An Improved, Versatile, and Easily Scalable Synthesis of Sphingomyelins: Application to Stable Isotope Labeling

Nicolas Philippe* 0000-0003-0162-1645
Serge Pérand*
Franck Le Strat
Jörg Blankenstein
Sébastien Roy
Sanofi-Aventis R & D, Integrated Drug Discovery, Isotope Chemistry Department, 13 quai Jules Guesde, 94403 Vitry-sur-Seine, France
nicolas.philippe-ICMS@sanofi.com
serge.perard@sanofi.com

Abstract
With a view to make conveniently labeled mass spectrometry standards available, a set of deuterated sphingomyelins were prepared by a new expedient, flexible, robust, scalable, and high-yielding synthetic scheme starting from 2-azido-3-O-benzoylsphingosine as the key intermediate. Unlike previously published procedures, this work emphasizes the benefit arising from the choice of the azido function as a masking group for the reactive primary amine during the troublesome, though crucial, phosphorylation step.

Key words sphingomyelin, sphingosine, stable isotope, deuterium, mass spectrometry standard, chemical synthesis

Sphingomyelins are the main components of mammalian sphingolipids. Indeed, they are found in all cell membranes and especially in the central nervous system at the level of myelin sheath. These complex sphingolipids are made up of both hydrophobic and hydrophilic groups. The nonpolar part comprises the sphingoid base, which is amidified by a fatty acid whereas the polar head consists of a phosphocholine moiety (Figure 1). This amphipathic arrangement allows sphingomyelins to play also a role in various cellular events such as signal transduction and apoptosis.1 Furthermore, an anomaly in sphingomyelin metabolism triggers severe illnesses such as the rare hereditary Niemann–Pick disease.2,3 This well-known example of lysosome storage disorder (LSD) is the result of a deficiency in the hydrolytic enzyme acid sphingomyelinase (ASM), leading to the accumulation of sphingomyelin in vital organs, including brain, and inflicting, among others, irreversible neurological damages.

Therefore, it is easy to understand that specific quantification of sphingomyelins in biological materials is of paramount importance for any pharmacological study. The use of stable isotope labeled (SIL) standards has been recognized as the method of choice for this purpose.4 Ideally, SIL standards should hold the very structure of the molecule to be analyzed by mass spectrometry (LC-MS/MS). The synthesis of these isotopologues has been a major concern for the pharmaceutical industry and was highlighted recently.5 Indeed, the commercial availability of these standards is rather scarce and several strategies have been developed to allow a realistic access to the target molecules.6,7

SIL sphingomyelins can be prepared using conventional organic synthesis. To the best of our knowledge, only a few preparations of deuterated sphingomyelins were spreaded out over the literature. Byun and Bittman8 described the total synthesis of 3-deuterio-D-erythro-sphingomyelin, an isotopomer failing to achieve the degree of labeling generally requested by bioanalysis scientists. Bartels et al.9 very succinctly (neither analytical data nor yield given) mentioned the preparation of N-perdeuteropalmitoyl-D-erythro-sphingomyelin (d31) by acylation of the corresponding amine (lysosphingomyelin). Matsumori et al. claimed the total synthesis of many kinds of deuterated (d to d3) site-
specific labeled stearoylsphingomyelins\textsuperscript{10} and 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholines\textsuperscript{11} specifically designed for \textsuperscript{2}H NMR biophysical studies. Mehnert et al.\textsuperscript{12} touched upon the preparation of three analogous derivatives (C2-d2-palmitoylsphingomyelin, C3-d2-palmitoylsphingomyelin and d31-palmitoylsphingomyelin). Finally, Cui et al.\textsuperscript{13} were brought to prepare 2-(d9-trimethylammonio)ethylphosphate-stearyl-D-erythro-sphingomyelin, as a probe for lipid rafts Raman imaging, by nucleophilic substitution of a 2-bromoethylphosphate-sphingosine precursor.

We now describe a refined, efficient, and more versatile methodology to provide an easy access to any labeled or unlabeled sphingomyelin variant, starting from 2-azido-3-O-benzoylsphingosine (1) and fatty acids, deuterated where needed.

This key intermediate 1 was synthesized in 8 steps from D-arabitol based on the method of Demchenko.\textsuperscript{14} After optimization of the experimental conditions to secure the overall yield and isomeric purity, this compound was obtained at several tens of gram scale.

Our first tested access to labeled sphingomyelin consisted in synthesizing first the ceramide, composed of sphingosine and a labeled fatty acid, before introducing the phosphocholine moiety. The ceramide was obtained in three steps from 1, after deprotection of the secondary alcohol, reduction of the azido group and N-acylation\textsuperscript{15} in good yield.

According to the literature,\textsuperscript{16} sphingomyelin could be obtained in moderate to good yield from ceramides displaying either a free or protected secondary alcohol function by using different phosphorylation reagents such as 2-chloro-2-oxo-1,3,2-dioxaphospholane or 2-chloro-1,3,2-dioxaphospholane. Unfortunately, our attempts to introduce the phosphocholine moiety under these conditions were unsuccessful as we faced deplorable low (at best 10%) and inconsistent yields.

Another access to sphingomyelin consists first in performing the delicate step, that is, the primary alcohol phosphorylation, leading to lyso-sphingomyelin before adding the fatty acid chain. In the literature, sphingosin's phosphorylation required to protect the amine as N-Boc derivatives,\textsuperscript{8,10,11b,13,17} whereas the secondary alcohol function could be left free. However, this strategy suffered the disadvantage of involving additional synthetic steps.

Nevertheless, Cairo et al.\textsuperscript{18} demonstrated that the phosphorylation step can be carried out with an azide acting as a protected amino group, enabling them to synthesize sphingomyelin analogues with modified choryl headgroups. The latter were obtained using modified phosphocholines as reagents. We choose to adapt this strategy to finally get

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
Scheme 1 & \\
\hline
\end{tabular}
\end{table}
sphingomyelin itself in only three steps featuring a short, versatile, scalable and robust synthetic pathway as depicted in Scheme 1.

The phosphocholine moiety was introduced via a one-pot reaction by reacting 1 and 2-chloro-2-oxo-1,3,2-dioxaphospholane in acetonitrile overnight following by addition of trimethylamine in THF at 50 °C for 2 days to give the azidophosphodiester 3 in 79% yield (Scheme 1). For this reaction, as the dioxaphospholane intermediate 2 is very sensitive to moisture,

1 it was important to work under an inert atmosphere with carefully dried solvents and compounds to ensure an excellent reproducibility. The O-benzoyl derivative 3 was deprotected by sodium methoxide in methanol to give the secondary alcohol 4 nearly quantitatively. The azide 4 was reduced with propanedithiol in the presence of trimethylamine in methanol to afford the lyso-sphingomyelin 5 in 73% yield. In the last step, the amine 5 was reacted with labeled p-nitrophenyl palmitate 6 or linoleate 7 in pyridine to provide the corresponding sphingomyelins 8 and 9 in greater than 70% yield after crystallization (Scheme 1).

The relative configuration (erythro or threo) of sphingolipids 8 and 9 can be most conveniently deduced from their 1H NMR spectra, on the basis of data published by Bruzik. 


© 2020. Thieme. All rights reserved. Synthesis 2020, 52, A–E
CHCl3/MeOH/H2O, 70:30:4). After cooling to rt, the mixture was concentrated under vacuum. The crude product was purified by flash chromatography on silica gel (first elution with CHCl3/MeOH/H2O, 90:10:1 then with CHCl3/MeOH/H2O, 70:30:4) to afford 3 as a white amorphous solid; yield: 1.1 g (79%); Rf = 0.3 (SiO2, CHCl3/MeOH/H2O, 70:30:4).

1H NMR (500 MHz, CDCl3 + CD3OD 50:50): δ = 5.91 (dt, J = 15.0, 6.9 Hz, 1 H), 5.63 (ddt, J = 15.3, 7.6, 1.4 Hz, 1H), 4.42 (br m, 2 H), 4.12 (m, 3 H), 3.77 (br m, 2 H), 3.37 (s, 9 H), 2.95 (br m, 2 H), 2.23 (qdd, J = 6.9, 1.1 Hz, 2 H), 1.56 (m, 2 H), 1.51–1.38 (br m, 20 H), 1.04 (t, J = 6.8 Hz, 3 H).

13C NMR (125 MHz, CDCl3 + CD3OD 50:50): δ = 137.01, 131.79, 75.38, 68.69 (d, J = 5.8 Hz), 68.51 (m), 61.09 (d, J = 4.9 Hz), 57.77, 55.94, 55.91, 55.88, 34.44, 33.95, 31.69, 31.66, 31.65, 31.54, 31.37, 31.34, 31.28, 24.66, 15.73.


4-Nitrophenyl [13,13,14,14,15,15,16,16-2H9]Hexadecanoate (6)

300 mg, 1.1 mmol) in CHCl3 (20 mL) was treated with 1,3-propanedithiol (4.0 mL, 39.5 mmol) and Et3N (7.5 mL, 53.5 mmol) under argon and the mixture was stirred at rt for 3 days. The reaction was monitored by TLC (SiO2, CHCl3/MeOH/H2O, 70:30:4) and after completion, the mixture was concentrated under vacuum. The residue purified by flash chromatography using a silica gel column (elution successively with CHCl3/MeOH/H2O, 90:10:1, 70:30:4, 50:50:4, then with MeOH/H2O, 100:4) to afford 5 as a white solid; yield: 540 mg (73%).

© 2020. Thieme. All rights reserved. Synthesis 2020, 52, A–E

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.
CHCl₃/MeOH/H₂O, 90:10:1, then with CHCl₃/MeOH/H₂O, 70:30:4. The compound was dissolved in a minimum amount of a CHCl₃/MeOH mixture, then crystallized by addition of acetone to give 8 as a white solid; yield: 270 mg (69%); mp 211 °C; IR (ATR): 3283 (br), 2916, 2849, 1644, 1547, 1478, 1378, 1226, 1085, 904, 828, 875, 753, 720, 504 cm⁻¹ (br).

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0039-1690863.

References