids lacks the primary hydroxyl group of canonical sphingoid bases which prevents the attachment of a polar head group and the formation of complex sphingolipids. As a consequence, 1-deoxysphinglipids cannot be degraded over the canonical pathway as the catabolic intermediate sphingosine-1-phosphate (S1P) cannot be formed. Instead, 1-deoxysphingolipids seem to follow different metabolic routes. Recent studies showed that the LC retention time of a synthetic 1-deoxysphingosine standard bearing the canonical Δ4-5 trans double bond did not match with the naturally occurring 1-deoxysphingosine. However, both lipids were isobaric, suggesting an altered double bond position in 1-deoxysphingosine compared to its canonical counterparts.[17] By comparing the retention time of natural 1-deoxysphingosine with those of synthetic standard compounds with incrementally shifted double bond position, we observed a step-wise approximation of retention times and finally identified the structure of deoxysphingosine as 2*S*,3*R*,14*Z*-2-aminooctadec-14-ene-3-ol. This structure was further confirmed by MS fragmentation studies on dimethyl disulfide derivatized 1-deoxysphingosine and ozonization-MS.[17]

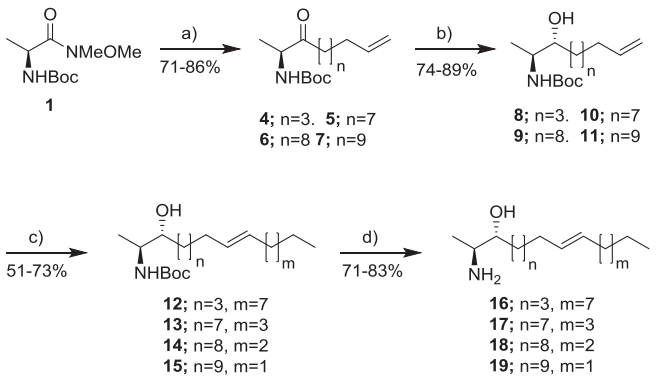
In the present report, we describe the concise and efficient synthesis of a set of deoxysphingosine derivatives, which can be used as potential biomarkers for human disease and different pathogens. Most of these compounds either have been confirmed as naturally-occurring metabolites or are currently under further investigation.

2. Results and discussion

The canonical sphingosine is synthesized from an activated fatty acid and L-serine. In seminal works, S. Garner and coworkers synthesized the protected serinal derivative known as “Garner’s aldehyde”,[18] which can be nucleophilically attacked by metallated terminal alkynes to form the respective substituted propargyl alcohols.[19] Partial hydrogenation of the latter to the corresponding *E*-alkenes yields the protected sphingosines in good yields. The diasteroselectivity of the nucleophilic addition with concomitant hydroxyl group formation was later improved by the use of HMPA and other auxiliaries.[20] An alternative strategy to synthesize the canonical sphingosine has been developed by Yamamoto *et al.*[21] The group reacted the Weinreb amide of protected L-serine with vinyl magnesium bromide to form the respective α,β unsaturated ketone. The latter was then stereospecifically reduced to the corresponding substituted allyl alcohol, followed by olefin metathesis. The great advantage of this approach is the free choice of distal residues from one common precursor molecule. Therefore, this approach has been widely used since then to synthesize sphingolipid derivatives modified in the sphingosine portion.[22–24] Following the same rationale, the synthesis of a few deoxysphingosine derivates starting from L-alanine instead of L-serine have been previously reported.[25–27] Indeed, in the present manuscript, all deoxysphingolipids were derived from the common precursor **1**, respectively. Analogously, the deoxymethylsphingosine derivative **50** was derived from the respective glycine derivative **47**.

In the course of our investigations, we envisioned to synthesize the most important intermediates and products of the deoxysphingolipid metabolism in humans. Therefore, we first synthesized 3-keto-1-deoxysphinganine **3**, the direct product of the SPT reaction by reaction of the precursor **1** with *in situ* generated pentadecane magnesium bromide, followed by acid-mediated deprotection (Scheme 1).

During our biochemical investigations, we noticed that the HPLC retention time of the orthopthalimide (OPA) derivative of deoxysphingosine taken from patient cells was slightly different from the retention time of the commercial 4*E*-deoxysphingosine.[17] Since the *m/z* values were identical, we reasoned that the position of the double bond in “natural” deoxysphingosine from patients might be shifted. Noteworthy, during sphingolipid biosynthesis, the double bond is introduced only after *N*-acylation of the saturated sphinganine (equal to dihydrosphingosine) to form ceramide, which then can be hydrolysed again to form sphingosine. Therefore, an altered binding of *N*-acyl-deoxysphinganine to the respective desaturase could result in a shifted position of the double bond. To follow this hypothesis, we aimed at synthesizing deoxysphingosine isomers differing in the position of the double bond. Towards this goal, **1** was reacted with different ω-alkenyl Grignard reagents, which were freshly prepared from the respective alkenyl bromides (Scheme 2). The resulting ω-desaturated α-amino ketones were subjected to reduction with TBLAH. The bulky *N*-Boc substituent at the pre-defined stereo centre allows stereo-selective reduction of resulting α-aminoketones to the desired D-*erythro* configured 2-amino-3-hydroxy motif. Next, the latter intermediates were reacted with terminal olefins of different length in a metathesis reaction to yield the desired C-18 deoxysphingosine isomers **16–19** after acid-mediated deprotection (Scheme 2). Very recently, this set of deoxysphingosine isomers was used to systematically explore the strengths and limitations of differential mobility spectrometry (DMS) and other key techniques for mass spectrometry based lipidomics, for differentiation of subtle structural changes between different lipid isomers.[28]



Scheme 2. Synthesis of novel *E*-1-deoxysphingosine analogues. Reagents and conditions: (a) (i) Mg, 1,2-DBE (drops), 1-bromo-alkene, Et_2O, reflux, 2 h, and then (ii) **1**, MeMgBr, DCM:Et_2O, 0 °C-rt, 3–4 h. (b) TBLAH, EtOH, −78 °C, 2–3 h, (*anti*:*syn* 4:1–9:1). (c) Cross partner alkene, *p*-benzoquinone (10 mol%), Grubbs 2nd generation cat., deuterated-chloroform, reflux, 4–12 h. (d) 4 M HCl-Dioxane, 0 °C-rt, 1–2 h.