diethyl ether (3 × 5 ml). The diethyl ether extracts are combined and dried over anhydrous sodium sulfate, the solvent is evaporated under a stream of dry nitrogen, and the dry material is dissolved in 500 μl of dry DMF followed by the addition of dicyclohexylcarbodiimide (37 μmol in 100 μl DMF) and N-didroxsuccinimide (37 μmol in 100 μl DMF). The reaction is allowed to proceed for 2 days at room temperature in the dark with continual stirring. The insoluble N,N-dicyclohexylurea is sedimented by centrifugation, and analysis of the supernatant using TLC-1 in solvent F shows formation of the NHS ester and the presence of the unreacted hexanoic acid in the proportion ~9:1. The NHS ester of hexanoic acid is purified by TLC-2 in solvent F (NHS ester: Rf = 0.8, hexanoic acid: Rf = 0.4). The area corresponding to the product is scraped from the plate and eluted quantitatively with chloroform–methanol (1:1, v/v) to provide the NHS ester of [14C]hexanoic acid with an 80% yield.

Practical Synthesis of N-Palmitoylsphingomyelin and N-Palmitoyldihydrosphingomyelin

By Anatoliy S. Bushnev and Dennis C. Liotta

Introduction

Sphingolipids play a significant role in the structural organization of biological membranes, and numerous intra- and intercellular processes are dependent on their metabolism and catabolism. Over the last decade, sphingolipids and their metabolites have generated a great deal of interest due to the discovery of the sphingomyelin cycle\(^1\) in which ceramide plays an important role in the regulation of cell growth, differentiation, and cell death. Sphingolipids have also been implicated in the development of general types of tumors, and there are several reports that the dietary sphingolipids can affect and even reverse the development of carcinogenesis.\(^2\)

The success of these and many other investigations depends on the accessibility of sphingolipids, which are used as substrates. Many kinds of sphingolipids can be isolated easily from natural sources; however, these compounds are not chemically homogeneous with respect to sphingosine bases or fatty acids. Furthermore, it is clear that the heterogeneity of

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these moieties affects both physicochemical characteristics and biological functions of the sphingolipids. The semisynthetic preparation of sphingomyelins and other sphingolipids through their lyso derivatives provides a way to obtain compounds in which the fatty acid is homogeneous, while the sphingosine base composition remains nonhomogeneous. Prepared by this method, substrates may be contaminated with the \textit{threo} isomers due to epimerization at the C-3-atom in the process of acidic deacylation of the natural materials.\textsuperscript{3} Moreover, commercial sphingolipids can be contaminated with other biologically active compounds, which can exhibit the same or different activity.\textsuperscript{4}

Total chemical synthesis allows the preparation of individual homogeneous natural compounds with a completely defined structure in any desirable quantities for biophysical and biochemical studies. Furthermore, chemically prepared substrates with variations in a core structure may be used for elucidating variations in the structure-dependent biochemical and biophysical properties. With this in mind, we undertook the preparative total syntheses of N-palmitoylsphingomyelin (1a) and N-palmitoyldihydrosphingomyelin (1b):

![Chemical structure of 1a and 1b](image)

1a: $X \cdot X = \text{trans-CH} = \text{CH}$
1b: $X \cdot X = \text{CH}_2\text{CH}_2$

Problems Related to Sphingomyelin Synthesis

The total chemical synthesis of sphingomyelins consists of two main stages: (1) the synthesis of 3-O-protected ceramides and (2) the introduction of the phosphocholine moiety into 3-O-protected ceramides, followed if necessary by removal of the protecting group(s) from the ceramide counterpart and phosphocholine residue. Buyn \textit{et al.}\textsuperscript{5} utilized unprotected N-octanoylceramide in the synthesis of the short chain N-octanoylsphingomyelin, but this approach could not be employed in the syntheses of long chain sphingomyelins due to limited solubility of the corresponding ceramides.

To date, numerous sphingomyelin syntheses have been reported, and modern approaches employ enantioselective methods for the preparation of sphingoid bases (3-O-protected ceramides)\(^6\)–\(^{11}\) and the highly efficient phosphoramidite (or phosphitetriester) technique of phosphorylation.\(^5\)–\(^7\)\(^{9}\)–\(^{12}\) However, these methods involve many steps\(^8\)–\(^{10}\) or lack \textit{erythro/threo} diastereselectivity\(^6\)\(^7\) in the preparation of 3-O-protected ceramides.

For these reasons, we developed another synthetic approach\(^{13}\)–\(^{16}\) based on the attachment of long chain alkynes to an N,O-diprotected L-serine aldehyde, or the (S)-Garner aldehyde [(S)-4-formyl-2,2-dimethyl-3-oxazolidinecarbonic acid \textit{tert}-butyl ester], to form the C-3–C-4 bond of (2S,3R)-sphingosine bases. These methods have a minimal number of steps and are highly efficient. Moreover, the synthesis of our target compounds could be started with \textit{l}-serine or the commercially available N-Boc-L-serine methyl ester or (S)-Garner aldehyde. It is thus possible to obtain sphingosine bases in either five or three steps, respectively. High yields (70–93%)\(^{13,15}\) and very good \textit{erythro/threo} selectivity (ca. 20:1) of the key addition reaction are ensured by the addition of hexamethylphosphoramide (HMPA).\(^{15}\) Furthermore, reduction of the intermediate propargyl alcohols with lithium in liquid ammonia leads to the protected sphingosine,\(^{15}\) whereas the same reaction in ethylamine immediately yields sphingosine bases,\(^{14,17,18}\) thus reducing the length of the synthesis by one more step. This method appears to be universal, as shown by the conversion of (S)-Garner aldehyde to unnatural (2S,3S)-\textit{threo-}sphingosine by the Mitsunobu isomerization of the intermediate propargylic alcohol\(^{16}\) or by direct conversion of (S)-Garner aldehyde into (2S,3S)-\textit{threo-}sphingosines through the reactions with corresponding alkydes in the presence of zinc bromide.\(^{13}\) If \textit{d}-serine or the


corresponding (R)-Garner aldehyde\textsuperscript{19–23} was chosen as the starting material, the unnatural (2R,3S)-erythro-sphingosine bases could be prepared.\textsuperscript{16}

The problem of the synthesis of dihydrosphingosine and derivatives may be solved rather simply by employing the reduction of the triple bond in the propargylic intermediate(s) via the hydrogenation over platinum oxide, as exemplified in syntheses of the cerebroside symbioramide.\textsuperscript{24–26}

There were several possibilities for introducing the phosphocholine moiety. A thorough review of the chemical literature on the syntheses of sphingomyelins\textsuperscript{5–7,9–12} and other sphingophospholipids—ceramide-1-phosphonucleosides,\textsuperscript{27,28} ceramide-1-phosphoinositol,\textsuperscript{9,29–32} ceramide-1-phosphates,\textsuperscript{10} sphingosine-1-phosphocholine,\textsuperscript{10} N,N-dimethylsphingoethanolamine,\textsuperscript{12} and sphingosine-1-phosphates and analogs\textsuperscript{33–35}—suggests that the application of phosphoramidite (phosphitetriester) methodology to the phosphorylation of 3-O-protected ceramides should proceed in a similar fashion to syntheses of other natural phosphoesters.\textsuperscript{36–40} In this approach, building of the phosphodiester structures includes four steps: (1) phosphorylation of alcohols with the appropriate phosphitylation reagents; (2) condensation of the resulting phosphoramidites with the second alcohol

component in the presence of 1H-tetrazole; (3) oxidation of the forming phosphitetriesters into the phosphatetriesters (i.e., the completely protected target compounds); and (4) deprotection of the phosphoric acid residue. Despite the apparent complexity and number of steps, these processes are easily conducted due to high reactivity of both phosphitylation reagents and intermediate phosphate derivatives. Under careful thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR) monitoring, these conversions could be carried out without isolation of the intermediates in pure form. As a whole, this approach leads to desired phosphodiesters with good efficiency.

In summary, we have designed a sphingomyelin synthesis \([\text{D-erythro-N-palmitoylsphingomyelin and D-erythro-N-palmitoyldihydrosphingomyelin (1a,b)}]\) based on the enantioselective synthesis of 3-O-benzoylceramides \((9a,b)\) from N-Boc-L-serine methyl ester \((2)\) (Scheme 1) and the introduction of phosphorylcholine moiety using phosphitetriester methodology (Scheme 2).

**Preparation of 3-O-Benzoylceramides (9a,b)**

Scheme 1 outlines a seven-step synthesis of these compounds. Preparation of the (S)-Garner aldehyde \((4)\) and condensation of this compound with lithium pentadecyne are the key steps in this process. The (S)-Garner aldehyde synthesis was performed according to published procedures\(^{19,20}\), however, commercially available N-Boc-L-serine methyl ester \((2)\) was used as the starting material.

The synthesis of the propargylic intermediate \(5\), common for the syntheses of both unsaturated \(9a\) and saturated \(9b\) 3-O-benzoylceramides, was accomplished by known methods. This stage is very critical for the overall success of the synthesis. Addition of HMPA\(^{13}\) increases the erythro/threo ratio in favor of the erythro isomer of the propargylic alcohol \(5\) (ca. 20:1). As result, isolation and purification of erythro-5 by column chromatography are facilitated. The yields of this reaction are reported to be 70–93%\(^{13,15}\). Our average yields of this product were approximately 60%. To convert propargylic alcohol \(5\) to olefinic derivative \(6a\), reduction with lithium in liquid ammonia was employed.\(^{15}\) This method has the advantage of being simple to perform and work up. The crude product \(6a\) was adequate for use in the next step without additional purification. Transformation of \(5\) into saturated \(6b\) was conducted by hydrogenation over platinum oxide in ethyl acetate using known procedures.\(^{25,26}\) The last compound was also used in the next step without purification. The presence of single unprotected hydroxy groups in \(6a\) and \(6b\) allowed facile conversion to 3-O-benzoylceramides \((9a)\) and \((9b)\). By direct benzyolation with three equivalents of
Scheme 1. Synthetic route to 3-O-benzoylceramides (9a, b) through (S)-Garner aldehyde (4): (a) Me₂C(OMe)₂TsOH; (b) DIBAL, toluene; (c) pentadecyne/HMPA, THF; (d) Li/NH₃ or H₂/PtO₂; (e) PhCOCl/PyCH₂Cl₂; (f) Me₃Sil/CH₂Cl₂; and (g) PalmCl/NaOAc/THF.

benzoyl chloride in the presence of pyridine, a benzoyl group was introduced at C-3 to give the fully protected sphingosine bases 7a and 7b, which were purified by column chromatography. From this point, the synthesis of 3-O-benzoylceramides (9a) and (9b) follows Shapiro's synthesis of these compounds⁴¹,⁴²: transformation of 7a and 7b into the corresponding 3-O-benzoylsphingosine (8a) and 3-O-benzoyldihydrosphingosine (8b), followed by acylation with palmitoyl chloride in the presence of sodium acetate. To obtain 8a and 8b from the fully protected bases 7a and 7b, tert-Boc and isopropylidene protecting groups were removed simultaneously by treatment with trimethylsilyl iodide in methylene chloride, followed by quenching with a 4:1 mixture of methanol and water.⁴³ This reaction was carried out under ambient conditions, and no by-products were detected by TLC.

lard et al. after our work was completed), 5 N hydrochloric acid in refluxing dioxane was used to perform this operation. After the conversion of 7a and 7b was completed, the reaction mixture was evaporated to dryness, and crude 8a and 8b were used directly in the next step. To acylate 8a and 8b, a solution of palmitoyl chloride in THF and saturated aqueous sodium acetate were added simultaneously to a solution of these crude compounds in a mixture of 1 N AcOH and THF under vigorous stirring. TLC revealed complete conversion to the desired product with no by-products. 3-O-Benzoylceramides (9a) and (9b) were isolated by pouring the reaction mixture into water, filtering off the reaction mixture, drying the resulting precipitate, and recrystallizing from ethanol. The yields, over seven steps, of the 3-O-benzoylceramides (9a) and (9b) from 2 were 26 and 30%, respectively. This approach could also be employed for the preparation of other 3-O-protected ceramides (Ac, Piv, TBDMS, and TBDPS) used commonly in sphingophospholipid and glycosphingolipids syntheses.

Preparation of Sphingomyelins (1a,b) (Scheme 2)

In the section “Problems Related to Sphingomyelin Synthesis,” we discussed the basic principles of creating phosphodiester structures using phosphoramidite methodology. Commonly, two phosphitylating reagents are used for performing the first stages of phosphorylation of 3-O-protected ceramides: (i) bifunctional chloro(N,N-diisopropylamino)methoxyphosphine (14) and monofunctional bis(diisopropylamino)cyanoethoxyphosphine (15). Phosphitylation with reagent 14 is conducted in the presence of tertiary amines, usually triethylamine, to give corresponding phosphoramidites in yields greater than 90%. The resulting phosphoramidites are stable and can be isolated and purified by column chromatography. Reactions with reagent 15 were carried out in the presence of 1H-tetrazole or its diisopropylammonium salt and also resulted in very good yields. Both processes are monitored easily by TLC and are very rapid. However, when chloroamidite 14 and tertiary amines are used in the phosphitylation reactions, excess reagents can be removed easily by gentle distillation and the resulting crude products could be used without further purification.

In the present work, optimal results were obtained by the combination of the following procedures: phosphitylation of 3-O-benzoylceramides (9a) and (9b) with phosphitylating reagent (14) in the presence of triethylamine, condensation of the amidites 10a,b with an excess choline tosylate in the presence of 1H-tetrazol, further oxidation of the corresponding phosphitetriesters 11a and 11b with tert-butyl hydroperoxide, and removal of the blocking groups in the resulting phosphoric acid residues of 12a and 12b with tert-butylamine and then the hydroxyl group in C-3 with methanolic
Schematic 2. Synthetic transformation of 3-O-benzoylcereamides (9a,b) to the target N-palmitoylsphingomyelins (1a,b) using the phosphoramidite approach: (a) 14, Et₃N, CH₂Cl₂, rt, 1 hr; (b) choline tosylate/1H-tetrazole, MeCN-THF, rt, 4.5 hr; (c) tBuOOH, CH₂Cl₂, rt, overnight; (d) tBuNH₂, CH₂Cl₂, rt, overnight; and (e) MeONa, MeOH, rt, 2 hr.

sodium methoxide. As observed in the literature, the products of the phosphitylation—amidites (10a,b)—were the only reaction products and were taken into the condensation with choline tosylate immediately after the excess reagents and solvents were removed. After TLC showed that the transformation of 10a,b to 11a,b was complete, excess tert-butyl hydroperoxide (5.0–6.0 M solution in 2,2,3-trimethylpentane) was added to the same reaction. After several hours, the reaction mixtures containing phosphotriesters 12a or 12b were concentrated in vacuo, dissolved in methylene chloride, and partially deprotected with excess tert-butylamine. The resulting 3-O-benzoylsphingomyelins (13a,b) were isolated as pure compounds after column chromatography on silica gel and were deionized with Amberlyte MB-3. The yields of desired products were 67–72% and were comparable to the yields described in the literature. These procedures allowed us to prepare 3-O-benzoylsphingomyelins (13a) and (13b) on a gram scale. The final compounds sphingomyelins (1a) and (1b) were ob-
tained in yields 92–97% after deprotection of the ceramide portions with sodium methoxide in methanol, column chromatography (for \( \text{Ia} \)), and treatment with Amberlyte MB-3. The purity of intermediate and final products was established by the specific optical rotation, IR, NMR spectroscopy, and mass spectrometry, and the overall yields of \( \text{Ia} \) and \( \text{Ib} \) from \( N\)-Boc-\( L\)-ser-OMe \( (2) \) were 17 and ca. 20\%, respectively.

Materials

Palmitoyl chloride, benzyol chloride, \( N\)-Boc-\( L\)-serine methylate \( (2) \), chloro\((N,N\)-diisopropylamino)methoxyphosphine \( (14) \), \( 1H\)-tetrazole (sublimated), triethylamine, were purchased from Aldrich and used without further purification. \( tert\)-Butyl hydroperoxide \( (5.0–6.0 \text{ M solution in 2,2,3-trimethylpentane, purity 85\%}) \), diisobutylaluminium hydride \( (\text{DIBAL}) \) \( (1.5 \text{ M solution in toluene}) \), and \( n\)-BuLi \( (1.6 \text{ M solution in hexanes}) \) were purchased from Aldrich. 1-Pentadecylye was purchased from Lancaster. Choline tosylate was purchased from Sigma or was prepared from choline hydroxide \( (\text{from Aldrich}) \) and \( p\)-toluenesulfonic acid.\(^ {45} \) THF, methylene chloride, and acetonitrile were stored over molecular sieves \( (5 \text{ Å}) \) and activated in a microwave oven \( (\text{high power}) \) for 4–5 min.

General Methods

All reactions that require dry conditions are run under an argon atmosphere using anhydrous solvents. The melting points are uncorrected. Infra-red spectra are recorded on sodium chloride disks with an Impact Model 400 spectrophotometer, and only the structurally important peaks are listed. Positive and negative ion FAB mass spectra are taken on a JEOL spectrometer. Merck silica gel 60 TLC plates \( (20 \times 50 \text{ mm of 0.25 mm thickness}) \) are used to monitor the reactions, with visualization by heating with orcinol spray reagent \( (0.5\% \text{ solution of orcinol in diluted sulfuric acid, 1:9}) \) or with Sigma phosphate-specific spray reagent Molybdenum blue. Flash column chromatography is performed using silica gel 60 \( (\text{Merck, 230–400 mesh ASTM}) \). Solvent systems for TLC and preparative flash column chromatography are: \( \text{I, II, and III hexane–ethyl acetate, 6:1, 9:2, and 9:1, respectively; IV, hexane–triethylamine, 20:1; V, chloroform–methanol–acetone, 10:0.5:0.5; VI chloroform–methanol–25\% \text{ ammonium hydroxide, 6:1:0.2; VII, chloroform–methanol–25\% \text{ ammonium hydroxide, 7:1:0.1; VIII, chloroform–methanol–water, 165:35:4, IX chloroform–methanol–water, 65:35:4; and X, chloroform–methanol–acetic acid–water, 100:50:16:8.} \)

All proportions of solvents are v/v. Optical rotations are measured in 1 dm tube with a Perkin-Elmer Model 241C polarimeter. Concentrations of the solutions are conducted by rotary evaporation with bath temperature kept below 30°; when concentrations are for drying purposes, an oil pump with a CO₂-acetone trap is employed.

Procedures

Preparation of 3-O-Benzoylceramides (9a, b)

According to the method of Garner and Park, the synthesis of all-protected l-serine methyl ester (3) from N-Boc-L-ser-OMe (2) is carried out with a yield of 76-78%, [α]D 25° = -57.63° (c 1.30, CHCl₃). Lit. [α]D = -46.7° (c 1.30, CHCl₃). The reduction of 3 with DIBAL leads to Garner aldehyde (4) in 84-85% yield, [α]D 23° = -96.40° (c 1.34, CHCl₃). Lit. [α]D = -91.7° (c 1.34, CHCl₃). The condensation of pentadecyne to aldehyde 4 and characterization of the resulting (4S,1'R)-4-(1'-hydroxy-2'-hexadecynyl)-2,2-dimethyloxazolidine-3-carboxylic acid tert-butyl ester (5) are performed as described in the literature. Yield 58.0%, [α]D 25° = -39.0° (c 1.43, CHCl₃). Lit.: [α]D = -39.7° (c 1.41, CHCl₃).

(4S,1'R)-4-(1'-Benzoxy-2'-hexadecenyl)-2,2-dimethyloxazolidine-3-carboxylic acid tert-butyl ester (7a). According to the protocol of Radunz et al., a solution of derivative 5 (6.00 g, 13.71 mmol) in THF (400 ml) is added dropwise to a stirring solution of Li (5.0 g) in liquid ammonia (500 ml) at -75° over a period of 1.5 hr, and the reaction mixture is allowed to stir at -75-78° for an additional 1.5 hr. Ammonium chloride (120 g), ethyl acetate (600 ml), and water (400 ml) are then added sequentially. The reaction mixture is transferred into a separatory funnel, the organic phase is separated, and the aqueous phase is extracted with ethyl acetate (2 x 500 ml). The organic solutions are combined, dried over magnesium sulfate, and concentrated in vacuo. The crude (4S,1'R)-4-(1'-hydroxy-2'-hexadecenyl)-2,2-dimethyloxazolidine-3-carboxylic acid tert-butyl ester (6a) [6.00 g, ~100%, R₆ 0.43 (II), MS: m/z 440.3741. (M + H)+ C₂₆H₉₀NO₄ requires 440.3740] is dissolved in a mixture of methylene chloride (100 ml) and pyridine (10 ml). To this is added benzoyl chloride (5.0 ml, 6.1 g, 43 mmol) at 0° over a period of 15-20 min. The reaction mixture is allowed to warm to room temperature over a period of 6-7 hr. Saturated sodium bicarbonate solution (3 ml) is then added and stirring is continued overnight. The reaction mixture is diluted with ether (1 liter), and the ether solution is washed sequentially with 10% sodium bicarbonate solution (2 x 100 ml), brine (2 x 100 ml), dried over magnesium sulfate, and concentrated in
vacuo. The residue is chromatographed on silica gel (300 g) using solvent mixture III as eluent. Yield 7.20 g (96.6%). \( R_f 0.56 \) (I), [\( \alpha \])\( \text{D} \)\( ^{23} \) \(-32.9^\circ \) (c 1.00, CHCl\(_3\)). IR (neat): 1723 (C=O of benzoate), 1702 (C=O of carbamate), 1268 (benzoate) and 968 (\( \text{trans} \) double bond) (cm\(^{-1}\)). MS: \( m/z \) 550.4080 (M + Li)\( ^+ \) \( C_3H_5NO_3Li \) requires 550.4084.

\((4S,1'R)-4-(1'-\text{Benzoxyhexadecyl})-2,2\text{-dimethyloxazolidine-3-carboxylic acid tert-butyl ester} \) (7b). According to the literature, the propargyl alcohol 5 (16.80 g, 38.39 mmol) is hydrogenated at room temperature in ethyl acetate (200 ml) over platinum oxide with hydrogen at atmospheric pressure for 2 hr to yield \(-100\%\) of the compound 6b. \( R_f 0.46 \) (II), [\( \alpha \])\( \text{D} \)\( ^{25} \) \(-13.7^\circ \) (c 0.84, CHCl\(_3\)). IR (neat): 3434 (OH), 1697 and 1675 (C=O of carbamate) (cm\(^{-1}\)), MS: \( m/z \) 442.3896. (M + H)\( ^+ \) \( C_{26}H_{52}NO_4 \) requires 442.3896. Lit.: [\( \alpha \])\( \text{D} \)\( ^{25} \) \(-13.0^\circ \) (c 1.03, CHCl\(_3\)), \( R_f 0.55 \) (I), [\( \alpha \])\( \text{D} \)\( ^{23} \) \(-48.9^\circ \) (c 1.19, CHCl\(_3\)). IR (neat): 1722 (C=O of benzoate), 1700 (C=O of carbamate), 1270 (benzoate) (cm\(^{-1}\)). MS: \( m/z \) 546.4141. (M + H)\( ^+ \) \( C_{33}H_{56}NO_5 \) requires 546.4158.

\( \text{d-erythro-3-O-Benzoyl-2-N-palmitoylsphingosine} \) (3-O-benzoylceramide) (9a). To a solution of the benzoyl derivative 7a (6.75 g, 12.4 mmol) in methylene chloride (150 ml) is added via syringe trimethylsilyl iodide (2.2 ml, 3.1 g, 15.5 mmol). The reaction mixture is allowed to stir at 20–25\(^\circ\) for 2–3 hr. After this time, TLC shows the disappearance of the starting material, a mixture of water–methanol (1:4, 10 ml) is added, and the mixture is stirred for 15 min. The reaction mixture is concentrated in vacuo, and then the residue, crude 3-O-benzoylsphingosine (8a) \( R_f 0.58 \) (VII), IR (mineral oil): 3429 (OH, NH\(_2\)), 1720 (C=O of benzoate) and 971 (\( \text{trans} \) double bond) (cm\(^{-1}\)) is taken up into a mixture of THF (160 ml) and 1 M acetic acid (60 ml). A solution of palmitoyl chloride (3.70 g, 13.5 mmol) in THF (30 ml) and a saturated solution of sodium acetate (160 ml) are added simultaneously over a period of 5–10 min, and the reaction mixture is stirred at 20–25\(^\circ\) for 2 hr. After this reaction is complete, the reaction mixture is diluted with water (250–300 ml), the precipitate is separated by filtration, washed sequentially with 5% sodium bicarbonate and water, dried carefully in air, and crystallized from ethanol (150 ml). The yield is 5.71 g (71.7%). mp 88.0–88.5\(^\circ\), [\( \alpha \])\( \text{D} \)\( ^{23} \) +21.0\(^\circ\) (c 0.97, CHCl\(_3\)). \( R_f 0.52 \) (V). IR (mineral oil): 3414 (OH), 3328 (NH), 1720 (C=O of benzoate), 1630 (amide I), 1535 (amide II), 1266 (benzoate), 970 (\( \text{trans} \) double bond) (cm\(^{-1}\)). Lit.: mp 88–90\(^\circ\) or 90–92\(^\circ\) (c = 1.05, CHCl\(_3\)) or [\( \alpha \])\( \text{D} \)\( ^{22} \) +17.8\(^\circ\) (c = 0.56, CHCl\(_3\)).

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Starting from the benzoyl derivative 7b (4.63 g, 8.48 mmol), 3-O-benzoylceramide (9b) is prepared in 82–83% yield using the previous method. mp 75.0–75.5° (MeOH), [α]_D^{25} +32.56° (c 1.26, CHCl_3). R_f 0.59 (V). IR (mineral oil): 3427 (OH), 3300 (NH), 1717 and 1693 (C=O of benzoate), 1638 (amide I), 1537 (amide II), 1276 (C benzoate) (cm^{-1}). MS: m/z: 644.5638. (M + H)⁺ C_{41}H_{74}NO_{4} requires 644.5618. Lit.: \textit{ml}, 42 mp 78–80° and [α]_D^{+} +26.4° (c = 1.25, CHCl_3).

**Synthesis of Sphingomyelins (1a) and (1b)**

\textit{d-erythro-3-O-Benzoyl-2-N-palmitoyldihydrospingosine (3-O-Benzoyldihydroceramide) (9b)}. To a solution of the carefully dried \textit{in vacuo} 3-O-benzoylceramide (9a) (2.79 g, 4.34 mmol) in methylene chloride (25 ml) under argon is added via syringe triethylamine (1.40 ml, 1.02 g, 10.08 mmol) and phosphitylating reagent (14) (1.20 g, 1.18 ml, 6.1 mmol), and the resulting mixture is allowed to stir at 20–25° for 1 hr. After this time TLC shows the disappearance of 9a, R_f ~0.5 in solvent system V, and the formation of a new product 10a with R_f ~0.45 in solvent system IV. The reaction mixture is concentrated \textit{in vacuo}, and the residue is dissolved in dry toluene and then \textit{in vacuo}. This crude amide 10a is dissolved in THF (20 ml), and to this solution under argon is added choline tosylate (4.60 g, 16.7 mmol) in acetonitrile (35 ml) and 1H-tetrazole (1.20 g, 16.9 mmol) in acetonitrile (25 ml). The reaction mixture is allowed to stir at 20–25° for 2.5 hr. After this reaction appears to be complete (TLC shows the disappearance of 10a with R_f ~0.45, solvent system IV, and the formation of the phosphitetriester 11a with R_f ~0.5, solvent system VI), \textit{tert}-butyl hydroperoxide (5.0–6.0 M solution in 2,2,3-trimethylpentane) (2.5 ml) is added and the reaction mixture is allowed to stir at 20–25° for 2 hr. After this time, TLC shows the disappearance of compound 11a with R_f ~0.5 (solvent system VI) and the formation of the phosphatetriester 12a with R_f ~0.4 (solvent system VI). The reaction mixture is concentrated \textit{in vacuo}, and the residue is dissolved in methylene chloride (20 ml). \textit{tert}-Butylamine (10 ml) is then added to this solution, and the reaction mixture is allowed to stir at 20–25° overnight. The mixture is then concentrated \textit{in vacuo}, and the residue is chromatographed on silica gel (175 g) using solvent mixture IX. The resulting product is purified further using an Amberlyte MB-3 column with a mixture of chloroform–methanol–water (7:7:1) as eluent to yield 2.35 g (65.6%) of amorphous 3-O-benzoylsphingomyelin (13a) (monohydrate), R_f 0.50 (X), [α]_D^{23} +6.08° (c 1.15, CHCl_3) IR (film): 3500, 3300, 1715 (C=O of benzoate), 1645 (amide I), 1540 (amide II), 1262...
(benzoate), 1119–1034 (PO₃), 966 (trans double bond) (cm⁻¹). MS: m/z: 807.6023. (M + H)⁺ C₄₆H₈₄N₂O₇P requires 807.6016.

\( \text{d-erythro-2-N-Palmitoylsphingosine-1-phosphorylcholine (N-Palmitoylsphingomyelin) (1a).} \)

To a solution of 3-O-benzoylsphingomyelin (13a) (2.06 g, 2.55 mmol) in methanol (25 ml) is added 2 M sodium methoxide solution in methanol (2.5 ml), and the reaction mixture is allowed to stir at 20–25° for 2 hr. The reaction mixture is neutralized with acetic acid, concentrated in vacuo, and the residue is chromatographed on silica gel using solvent mixture IX to yield 1.74 g (97.2%) of sphingomyelin (1a). mp ~ 190°, Rᵣ 0.36 (X). [α]D²² +5.6° (c 1.02, CHCl₃–MeOH, 1:1) or +4.3° (c 1.07, CHCl₃–MeOH–H₂O, 65:35:4). MS: m/z: 703.5767 (M + H)⁺ C₃₉H₆₀N₂O₆P requires 703.5754. Lit.: mp 215–217°, [α]D²⁵ +6.1° or [α]D +6° (c 1, CHCl₃–MeOH, 1:1).¹⁰ IR (film): 3285, 1641 (amide I), 1575 (amide I), 1009–1058 (PO₃) (cm⁻¹).

\( \text{8-erythro-3-O-Benzoyl-2-N-pahnitoyldihydrosphingosine-1-phosphorylcholine (3-O-Benzoyldihydrosphingomyelin) (13b).} \)

Using the same procedure employed for converting 9a into 13a, 13b (amorphous monohydrate) is prepared from 3-O-benzoyldihydroceramide (9b) (4.84 g, 7.51 mmol). Yield 4.39 g (71.9%), Rᵣ 0.50 (X). IR (mineral oil): 3343, 1704 (C=O of benzoate), 1651 (amide I), 1531 (amide II), 1277 (benzoate), 1180 and 1117 (PO₃) (cm⁻¹). MS: m/z: 809.6171 (M + H)⁺ C₄₆H₈₄N₂O₇P requires 807.6173.

\( \text{d-erythro-3-O-Benzoyl-2-N-palmitoyldihydrosphingosine-1-phosphorylcholine (3-O-Benzoyldihydrosphingomyelin) (13b).} \)

3-O-Benzoyldihydrosphingomyelin (13b) (1.97 g, 2.38 mmol) is deprotected as described earlier to yield 1b, 1.58 g (91.9%), monohydrate. mp 220–221°, Rᵣ 0.36 (X). [α]D²⁵ +24.04° (c 0.92, CHCl₃–MeOH, 1:1). IR (mineral oil): 3286 (NH and OH), 1641 (amide I), 1549 (amide II), 1087 and 1059 (PO₃) (cm⁻¹). MS: m/z: 705.5928. (M + H)⁺ C₃₉H₆₂N₂O₆P requires 705.5911. Lit.: mp 222–223° and [α]D²⁵ +22.5°.

[46] Synthesis and Biological Activity of Glycolipids, with a Focus on Gangliosides and Sulfatide Analogs

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Introduction

Glycosphingolipids, which are composed of a complex carbohydrate chain and a hydrophobic ceramide, are localized primarily on outer cell