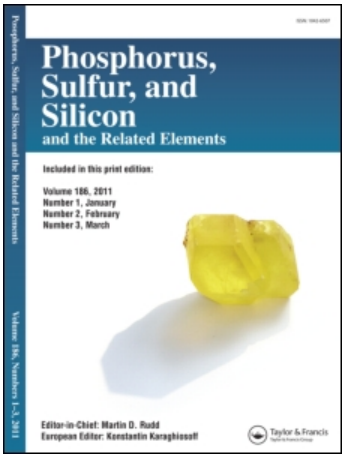

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FROM OLIGONUCLEOTIDES TO PHOSPHOLIPIDS: CROSS-FERTILIZATION THROUGH COMPLEMENTARY CHEMISTRY

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With expanded research efforts targeted towards developing gene therapies, oligonucleotide chemistry is enjoying a parallel renaissance. Oligonucleotides and their congeners are utilized as complementary sequence-specific strands in DNA and/or RNA hybridization techniques and, therefore, can also be utilized in vivo to modify the course of various oncological, viral, parasitic, and genetic diseases. This paper illustrates how advances in oligonucleotide chemistry have been applied to the seemingly unrelated area of phospholipid chemistry. A specific example to be addressed is the synthesis of C-14-radiolabeled natural N-palmitoyl D-erythro-sphingomyelin using methods adopted from oligonucleotide chemistry. This and related compounds are valuable analytical tools for the investigation of Niemann-Pick disease, a sphingolipidosis characterized by an inherited sphingomyelinase deficiency. The cross-fertilization of these two chemistries and utilization of the resultant novel approaches in design and synthesis are demonstrated.

Sphingomyelin (N-acyl sphingosyl phosphorylcholine, SPM, is an important constituent of biological membranes and blood plasma lipoproteins. The stereochemical configuration of sphingosyl fragment of naturally occuring SPMs is D-*erythro* and usually 18 carbon atoms long. The chemical structure of sphingosine is defined as (2S, 3R, 4E)-2-amino-4-octadecen-1,3-diol. The precise composition of natural SPMs, which is a multi-compound mixture, depends on their biological source and usually varies in their N-acyl long chain residues.

Abnormalities in metabolism of SPMs is the cause of Niemann-Pick disease and has been associated with atherosclerosis and cancer. Recently, it was suggested that SPM plays an important role in cell regulation and intracellular signal transduction. Niemann-Pick disease (NPD) is a lysosomal storage disorder which result is caused by a profound deficiency of the enzyme sphongomyelinase that catalyzes the hydrolytic cleavage of sphingomyelin to ceramide and phosphocholine, which has a reported high incidence rate in the Ashkenazic Jewish population. Type A NPD is a fatal disorder of infancy characterized by failure to thrive, hepatosplenomegaly and a rapidly progressive neurodegenerative course that leads to death by 2 to 3 years of age. In contrast, type B NPD is a phenotypically variable disorder that is usually diagnosed in childhood by the presence of hepatosplenomegaly. Most type B patients have little or no neurologic involvement and survive into adulthood. Compared to the general population, Ashkenazic Jewish individuals have a higher incidence of type A NPD 1:120; the estimated carrier frequency for type A NPD in this population is about 1:60.

Although the biological significance of SPM has been increasingly recognized, it is well documented that the sphingomyelins isolated from natural sources are heterogeneous in regard to the N-acyl chain and the length of the sphingosyl backbone fragment. Although most of the

* This lecture is dedicated to the memory of the late Prof. David Shapiro, the pioneer of sphingolipid chemistry.

earlier biochemical studies have been performed with natural mixture of sphingomyelins, semi-synthetic **(1)** or fully synthetic racemic, (±) - erythro-sphingomyelin **(2)**, precise biophysical studies usually require highly pure homogeneous sphingomyelin. The evident shortcomings is in reproduceability of the biophysical parameters of natural and semi-synthetic sphingomyelins, since natural materials are a multicomponent mixture dependent on source and the semi-synthetic material is usually a not-well-defined mixture of enantiomers as a result of epimerisation during the hydrolytic deacylation. The fully synthetic racemic material not only has a 50% ballast of unnatural material, but also often negatively influences the biochemical measurements.

Therefore, it is obvious that there is a demand for an efficient synthetic procedure leading to enantiomerically pure, high quality, homogenous D-*erythro*-sphingomyelin, which could also be adapted to isotopic labeling of the sphingomyelins.

In order to precisely evaluation the specific activity of enzymes necessary for the treatment of patients with NPD, a highly pure radiolabeled substrate must be synthesized.

In 1986 -88, we were approached by several research groups interested in multigram quantities of highly pure homogenic synthetic sphingomyelins having natural D-erythro configuration and containing one of three fatty acid residues: palmitoyl (C16:0), stearoyl (C18:0) or lignoceryl (C24:0). At the same time, the National Institutes of Health was soliciting proposals for synthesis of ^{14}C labeled pure sphingolipids, including radiolabeled N-palmitoyl D-erythro-sphingomyelin.

The first synthetic sphingomyelin, although racemic, was elegantly prepared by D Shapiro and co-workers in the early sixties. This material was also synthesized in radioactive form. In the mid-80s, our lab, along with others (ref), synthesized pure D-*erythro* sphingosine and established a platform for preparation of synthetic enantiomerically pure sphingolipids.

As part of our program directed toward synthesis of ^{14}C labeled enantiomerically pure, homogeneous sphingomyelin, we prepared N-palmitoyl-3-*O*-t-butyldiphenylsilyl ceramide. Three sources of radioactive starting materials were identified : ^{14}C methyl iodide; ^{14}C trimethylamine hydrochloride; and ^{14}C-choline hydrochloride. . Basing ourselves on retrosynthetic analysis and literature data, several synthetic schemes were designed for the preparation of ^{14}C labeled *N*-palmitoyl sphingomyelin. The first procedure was an improved Shapiro method utilizing enantiomerically pure ceramide. This process was unsuitable for radioactive synthesis because of the high cost and low-yield of sphingomyelin, as well as the difficulties related to handling of the ^{14}C labeled trimethyl amine,

Our strong interest and involvement at that time in oligonucleotide synthesis for antisense/triplex applications in gene therapyled to the design of two schemes to prepare sphingomyelin from ceramide using the phosphoramidite technology. While this coupling approach could, in principle, be applied to sphingomyelin synthesis, it was considerably refined and optimized so that a 95% yield was achieved in each reaction step (3).

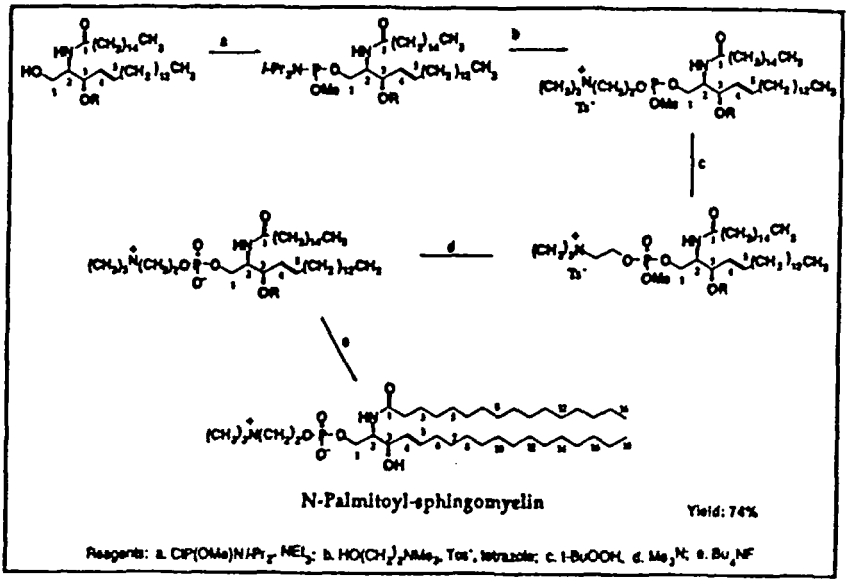
The first scheme utilized ceramide and dimethylaminoethanol as substrates for phosphoramidite synthesis, followed by methylation using methyl iodide to achieve sphongomyeline.This methodology was expected to enable ^{14}C labeling by using radioactive methyl iodide in the methylation step. To our surprise, the usually quantitative phosphoramidite coupling reaction gaave several products and an isolated yeild of about 55%. The methylation procedure required an excess of methyl iodide; and, based on the cost and difficulties in purification, this synthesis was not feasible for preparation of radiolabeled material.

The second scheme utilized choline tosylate (which can be obtained in radioactive form from commercially available choline hydrochloride) as a second component of the phosphoramidite synthesis.

In parallel to our investigations, two papers appeared in the literature using the same concept for synthesis of sphongomyelin (4). Reproduction and optimization of Bruzik's synthetic scheme allowed us to prepare ^{14}C labeled sphongomyelin in 79% yeild starting from ceramide.

The synthesis of *D-erythro*-SPM and its analogues is outlined in the Scheme below.

^{14}C Labeled Sphingomyelin



D-*erythro*-3-*O*-(diphenyl-t-butylsilyt)-2-*N*-stearoylsphingosine was separately treated with chloro-*N,N*-di-isopropylamino-methoxy-phosphine in the presence of triethylamine in chloroform. The resulting pohsphoramidite was treated with a mixture of choline tosylate and tetrazone in the acetonitrile-THF. The phosphite obtained in this way was oxidized with t-butyl hydroperoxide in THF to give the corresponding phosphate. The desired phosphodiester was obtained by dimethylation of its respective triester with anydrous trimethylamine in toluene. The final product, D-*erythro* \SPM was formed in the desilylation reaction of 3-*O*-silyl-protected derivative with tetrabutylammonium fluoride and yielded the desired sphingomyelin in 79 percent. This one-pot procedure did not involve isolation of the intermediate products and was therefore adopted as an efficient radiolabeling approach.

Subsequently two facile synthetic approaches to sphingomyelin were developed, one using 2-chloro-2-oxo-1,3,2-dioxaphospholane and trimethylamine (5) and the other involving $POCl_3$ and choline tosylate (6).

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