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SYNTHESIS AND IMMOBILIZATION OF CERAMIDE ANALOGS ON SILICA PARTICLES

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Abstract: Ceramides are the major lipid components of the stratum corneum, the major permeability barrier of the skin. Here we report a chemical synthesis of ceramide analogs covalently bonded on the silica particles, that can be used to predict the skin permeability of chemicals via HPLC methods.

The skin is the largest organ of the human body, a primary area contacted with the environment, and a route by which many chemical substances enter the body. Research has demonstrated that drug delivery through the skin is feasible for many simple potent drug molecules (less than 1000 Da) via transdermal drug delivery systems.[1] Seven drugs marketed in United States including clonidine, estradiol, fentanyl, nicotine, nitroglycerin, scopolamine, and testosterone[2] are delivered by transdermal systems. Through transdermal drug delivery systems, steady-state plasma concentrations of a drug can be achieved without the high peak levels associated with oral therapy. The avoidance of high peak levels may help minimize the side effects of certain drugs. Meanwhile, dermal exposure to toxic substances represents a major occupational hazard; successful anticipation of potential risk could significantly reduce the incidence of chronic health and environmental problems. The interest in the skin has encouraged research in the field and has led to a better understanding of the skin structure and composition of human skin. The major permeability barrier of human skin is provided by its outer layer—the stratum corneum (SC).[3] The stratum corneum consists of dead cells surrounded by an extracellular matrix containing lipid lamellae. The major lipid components of this extracellular matrix are ceramides which comprise 50% of the total lipid and consist of six structurally heterogeneous ceramides.[4-8] Among them, ceramide 2 comprises 40% of the total ceramides. Ceramide 2 (Fig. 1) consists of mainly 24- through 28- carbon fatty acids amide-linked to sphingosine and dihydrosphingosine bases.

Studies of model systems capable of predicting plasma levels following topical administration are continuously being pursued.[9] These investigations aim at facilitating the rational selection of a transdermal drug candidate that can penetrate the skin barrier or predicting the potential risk of dermal exposure to toxic chemicals. Here we report the synthesis of immobilized ceramide which can be used to evaluate chemical skin interactions by

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conventional chromatographic method. This chromatographic model system requires less experimental effect than both octanol/water partition systems and in vivo skin permeability measurement. The ceramide ligand (N-[13-carboxyltridecanoyl]-D-*erythro*-sphingosine, **2**) used to prepare the ceramide silica surface contains the basic ceramide 2 functional moiety, and a free ω-carboxyl group. The free ω-carboxyl group is used to tether ceramide 2 to the silica propyl amine particles using 1,1′-carbonyldiimidazole (CDI) activation. The decanoic and propionic anhydrides are used to endcap excess amine groups on the silica propyl amine particles.

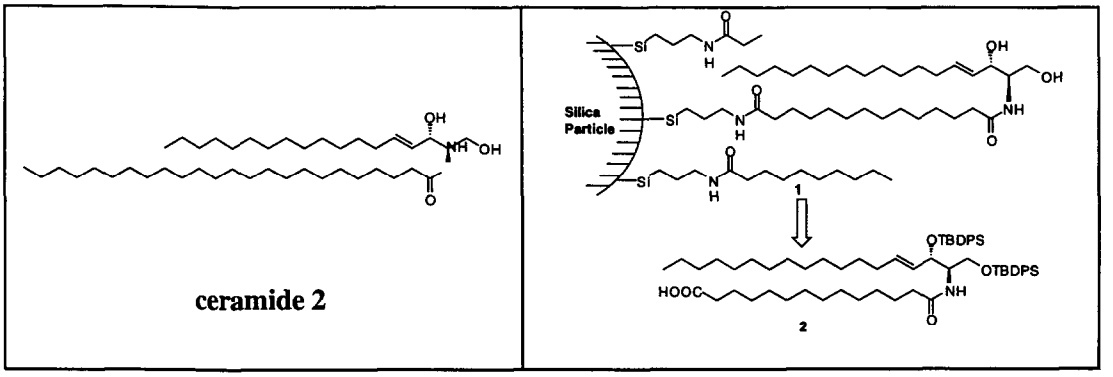
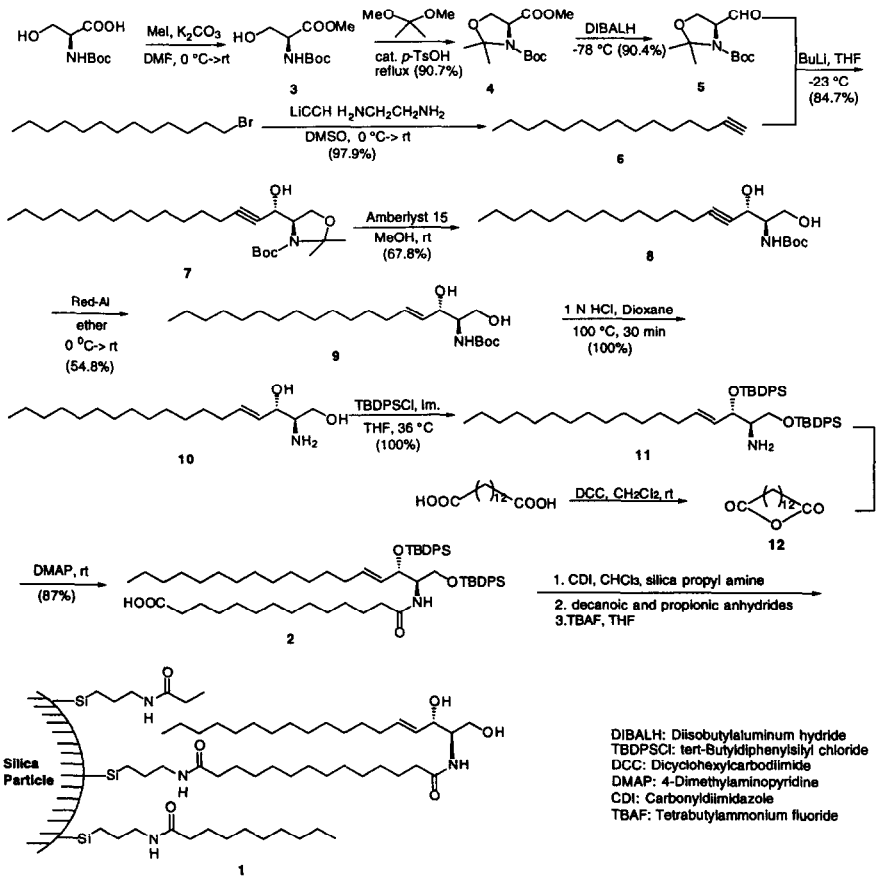


Figure 1

A representative synthesis of ceramide stationary surfaces is outlined in Scheme 1. The treatment of N-Boc-L-serine with methyl iodide gave N-Boc-L-serine methyl ester **3**, which was reacted with 2,2-dimethoxypropane under the catalysis of *p*-toluenesulfonic acid monohydrate to produce the acetal protected compound **4**, which was reduced to the aldehyde **5** under mild reduction (using diisobutylaluminum hydride (DIBALH) as reagent and running the reaction at –78 °C).[10] Meanwhile, 1-pentadecyne **6** was synthesized from 1-bromotridecane and lithium acetylide ethylenediamine complex in dimethyl sulfoxide (DMSO) solution.[11] Then oxazolidine aldehyde **5** was treated with lithium acetylide (from 1-pentadecyne **6** and *n*-butyl lithium) at –23 °C to produce the basic structure of sphingosine **7** in 84% yield after flash chromatography.[12] High *erythro*-selectivity (with diastereoselectivity (ds) ~ 89%) was observed in this reaction. Treatment of compound **7** with mild acid Amberlyst 15 in methanol at room temperature resulted in selective cleavage of the acetal protecting group, leading to the 1,3-diol **8**, which was converted to N-Boc-sphingosine **9** by selective reduction of the triple carbon bond with Red-Al in ether.[13] This reduction condition specifically converted the triple bond to the trans double bond. Cleavage of the carbamate moiety with 1.0 N HCl in dioxane at 100 °C resulted in D-*erythro*-sphingosine **10**, which was reacted with *tert*-butyldiphenylsily chloride (TBDPSCl) for protecting the two hydroxyl groups.[14] The TBDPS group is considerably more stable (about 100 times) than the *tert*-butyldimethylsilyl (TBDMS)



Scheme 1. Synthetic route of silica-immobilized ceramide stationary phase

group toward acidic hydrolysis[15] and therefore is appropriate for the immobilization reaction. Subsequent reaction of TBDPS-protected sphingosine **11** with dodecanedicarboxylic acid anhydride **12** (a cyclic anhydride, synthesized from diacid and dicyclohexylcarbodiimide (DCC)) in chloroform afforded N-[13-carboxyltridecanoyl]-D-*erythro*-sphingosine **2**[16] under catalysis of 4-dimethylaminopyridine (DMAP) in 87% yield after flash chromatography.[17] The ω-carboxyl group of ceramide analog **2** was activated with CDI in chloroform. Following the activation, the ceramide-imidazolides were bonded to silica propylamine (SPA) particles with 24 h of mild shaking.[18] After workup, the ceramide-based silica stationary phase was obtained and subjected to C10 and C3 end-capping of the residual amines on the silica particle surface. The TBDPS protecting groups were removed with tetrabutylammonium fluoride (TBAF) in THF.[19] The reactions were monitored by a Nicolet Magna 550 FT-IR spectrometer equipped with a Spectratech IR-Plan I microscope.[18] After deprotection, a monolayer of ceramide lipid membrane is generated bearing identical interfacial groups to the endogenous

ceramides found in the stratum corneum of human skin. The bonding density was calculated from elemental analysis. Finally these silica particles were packed into the stainless-steel columns and used for high performance liquid chromatography. Preliminary data (not shown) demonstrated that ceramide based stationary phases are suitable for the prediction of skin permeability constants of diverse chemicals. Solute capacity factors (k') measured on the columns correlated well with skin permeability coefficients (kp) or the percentage of percutaneous absorption (% Abs) measured through the excised human skin or the human subjects. These data will be reported elsewhere.

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16. [α]25D = -22.4° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.66–7.55 (8H, m), 7.42–7.26 (12H, m), 5.34 (1H, dd, *J* = 13.0 and 8.7 Hz), 5.24 (1H, dt, *J* = 13.0 and 6.0 Hz), 4.36 (1H, tr, *J* = 5.2 Hz), 4.16–4.07 (2H, m), 3.91 (1H, dd, *J* = 10.9 and 4.8 Hz), 3.69 (1H, dd, *J* = 10.9 and 4.8 Hz), 2.32 (2H, tr, *J* = 7.2 Hz), 1.87 (2H, m), 1.71 (2H, m), 1.64 (2H, m), 1.24 (38H, br), 0.99 (9H, s), 0.97 (9H, s), 0.86 (3H, tr, *J* = 6.9 Hz); FAB–MS (NBA matrix): calcd 1016 (M^+), found 1016; HR–FAB (NBA matrix): calcd for $C_{64}H_{97}NO_5Si_2$ (M^+) 1016.6984, found 1016.6952.
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