Convergent Synthesis of a Deuterium Labeled Serine Dipeptide Lipid for Analysis of Biological Samples

Christopher Dietz[a], Robert B. Clark[b], Frank C Nichols[c] and Michael B. Smith[a]*

[a] **Department of Chemistry, The University of Connecticut, Storrs, CT   06269**
[b] **Department of Immunology, The University of Connecticut Health Center, Farmington, CT 06030**
[c] **Division of Periodontology, The University of Connecticut Health Center, Farmington, CT 06030**

Abstract

Bacterial serine dipeptide lipids are known to promote inflammatory processes and are detected in human tissues associated with periodontal disease or atherosclerosis. Accurate quantification of bacterial serine lipid, specifically lipid 654 [((S)-15-methyl-3-((13-methyltetradecanoyl)oxy)hexadecanoyl)glycyl-L-serine, (3S)-L-serine] isolated from *Porphyromonas gingivalis*,[1] in biological samples requires the preparation of a stable isotope internal standard for sample supplementation and subsequent mass spectrometric analysis. This report describes the convergent synthesis of a deuterium-substituted serine dipeptide lipid, which is an isotopically labeled homologue that represents a dominant form of serine dipeptide lipid recovered in bacteria.

Introduction

The anaerobic Gram-negative organism, *Porphyromonas gingivalis*, is thought to be a major periodontal pathogen[2] associated with destructive periodontal disease in adults. These agonists are also produced by common oral and intestinal *Bacteroidetes,*[3] and they are recovered in chronically inflamed human tissues including destructive periodontal disease and atherosclerosis tissues.[2] The most commonly observed serine dipeptide mono-fatty acid species is composed of 3-OH iso-$C_{17:0}$ fatty acid moiety may form a 3-OH ester linkage to a saturated fatty acid, most frequently to iso-$C_{15:0}$ (see **1**).[4] Members of the *Bacteroidetes* phylum comprise a group of commensal organisms recovered in the human oral and intestinal microbiomes. Oral and intestinal *Bacteroidetes* organisms have recently been shown to produce novel serine dipeptide lipids identical to those described for specific Flavobacteria. The dominant serine lipid species isolated from *Porphyromonas gingivalis*, called Lipid 654 (**1**, ((*S*)-15-methyl-3-((13-methyltetradecanoyl)oxy)hexadecanoyl)glycyl-L-serine, (3*S*)-L-serine), acts as a bacterial virulence factor by engaging the innate immune receptor, Toll-like receptor 2 (TLR2). We have identified Lipid 654 in oral tissues from periodontal disease sites as well as in human blood and atherosclerotic lesions. In order to implicate this lipid class in human disease, accurate quantification of Lipid 654 requires supplementing biological samples with a stable isotope internal standard for subsequent quantification using conventional mass spectrometric approaches. The present report describes the preparation of a deuterated serine dipeptide internal standard (**2**) for use in quantifying **1** in biological samples. In addition to quantifying the bacterial lipid **1** in biological samples, the deuterated lipid standard **2** can also be used for the evaluation of enzymatic hydrolysis of the parent lipid by known esterases as well as peptidases. In addition, the deuterated lipid **2** can be used for evaluation of metabolic breakdown of **1** by cells important in chronic inflammatory disease processes associated with the accumulation of bacterial serine dipeptide lipids. Indeed, **13B**

will be used as a standard for the lipid 430 isolated from *Porphyromonas gingivalis* and **2B** will be used as a standard for 654 isolated from *P. gingivalis.* The isotopically labeled homologue **2B**, rather than the isotopologue of **1**, was chosen because of the cost effectiveness in the synthetic scheme, the requirement that the standard not co-elute with the substrates, and with the approval of our colleagues who plan to use the standards in their research. The racemic compounds are sufficient for mass spectral analysis and their planned use as standards. The racemic targets are deemed sufficient for the planned analyses, at present, but future studies may require the synthesis of enantioenriched or enantiopure standards.

Discussion

Deuterium labeled serine dipeptide lipid was successfully prepared for use in the analysis of biological samples. The first step in the synthesis, however, was to prepare the non-deuterated fatty ester dipeptide via procedures that mimic the convergent synthesis of lipids 430 and 654 in our recent article.[1] In order to mitigate the reactivity of the Grignard reagent with the acidic hydroxyl proton, we first protected the commercially available 11-bromoundecanol as the dimethyl(*t*-butyl)silyl derivative, in 61% yield. The Grignard reagent derived from 1-bromobutane was coupled with the protected alcohol, facilitated by lithium tetrachlorocuprate and NMP (*N*-methylpyrrolidone) to give the protected alcohol product, **4A**, in 50% overall yield from 11-bromoundecan-1-ol.[6c] Deprotection gave **5A** in 87% yield and oxidation with the oxoammonium salt known as Bobbitt's reagent[5] provided pentadecanal, **6A**, in 91% yield, without oxidation of the initially formed aldehyde to the carboxylic acid. Coupling with ethyl diazoacetate in the presence of a tin (II) chloride catalyst, using the protocol developed by Roskamp et. al.,[6a,b] gave **7A** (90%), our standard methodology.[6c] Subsequent reduction with NaBH₄, followed by saponification with LiOH, afforded β-hydroxy acid **9A** in 65% yield. Coupling of **9A** and the previously prepared

protected dipeptide **10**[1] using *N*-hydroxy succinimide for the conversion to the activated carboxylic acid derivative and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) to facilitate the coupling.[1] Removal of the *N*-Cbz group by catalytic hydrogenation gave **11A** in 67% yield. Deprotection of the ester of the protected serine afforded **12A** in 81% yield. Treatment of **12A** with LiOH gave **13A** in 84% yield. This sequence completed the first part of our synthesis.

Esterification of **9A** with 13-methyltetradecanoic acid gave **14A** in 63% yield and deprotection of the OTBDPS group with TBAF gave **15A** in 76% yield. Deprotection of the methyl ester group with potassium trimethlsilanolate gave **2A** in 65% yield. The unusual reagent used to convert the methyl ester to the carboxylic acid was necessitated by the sensitivity of the fatty ester linkage to the usual conditions used to cleave the methyl ester. The overall conversion for the linear sequence of 10 steps to **13A** was 13% overall yield, whereas the linear sequence of 11 steps gave **2A** in 9% overall yield.

Following a similar synthetic methodology, the synthesis initially focused on the fatty acid portion of the lipid and the preparation of **9B**.[1] To avoid undesired reactivity of expensive deuterated Grignard reagent with the acidic hydroxy proton, commercially available 11-bromoundecan-1-ol was protected with *tert*-butyl dimethyl silyl chloride to form the silyl ether. Coupling with the Grignard derivative of **3B,** prepared by bromination of the commercially available [^{2}H$_9$]-butan-1-ol, required a lithium tetrachlorocuprate complex with *N*-methylpyrrolidone (NMP) as an additive.[6c] The use of NMP as well as lithium tetrachlorocuprate insured that a minimum amount of 1-bromo-[^{2}H$_9$]-butane was used to produce deuterated silyl ether **4B** in 55% overall yield from 11-bromoundecanol.[7] Deprotection using TBAF generated alcohol **5B**. Oxidation of the alcohol with the oxoammonium reagent Bobbitt's salt[5] yielded aldehyde **6B** in 76% yield, with no over-

oxidation to the acid. β–Keto ester **7B** was prepared in 61% yield by the reaction of the aldehyde **6B** with ethyl diazoacetate in the presence of catalytic tin (II) chloride, using Roskamp's protocol.[6a,b] Selective reduction of the ketone moiety with sodium borohydride generated racemic β–hydroxy ester **8B** in 80% yield Saponification of this ester using lithium hydroxide yielded racemic β-hydroxy acid **9B**. The racemic compounds are sufficient for mass spectral analysis and their planned use as standards. The racemic targets are deemed sufficient for the planned analyses, at present, but future studies may require the synthesis of enantioenriched or enantiopure standards.

With nonadeuterated β-hydroxy acid **9B** in hand, the convergent synthesis proceeded by coupling with the previously prepared protected dipeptide **10** to give **11B** in 78% yield.[1] Activation of the acid with *N*-hydroxysuccinimide combined with the hydrogenolysis product from the dipeptide led to the protected lipid **11B** using EDC as the coupling agent.[8] Subsequent deprotection with TBAF gave 12B in 85% yield, followed by LiOH saponification gave the nonadeuterated lipid **13B** in 73% yield from the C_{17} fatty acid.

The other targeted lipid utilized esterification of the alcohol moiety **11B** with 13-methylpentadecanoic acid. An EDC/DMAP mediated coupling with **10** gave nonadeuterated-protected lipid **14B** in 80% yield. Deprotection proceeded via removal of the silyl ether using TBAF to liberate the serine hydroxyl group in **15B** in 85% yield The sensitivity of the ester linkage in the lipid required liberation of the methyl ester using the potassium trimethylsilanolate, which led to **2B** in 58% yield[9]

Conclusions

We have successfully incorporated nine deuterium atoms into **2B** from the commercially available 11-bromoundecan-1-ol using commercially available [^{2}H$_9$]-butan-1-ol as the deuterium source. The convergent synthesis prepared **13A** in 10 linear steps and 13% overall yield from 11-bromoundecan-1-ol and **2A** in 11 linear steps and 9% overall yield. Using an identical route we have prepared **13B** in 10 linear steps and 12% overall yield and **2B** in 10 linear steps and 8% overall yield. The **13B** will be used as a standard for the lipid 430 isolated from *Porphyromonas gingivalis* and **2B** will be used as a standard for 654 isolated from *P. gingivalis*.[1]

Experimental Section

All glassware was oven-dried, and all reactions were performed under a nitrogen atmosphere. All chemicals were purchased from the Sigma-Aldrich Chemical Co., and used without further purification unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl, methylene chloride (dichloromethane, DCM) was distilled from calcium hydride, and dimethylformamide (DMF) was distilled *in vacuo* from calcium hydride. Ethyl acetate (EtOAc), methanol (MeOH), and diethyl ether were used as obtained from the vendor. Bobbitt's salt[5] was provided as a gift from Dr. James Bobbitt (Department of Chemistry, The University of Connecticut)), but it is commercially available from Sigma-Aldrich as 4-acetamido-2,2,6,6-tetramethylpiperidine 1-oxyl. [^{2}H$_9$]-Butan-1-ol was purchased from Sigma-Aldrich and used without further purification.?

Thin-layer chromatography was done on Sorbent Technologies aluminum-backed TLC plates with fluorescent indicator and 0.2 mm silica gel layer thickness, and *p*-anisaldehyde or phosphomolybdic acid were used as developing agents. Column chromatography was done using 60 Å porosity, 32-63 μm silica gel. ^{1}H and ^{13}C NMR were collected on a Bruker Avance 300 (300.13 MHz ^{1}H, 75.48 MHz ^{13}C), Bruker DRX-400 (400.144 MHz ^{1}H, 100.65 MHz ^{13}C) or a Bruker Avance 500 (500.13 MHz ^{1}H, 125.65 MHz ^{13}C). Chemical shifts are reported in ppm downfield from tetramethylsilane (TMS) in the following format chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet). Coupling constants are reported in Hz. Mass spectrometry data was collected on a HP 5870B GC/MSD mass spectrometer with an HP-1 column, and high resolution MS and MS/MS spectra were obtained by direct infusion of the target lipids into a QTOF mass spectrometer, QSTAR Elite from Sciex (Foster City, CA) or directly by using AccuTOF™ DART, JEOL (Peabody, MA). Multiple reaction monitoring (MRM) transitions were selected based the MS/MS spectra of **1**. Lipid samples were injected into a triple quadrupole mass spectrometer, 4000 QTrap from Sciex (Foster City, CA). IR spectra were taken on an FT/IR-410/C031560585 JASCO or a Nexus 670 FT-IR E.S.P, neat, unless otherwise stated. All melting points to an upper limit of 270 °C were obtained using a Unimelt capillary melting point apparatus or Digimelt MPA160. For products described as waxy solid or semi-solids, melting points could not be obtained. The term brsm is 'based on recovered starting material.'

((11-Bromoundecyl)oxy)(*tert*-butyl)dimethylsilane

A flame-dried 100 mL round bottom flask was charged with 11-bromoundecan-1-ol (2.00 g, 7.96 mmol). The starting material was dissolved in CH_2Cl_2 (50 mL) and cooled on an ice bath. *tert*-Butyldimethylsilyl chloride (1.40 g, 9.15 mmol) was added and stirred for 15 min before the addition of imidazole (0.705 g, 10.4 mmol). The reaction was stirred overnight at room temperature. Upon completion the suspension was gravity filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (5% EtOAc:hexane) to yield ((11-bromoundecyl)oxy)(*tert*-butyl)dimethylsilane,[10] as a clear liquid (2.85 g, 7.78 mmol, 97.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 (t, J = 6.6 Hz, 2H), 3.41 (t, J = 6.9 Hz, 2H), 1.85 (p, J = 7.0 Hz, 2H), 1.51 (t, J = 6.8 Hz, 2H), 1.42 (t, J = 7.4 Hz, 2H), 1.28 (s, 12H), 0.90 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 63.38, 34.09, 32.94, 32.91, 29.63, 29.53, 29.47, 28.82, 28.24, 26.05, 25.85, 18.44, -5.19. ppm HRMS (AccuTOF): [MH]+ Calc'd for $C_{17}H_{37}BrOSi$ *m/z* 365.1875. Found: *m/z* 365.1869.

1-Bromobutane, 3A

A solution of 48% HBr (2.50 g) was added to a flame dried 25 mL round bottomed flask. Dropwise addition of H_2SO_4 (0.40 mL) was followed by stirring until the flask was cool to the touch. Dropwise addition of butan-1-ol (1.00 g, 13.5 mmol) was followed by treatment with H_2SO_4 (0.35 mL). A reflux condenser was attached and the solution was heated for 3 h. A vacuum distillation apparatus was set up through the top of the original reflux condenser. The product was gently vacuum-distilled into an ice-chilled flask while the vertical condenser was slowly warmed. The distillate was dried over $CaCO_3$ to yield 1-bromobutane,[11] **3A**, as a clear liquid (1.13 g, 8.25 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$)

δ 3.42 (t, J = 6.8 Hz, 2H), 1.84 (p, J = 6.9 Hz, 2H), 1.48 (hept, J = 7.5 Hz, 2H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 34.83, 33.72, 21.37, 13.23.

1-Bromo-[^{2}H$_9$]-butane, 3B

A solution of 48% HBr (2.50 g) was added to a flame-dried 25 mL round bottomed flask. Dropwise addition of H$_2$SO$_4$ (0.40 mL) was followed by stirring until the flask was cool to the touch. Dropwise addition of commercially available [^{2}H$_9$]-butan-1-ol (1.00 g, 12.0 mmol), was followed by addition of H$_2$SO$_4$ (0.35 mL). A reflux condenser was attached and the solution was heated at reflux for 3 h. A vacuum distillation apparatus was set up through the top of the original reflux condenser. The product was vacuum distilled into an ice-chilled flask while the vertical condenser was slowly warmed. The distillate was dried over CaCO$_3$ to yield 1-bromo-[^{2}H$_9$]-butane,[12] **3B**, as a clear liquid (0.99 g, 6.78 mmol, 56.4%).

tert-Butyldimethyl(pentadecyloxy)silane, 4A

Magnesium turnings (33 mg, 1.37 mmol) were added to a 25 mL round bottomed flask that was fitted with a stirbar and the apparatus was flame-dried under a nitrogen atmosphere. After cooling, the Mg turnings were suspended in dry THF (5 mL) and 1-bromobutane (**3A**, 0.13 mL, 1.20 mmol) was rapidly added with vigorous stirring. The reaction was stirred until the flask cooled to ambient temperature and the flask was subsequently chilled to 0 °C. The Grignard reagent was added to a mixture of ((11-bromoundecyl)oxy)(*tert*-butyl)dimethylsilane (**2**, 0.250 g, 0.684 mmol), LiCl (0.88 mg, 0.021 mmol), copper (II) chloride (2.8 mg, 0.021 mmol), and *N*-methyl-2-pyrrolidone (NMP, 0.30 mL, 3.11 mmol) dissolved in THF (5 mL) and held at 0 °C. The mixture was slowly warmed to ambient temperature and stirred for 1 h. The reaction was quenched on ice with 1M HCl

(10 mL) before being transferred to a separatory funnel with EtOAc (15 mL) and H_2O (10 mL). The layers were separated and the aqueous layer was extracted once with EtOAc (15 mL). The organic layers were combined and then washed with 1M HCl (10 mL), H_2O (2 X 10 mL), and brine (10 mL). The organic phase was dried with $MgSO_4$, gravity filtered, and solvents removed *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane) to yield *tert*-butyldimethyl(pentadecyloxy)silane, **4A**, as a clear liquid (0.218 g, 0.636 mmol, 93.0%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.60 (t, J = 6.6 Hz, 2H), 1.52 (d, J = 6.8 Hz, 2H), 1.26 (s, 24H), 0.96–0.79 (m, 12H), 0.05 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 63.41, 32.97, 31.99, 29.75, 29.72, 29.51, 29.42, 26.05, 25.87, 22.75, 14.18, -5.19. HRMS (AccuTOF): [MH]+ Calc'd for $C_{21}H_{46}OSi$ *m/z* 343.3396. Found: *m/z* 343.3373.

tert-Butyldimethyl([12,12,13,13,14,14,15,15,15-^{2}H$_9$]-pentadecyloxy)silane, 4B

Magnesium turnings (133 mg, 5.48 mmol) were added to a 50 mL round bottom flask fitted with a stirbar and the apparatus was flame-dried under a nitrogen atmosphere. After cooling, the Mg turnings were suspended in dry THF (10 mL) and 1-bromo-[^{2}H$_9$]-butane (**3B**, 0.52 mL, 4.80 mmol) was rapidly added with vigorous stirring. The reaction was stirred until the flask cooled to ambient temperature and the temperature was lowered to 0 °C. The Grignard reagent was added to a mixture of ((11-bromoundecyl)oxy)(*tert*-butyl)dimethylsilane (1.00 g, 2.74 mmol), LiCl (5.8 mg, 0.14 mmol), copper (II) chloride (9.2 mg, 0.069 mmol), and NMP (1.98 mL, 20.6 mmol) dissolved in THF (10 mL) and the reaction mixture was maintained at 0 °C. The mixture was slowly warmed to ambient temperature and stirred for 1 h. The reaction was quenched on ice with 1M HCl (25 mL) before being transferred to a separatory funnel with EtOAc (50 mL) and H_2O (50 mL). The layers were separated and the aqueous layer was extracted once with EtOAc (50 mL). The organic layers were combined and washed with 1M HCl (25 mL), H_2O (2 X 25 mL), and

brine (25 mL). The organic phase was dried with $MgSO_4$, gravity filtered, and solvents removed *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane) to yield *tert*-butyldimethyl([12,12,13,13.14,14,15,15,15-[2]H$_9$]-pentadecyloxy)silane, **4B**, as a clear liquid (0.949 g, 2.69 mmol, 98.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 (t, J = 6.6 Hz, 2H), 1.51 (t, J = 6.8 Hz, 2H), 1.25 (s, 18H), 0.89 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 63.42, 32.96, 29.76, 29.72, 29.68, 29.52, 29.47, 26.05, 25.87, 18.44, -5.19. HRMS (AccuTOF): [MH]+ Calc'd for $C_{21}H_{37}D_9OSi$ *m/z* 352.3961. Found: *m/z* 352.3949.

Pentadecan-1-ol, 5A

 tert-Butyldimethyl(pentadecyloxy)silane (**4A**, 678 mg, 1.98 mmol) was dissolved in 25 mL of dry THF under nitrogen. The solution was cooled to 0 °C and was treated with 1 M TBAF (4.95 mL, 4.95 mmol). The reaction was slowly warmed to ambient temperature and stirred for 6 h. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (15% EtOAc:Hexanes) to yield ethyl 3-hydroxyheptadecanoate,[13] **5A**, as a white solid (395 mg, 1.73 mmol, 87.4 %), MP 44-46 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (t, J = 6.6 Hz, 2H), 1.57 (t, J = 7.2, 6.7 Hz, 2H), 1.35–1.23 (m, 25H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 63.17, 32.88, 31.98, 29.74, 29.72, 29.66, 29.49, 29.41, 25.80, 22.75, 14.17. HRMS (AccuTOF): [M2H]+ Calc'd for $C_{15}H_{32}O$ *m/z* 457.4985. Found: *m/z* 457.4998.

[12,12,13,13.14,14,15,15,15-²H₉]-Pentadecan-1-ol, 5B

tert-Butyldimethyl([12,12,13,13.14,14,15,15,15-²H₉]-pentadecyloxy)silane (**4B**, 961 mg, 2.73 mmol) was dissolved in 25 mL of dry THF under nitrogen. The solution was cooled to 0 °C and was treated with 1 M TBAF (6.83 mL, 6.83 mmol). The reaction was slowly warmed to ambient temperature and stirred for 6 h. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (15% EtOAc:Hexanes) to yield [12,12,13,13.14,14,15,15,15-²H₉]-pentadecan-1-ol, **5B**, as a white solid (643 mg, 2.71 mmol, 99.1 %), MP 42-45 °C. ^{1}H NMR (400 MHz, CDCl₃) δ 3.64 (t, J = 6.6 Hz, 2H), 1.57 (p, J = 6.9 Hz, 2H), 1.39–1.22 (m, 19H). ^{13}C NMR (101 MHz, CDCl₃) δ 63.18, 32.89, 29.72, 29.67, 29.49, 25.80. HRMS (AccuTOF): [M-OH]+ Calc'd for $C_{15}H_{23}D_9O$ *m/z* 220.2991. Found: *m/z* 220.2975.

Pentadecanal, 6A

4-(Acetyamino)-2,2,6,6-tetramethyl-1-oxo-piperidinium tetrafluoroborate[5] (Bobbit's salt, 3.552 g, 11.84 mmol) was added to pentadecan-1-ol (**5A**, 2.351 g, 10.29 mmol) dissolved in 200 mL of dry DCM. An equal mass of silica was slowly added to the stirring mixture. The bright yellow solution stirred at ambient temperature until the color faded, giving a solution with a pale yellow tint. The solution was filtered to remove the silica, and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography (5% EtOAc:hexanes) to yield pentadecanal,[14] **6A**, as a clear solid, (2.116 g, 9.346 mmol, 90.8%), MP ~25 °C. ^{1}H NMR (400 MHz, CDCl₃) δ 9.76 (t, J = 1.9 Hz, 1H), 2.41 (td, J = 7.3, 1.9 Hz, 2H), 1.63 (p, J = 7.3 Hz, 2H), 1.35–1.22 (m, 22H), 0.88 (t, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, CDCl₃) δ 203.01, 43.98, 31.98, 29.70, 29.63, 29.48, 29.41, 29.23, 22.74, 22.16, 14.17. HRMS (AccuTOF): [M-H]+ Calc'd for $C_{15}H_{30}O$ *m/z* 225.2218. Found: *m/z* 225.2216.

[12,12,13,13.14,14,15,15,15-²H₉]-Pentadecanal, 6B

4-(Acetyamino)-2,2,6,6-tetramethyl-1-oxo-piperidinium tetrafluoroborate[5] (Bobbit's salt, 829 mg, 2.76 mmol) was added to [12,12,13,13.14,14,15,15,15-²H₉]-pentadecan-1-ol (**5B**, 625 mg, 2.63 mmol) dissolved in 100 mL of dry DCM. An equal mass of silica was slowly added to the stirring mixture. The bright yellow solution stirred at ambient temperature until the color faded, giving a solution with a pale yellow tint. The solution was filtered to remove the silica, and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography (5% EtOAc:hexanes) to yield [12,12,13,13.14,14,15,15,15-²H₉]-pentadecanal, **6B**, as a clear solid, (468 mg, 1.99 mmol, 75.6%), MP, ~25 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.76 (t, J = 2.0 Hz, 1H), 2.41 (td, J = 7.4, 1.9 Hz, 2H), 1.64 (p, J = 7.2 Hz, 2H), 1.32–1.20 (m, 16H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 203.01, 43.98, 29.73, 29.69, 29.64, 29.48, 29.46, 29.41, 29.23, 22.16. HRMS (AccuTOF): [MH]+ Calc'd for $C_{15}H_{21}D_9O$ *m/z* 234.2783. Found: *m/z* 234.2808.

Ethyl 3-oxoheptadecanoate, 7A

Tin (II) chloride dihydrate (52 mg, 0.231 mmol) was suspended in 20 mL of dry DCM. Ethyl diazoacetate (319 mg, 2.43 mmol) was added, dropwise via syringe, and the reaction was stirred for 15 min. Pentadecanal (**6A**, 524 mg, 2.31 mmol) was dissolved in 10 mL of DCM and added to the reaction, dropwise, via cannula. The mixture was stirred at ambient temperature for about 6 h, until nitrogen evolution ceased. The reaction mixture was diluted with 100 mL of DCM and washed with a saturated brine solution (2 X 50 mL). The aqueous layers were combined and extracted with DCM (2 X 25 mL). All organic layers were combined, dried with MgSO$_4$, filtered, and solvents evaporated at reduced pressure. The resulting crude product was purified via column chromatography (5% EtOAc:hexanes) to yield ethyl 3-oxoheptadecanoate,[15] **7A**, as a clear solid (642 mg, 2.07 mmol, 89.6%), MP

37-39 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.20 (q, J = 7.1 Hz, 2H), 3.42 (s, 2H), 2.53 (t, J = 7.4 Hz, 2H), 1.59 (p, J = 7.1 Hz, 2H), 1.34–1.22 (m, 25H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 203.03, 167.32, 61.38, 49.38, 43.12, 31.98, 29.71, 29.65, 29.50, 29.41, 29.09, 23.54, 22.75, 14.17. HRMS (AccuTOF): [MH]+ Calc'd for C$_{19}$H$_{36}$O$_3$ *m/z* 313.2743. Found: *m/z* 313.2724.

Ethyl 3-oxo-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoate, 7B

Tin (II) chloride dihydrate (46 mg, 0.206 mmol) was suspended in 15 mL of dry DCM. Ethyl diazoacetate (0.275 mL, 2.27 mmol) was added, dropwise via syringe, and the reaction was stirred for 15 min. The [12,12,13,13.14,14,15,15,15-^{2}H$_9$]-pentadecanal (**6B**, 486 mg, 2.06 mmol) was dissolved in 10 mL of DCM and added to the reaction, dropwise, via cannula. The mixture was stirred at ambient temperature for about 6 h, until nitrogen evolution ceased. The reaction mixture was diluted with 100 mL of DCM and washed with a saturated brine solution (2 X 50 mL). The aqueous layers were combined and extracted with DCM (2 X 25 mL). All organic layers were combined, dried with MgSO$_4$, filtered, and solvents evaporated at reduced pressure. The resulting crude product was purified via column chromatography (5% EtOAc:hexanes) to yield ethyl 3-oxo-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoate,[15] **7B**, as a clear solid (534 mg, 1.66 mmol, 80.6%), MP 36-38 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.19 (q, J = 7.1 Hz, 2H), 3.42 (s, 2H), 2.52 (t, J = 7.4 Hz, 2H), 1.59 (t, J = 7.2 Hz, 2H), 1.29–1.24 (m, 19H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 203.05, 167.32, 61.38, 49.38, 43.12, 29.73, 29.70, 29.65, 29.50, 29.46, 29.41, 29.09, 23.54, 14.16. HRMS (AccuTOF): [MH]+ Calc'd for C$_{19}$H$_{27}$D$_9$O$_3$ *m/z* 322.3308. Found: *m/z* 322.3324.

Ethyl 3-hydroxy-heptadecanoate, 8A

A solution of ethyl 3-oxoheptadecanoate (**7A**, 620 mg, 1.98 mmol) in 2 mL of ethanol and 18 mL of THF was cooled to 0 °C on an ice bath. Sodium borohydride (60 mg, 1.59 mmol) was added and the resulting slurry was stirred vigorously for 1 h. The reaction was quenched by the slow addition of 10 mL of a 10 % aq. citric acid solution. Subsequent neutralization with satd. K_2CO_3 solution was followed by extraction with EtOAc (3 X 20 mL). The organic layers were washed with 10 mL of satd. brine solution, dried with $MgSO_4$, filtered, and the solvents were removed at reduced pressure. The crude product was purified by column chromatography (10% EtOAc:Hexanes) to yield ethyl 3-hydroxy-heptadecanoate, 8A, as a white solid (455 mg, 1.45 mmol, 77.6 % brsm) MP 41-43 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.17 (q, J = 7.1 Hz, 2H), 3.99 (tt, J = 7.9, 3.9 Hz, 1H), 2.90 (d, J = 4.0 Hz, 1H), 2.50 (dd, J = 16.4, 3.1 Hz, 1H), 2.39 (dd, J = 16.4, 9.0 Hz, 1H), 1.43 (dd, J = 8.5, 4.3 Hz, 2H), 1.38–1.19 (m, 27H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 173.19, 68.11, 60.71, 41.35, 36.59, 31.99, 29.74, 29.71, 29.64, 29.59, 29.42, 25.54, 22.75, 14.25, 14.18. HRMS (AccuTOF): [MH]+ Calc'd for $C_{19}H_{38}O_3$ *m/z* 315.2899. Found: *m/z* 315.2899.

Ethyl 3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoate, 8B

A solution of ethyl 3-oxo-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoate (**7B**, 515 mg, 1.60 mmol) in 2 mL of ethanol and 18 mL of THF was cooled to 0 °C on an ice bath. Sodium borohydride (48 mg, 1.28 mmol) was added and the resulting slurry was stirred vigorously for 1 h. The reaction was quenched by the slow addition of 10 mL of a 10 % aq. citric acid solution. Subsequent neutralization with satd. K_2CO_3 solution was followed by extraction with EtOAc (3 X 20 mL). The organic layers were washed with 10 mL of satd. brine solution, dried with $MgSO_4$, filtered, and the solvents were removed at reduced pressure. The crude product was purified by column chromatography (10% EtOAc:Hexanes)

to yield ethyl 3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoate, **8B**, as a white solid (392 mg, 1.21 mmol, 79.7 % brsm) MP 38-40 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.17 (q, J = 7.2 Hz, 2H), 4.00 (ddt, J = 12.0, 7.3, 4.0 Hz, 1H), 2.90 (d, J = 4.0 Hz, 1H), 2.50 (dd, J = 16.4, 3.2 Hz, 1H), 2.39 (dd, J = 16.4, 9.0 Hz, 1H), 1.47–1.39 (m, 2H), 1.39–1.12 (m, 21H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 173.19, 68.11, 60.72, 41.35, 36.59, 29.75, 29.71, 29.64, 29.59, 29.47, 25.54, 14.25. HRMS (AccuTOF): [MH]+ Calc'd for C$_{19}$H$_{29}$D$_9$O$_3$ *m/z* 324.3464. Found: *m/z* 324.2442.

3-Hydroxyheptadecanoic acid, 9A

Hydroxy ester **8A** (440 mg, 1.40 mmol) was dissolved in 2 mL of MeOH and 4 mL of THF and 1 M aqueous LiOH (1.68 mL, 1.61 mmol) were added. The mixture was stirred overnight at ambient temperature. The reaction was then acidified with 1 M HCl and extracted with EtOAc (3 X 15 mL). The organic layers were washed with 10 mL of brine, dried with MgSO$_4$, filtered, and the solvents were removed at reduced pressure. The crude solid was recrystallized from hexanes to yield 3-hydroxy-heptadecanoic acid, **9A,** as a white solid (335 mg, 1.17 mmol, 83.6%), MP 86-88 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.04 (tt, J = 8.1, 3.8 Hz, 1H), 2.59 (dd, J = 16.6, 3.2 Hz, 1H), 2.48 (dd, J = 16.6, 8.9 Hz, 1H), 1.55–1.43 (m, 3H), 1.40–1.15 (m, 24H), 0.88 (t, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 175.72, 68.07, 40.73, 36.62, 31.98, 29.75, 29.71, 29.63, 29.60, 29.52, 29.42, 25.48, 22.75, 14.18. HRMS (AccuTOF): [MH]+ Calc'd for C$_{17}$H$_{34}$O$_3$ *m/z* 287.2586. Found: *m/z* 287.2605.

3-Hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoic acid, 9B

Ethyl 3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoate, **8B** (390 mg, 1.21 mmol) was dissolved in 2 mL of MeOH and 4 mL of THF and 1 M aqueous LiOH (1.45 mL, 1.39 mmol) were added. The mixture was stirred overnight at ambient temperature. The reaction was then acidified with 1 M HCl and extracted with EtOAc (3 X 15 mL). The

organic layers were washed with 10 mL of brine, dried with MgSO$_4$, filtered, and the solvents were removed at reduced pressure. The crude solid was recrystallized from hexanes to yield 3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoic acid, **9B,** as a white solid (318 mg, 1.08 mmol, 89.3%), MP 79-81 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.03 (s, 1H), 2.58 (dd, J = 16.6, 3.2 Hz, 1H), 2.48 (dd, J = 16.6, 8.9 Hz, 1H), 1.58–1.40 (m, 3H), 1.25 (s, 18H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 175.79, 68.06, 40.76, 36.63, 29.71, 29.63, 29.60, 29.53, 29.47, 25.49. HRMS (-TOF MS): [M-H]- Calc'd for C$_{17}$H$_{25}$D$_9$O$_3$ *m/z* 294.2995. Found: *m/z* 294.2982.

Methyl-*O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-heptadecanoyl)glycyl)-L-serinate, 11A

N-Hydroxy succinimide (185 mg, 1.61 mmol) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (308 mg, 1.61 mmol) were added to a stirring solution of 3-hydroxyheptadecanoic acid (**9A**, 401 mg, 1.40 mmol) in 25 mL of dry DCM. The solution was stirred for 5 h at ambient temperature, filtered, and the filtrate was concentrated *in vacuo*. The resulting white solid was dissolved in 25 mL of methanol and this solution was added via cannula to a solution of methyl *N*-(((benzyloxy)carbonyl)glycyl)-*O*-(*tert*-butyldiphenylsilyl)-L-serinate (**10**, 960 mg, 1.75 mmol) and Pd/C (298 mg) in 50 mL of methanol. The reaction vessel was flushed with H$_2$ and the mixture was stirred overnight, filtered through Celite, and concentrated under reduced pressure. The crude product was purified via column chromatography (2% MeOH:DCM) to yield methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-heptadecanoyl)glycyl)-L-serinate, **11A**, as a clear oil (641 mg, 0.938 mmol, 67.0%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.58 (t, J = 7.0 Hz, 4H), 7.50–7.33 (m, 6H), 6.88 (dd, J = 28.7, 8.2 Hz, 1H), 6.39 (dt, J = 42.3, 5.5 Hz, 1H), 4.67 (dt, J = 7.7, 2.5 Hz, 1H), 4.19–3.76 (m, 5H), 3.75 (s, 3H), 3.43 (dd, J = 38.5, 3.6 Hz, 1H), 2.41 (dt, J = 14.6, 2.2 Hz, 1H), 2.27 (dd, J = 13.9, 9.4 Hz, 1H), 1.52–1.35 (m, 3H), 1.33–1.22 (m, 24H), 1.04 (s, 9H), 0.88 (t, J = 6.6 Hz,

3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 172.80, 172.75, 170.73, 168.71, 168.71, 168.53, 135.56, 135.52, 132.98, 132.93, 132.69, 132.64, 130.08, 127.97, 127.94, 127.90, 127.87, 68.93, 64.31, 64.27, 54.34, 52.63, 43.06, 43.02, 42.93, 42.81, 37.19, 37.15, 31.98, 29.75, 29.72, 29.64, 29.57, 29.42, 26.80, 25.49, 22.75, 19.33, 14.17. HRMS (AccuTOF): [MH]+ Calc'd for C$_{39}$H$_{62}$N$_2$O$_6$Si *m/z* 683.4455. Found: *m/z* 683.4432.

Methyl O-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl)-ʟ-serinate, 11B

N-Hydroxy succinimide (130 mg, 1.13 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (216 mg, 1.13 mmol) were added to a stirring solution of 3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoic acid (**9B**, 303 mg, 1.03 mmol) in 10 mL of dry THF. The solution was stirred for 5 h at ambient temperature, filtered, and the filtrate was concentrated *in vacuo*. The resulting white solid was dissolved in 25 mL of methanol and this solution was added via cannula to a solution of methyl *N*-(((benzyloxy)carbonyl)glycyl)-*O*-(*tert*-butyldiphenylsilyl)-ʟ-serinate (**10**, 563 mg, 1.03 mmol) and Pd/C (273 mg) in 50 mL of methanol. The reaction vessel was flushed with H$_2$ and the mixture was stirred overnight, filtered through Celite, and concentrated under reduced pressure. The crude product was purified via column chromatography (2% MeOH:DCM) to yield methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl)-ʟ-serinate, **11B**, as a clear oil (555 mg, 0.802 mmol, 78.2%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.59 (tt, J = 8.1, 1.6 Hz, 4H), 7.49–7.33 (m, 6H), 6.86 (dd, J = 26.2, 8.2 Hz, 1H), 6.34 (dt, J = 41.6, 5.4 Hz, 1H), 4.67 (dtd, J = 7.6, 2.9, 1.2 Hz, 1H), 4.19–3.78 (m, 5H), 3.75 (s, 3H), 3.41 (dd, J = 35.9, 3.6 Hz, 1H), 2.40 (dt, J = 14.6, 2.5 Hz, 1H), 2.26 (ddd, J = 14.6, 9.5, 2.1 Hz, 1H), 1.53–1.34 (m, 3H), 1.32–1.22 (m, 17H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 172.78, 172.73, 170.70, 168.66, 168.49, 135.57, 135.52, 132.98, 132.93,

132.70, 132.64, 130.09, 127.96, 127.93, 127.90, 127.87, 68.92, 64.31, 64.26, 54.34, 52.61, 43.05, 43.02, 42.94, 42.82, 37.19, 37.14, 29.72, 29.65, 29.57, 29.47, 26.80, 25.49, 19.33. HRMS (AccuTOF): [MH]+ Calc'd for $C_{39}H_{53}D_9N_2O_6$ m/z 692.5020. Found: m/z 692.5007.

Methyl (3-hydroxyheptadecanoyl)glycyl-L-serinate, 12A

Methyl O-(*tert*-butyldiphenylsilyl)-N-((3-hydroxy-heptadecanoyl)glycyl)-L-serinate (**11A**, 124 mg, 0.182 mmol) was dissolved in 10 mL of dry THF under nitrogen. The solution was cooled to 0°C and was treated with 1 M tetrabutylammonium fluoride (TBAF, 0.20 mL, 0.200 mmol). The reaction was slowly warmed to ambient temperature and stirred for 6 h. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (2.5 % MeOH:DCM) to yield methyl (3-hydroxyheptadecanoyl)glycyl-L-serinate **12A**, as a white solid, (65 mg, 0.146 mmol, 80.5 %), MP, 104-111 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.36 (d, J = 7.8 Hz, 1H), 6.71 (d, J = 5.5 Hz, 1H), 4.66 (dt, J = 7.3, 3.4 Hz, 1H), 4.22–3.80 (m, 6H), 3.79 (s, 3H), 3.55 (d, J = 23.0 Hz, 1H), 2.46 (dt, J = 13.9, 2.8 Hz, 1H), 2.36–2.24 (m, 1H), 1.52–1.36 (m, 3H), 1.32–1.24 (m, 24H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 173.30, 173.10, 171.07, 170.89, 169.39, 169.18, 69.59, 69.31, 62.92, 54.87, 54.83, 52.89, 43.69, 43.50, 43.46, 43.32, 37.48, 37.27, 31.98, 29.75, 29.72, 29.65, 29.55, 29.42, 25.61, 25.54, 22.75, 14.17. HRMS (AccuTOF): [MH]+ Calc'd for $C_{23}H_{44}N_2O_6$ m/z 445.3278. Found: m/z 444.3278.

Methyl (3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl-L-serinate, 12B

Methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl)-L-serinate, (**11B**, 192 mg, 0.277 mmol) was dissolved in 10 mL of dry THF under nitrogen. The solution was cooled to 0°C and was treated with 1 M tetrabutylammonium fluoride (TBAF, 0.30 mL, 0.304 mmol). The reaction was slowly warmed to ambient temperature and stirred for 6 h. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (2.5 % MeOH:DCM) to yield methyl (3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl-L-serinate, **12B**, as a white solid, (107 mg, 0.236 mmol, 85.1 %), MP, 95-102 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.47 (dd, J = 30.8, 7.7 Hz, 1H), 6.93 (d, J = 6.2 Hz, 1H), 4.65 (dt, J = 7.4, 3.5 Hz, 1H), 4.23–3.78 (m, 6H), 3.78 (s, 3H), 2.45 (dt, J = 14.0, 2.4 Hz, 1H), 2.36–2.25 (m, 1H), 1.54–1.37 (m, 3H), 1.33–1.23 (m, 17H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 173.36, 173.21, 171.17, 170.97, 169.54, 169.39, 69.62, 69.30, 62.76, 54.92, 54.87, 52.86, 43.72, 43.57, 43.35, 43.26, 37.49, 37.28, 29.76, 29.73, 29.67, 29.58, 29.47, 25.65, 25.59. HRMS (AccuTOF): [MH]+ Calc'd for C$_{23}$H$_{35}$D$_9$N$_2$O$_6$ *m/z* 454.3843. Found: *m/z* 454.3814.

(3-Hydroxyheptadecanoyl)glycyl-L-serine, 13A

A solution of 1 M LiOH (0.12 mL, 0.12 mmol) was added to a solution of methyl (3-hydroxyheptadecanoyl)glycyl-L-serinate (**12A**, 47 mg, 0.11 mmol) in 2 mL of methanol at 0 °C, and 4 mL of THF was subsequently added. The resulting solution was warmed to ambient temperature and stirred for 2 h. The solution was then neutralized with 10% HCl and diluted with 25 mL of H$_2$O. The solution was then extracted with EtOAc (3 X 25 mL) and the organic layers were concentrated *in vacuo*. The resulting crude material was recrystallized from hot EtOAc to yield (3-hydroxyheptadecanoyl)glycyl-L-serine, **13A**, as a crystalline solid

(38 mg, 0.088 mmol, 84 %), MP, 119-122 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.64–4.40 (m, 1H), 4.14–3.69 (m, 5H), 2.43–2.33 (m, 1H), 2.30–2.20 (m, 1H), 1.52–1.20 (m, 26H), 0.83 (t, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 171.95, 171.25, 168.89, 67.48, 61.38, 54.54, 51.82, 43.63, 41.83, 36.86, 31.27, 29.08, 29.03, 28.98, 28.68, 25.10, 22.07, 13.93. HRMS (-TOF MS): [M-H]- Calc'd for C$_{22}$H$_{42}$N$_2$O$_6$ *m/z* 429.2965. Found: *m/z* 429.2989.

(3-Hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl-L-serine, 13B

A solution of 1 M LiOH (0.28 mL, 0.28 mmol) was added to a solution of methyl (3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl-L-serinate (**12B**, 117 mg, 0.258 mmol) in 2 mL of methanol at 0 °C, and 4 mL of THF was subsequently added. The resulting solution was warmed to ambient temperature and stirred for 2 h. The solution was then neutralized with 10% HCl and diluted with 25 mL of H$_2$O. The solution was then extracted with EtOAc (3 X 25 mL) and the organic layers were concentrated *in vacuo*. The resulting crude material was recrystallized from hot EtOAc to yield ((3-hydroxy-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl-L-serine, **13B**, as a crystalline solid (83 mg, 0.19 mmol, 73 %), MP, 118-122 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.50 (dt, J = 11.0, 3.4 Hz, 1H), 4.17–3.65 (m, 5H), 2.37 (ddd, J = 13.6, 7.9, 2.8 Hz, 1H), 2.29–2.19 (m, 1H), 1.49–1.16 (m, 20H). ^{13}C NMR (101 MHz, DMSO) δ 171.78, 171.22, 168.96, 67.47, 61.31, 54.51, 51.83, 43.60, 41.75, 36.84, 29.08, 29.04, 28.99, 28.74, 25.10. HRMS (AccuTOF): [MH]+ Calc'd for C$_{22}$H$_{33}$D$_9$N$_2$O$_6$ *m/z* 440.3686. Found: *m/z* 440.3689.

(6S)-6-(Methoxycarbonyl)-2,2-dimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silaheptacosan-13-yl 13-methyltetradecanoate, 14A

13-Methyltetradecanoic acid (82 mg, 0.338 mmol) was dissolved in 15 mL of dry DCM and stirred at 0 °C. To the solution was then treated with DCC (87 mg, 0.423 mmol) and a catalytic amount of DMAP. After stirring for 15 min, methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-heptadecanoyl)glycyl)-L-serinate, (**11A**, 231 mg, 0.338 mmol) dissolved in 5 mL of dry DCM was added to the reaction via cannula. The resulting solution was warmed to ambient temperature and stirred overnight. The solvent was removed under reduced pressure and the resulting oil was redissolved in 20 mL of diethyl ether. The resulting precipitate was filtered, and the filtrate washed with satd NaHCO$_3$ (3 X 10 mL). The organic layer was dried, filtered, and solvents evaporated under reduced pressure. Purification via column chromatography (1% MeOH:DCM) yielded (6*S*)-6-(methoxycarbonyl)-2,2-dimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silaheptacosan-13-yl 13-methyltetradecanoate, **14A**, as a clear wax (123 mg, 0.136 mmol, 63.4% brsm). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.58 (t, J = 7.1 Hz, 4H), 7.46–7.37 (m, 6H), 6.54 (t, J = 6.7 Hz, 1H), 6.37 (d, J = 4.9 Hz, 1H), 5.16 (p, J = 6.3 Hz, 1H), 4.66 (d, J = 7.8 Hz, 1H), 4.14–3.82 (m, 5H), 3.74 (s, 3H), 2.55–2.42 (m, 2H), 2.29 (t, J = 7.5 Hz, 2H), 1.50 (hept, J = 6.6 Hz, 1H), 1.31–1.24 (m, 42H), 1.15–1.10 (m, 4H), 1.03 (s, 9H), 0.89–0.84 (m, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 173.45, 170.47, 169.98, 168.26, 135.56, 135.52, 132.82, 132.64, 130.11, 130.06, 127.97, 127.88, 71.12, 64.15, 54.28, 52.58, 49.26, 42.88, 41.43, 39.12, 34.56, 34.18, 34.00, 31.98, 30.01, 29.75, 29.62, 29.57, 29.42, 29.36, 29.21, 28.03, 27.48, 26.78, 25.67, 25.32, 25.05, 24.99, 22.75, 22.72, 14.17. HRMS (AccuTOF): [MH]+ Calc'd for C$_{54}$H$_{90}$N$_2$O$_7$Si *m/z* 907.6596. Found: *m/z* 907.6570.

(6*S*)-6-(Methoxycarbonyl)-2,2-dimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-sila-[24,24,25,25,26,27,27,27-²H₉]-heptacosan-13-yl 13-methyltetradecanoate, 14B

13-Methyltetradecanoic acid (130 mg, 0.535 mmol) was dissolved in 15 mL of dry DCM and stirred at 0 °C. To the solution was then treated with DCC (110 mg, 0.535 mmol) and a catalytic amount of DMAP. After stirring for 15 min, methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-[14,14,15,15,16,16,17,17,17-²H₉]-heptadecanoyl)glycyl)-L-serinate (**11B**, 322 mg, 0.465 mmol) dissolved in 5 mL of dry DCM was added to the reaction via cannula. The resulting solution was warmed to ambient temperature and stirred overnight. The solvent was removed under reduced pressure and the resulting oil was redissolved in 20 mL of diethyl ether. The resulting precipitate was filtered, and the filtrate washed with satd $NaHCO_3$ (3 X 10 mL). The organic layer was dried, filtered, and solvents evaporated under reduced pressure. Purification via column chromatography (1% MeOH:DCM) yielded (6*S*)-6-(methoxycarbonyl)-2,2-dimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-sila-[24,24,25,25,26,26,27,27,27-²H₉]-heptacosan-13-yl 13-methyltetradecanoate, **14B**, as a clear wax (216 mg, 0.236 mmol, 79.6% brsm). ¹H NMR (400 MHz, $CDCl_3$) δ 7.63–7.55 (m, 4H), 7.48–7.36 (m, 6H), 6.52 (d, J = 6.8 Hz, 1H), 6.33 (d, J = 5.0 Hz, 1H), 5.20–5.13 (m, 1H), 4.66 (dd, J = 7.6, 3.4 Hz, 1H), 4.13 (dd, J = 10.3, 2.9 Hz, 1H), 4.01–3.81 (m, 3H), 3.75 (s, 3H), 2.54–2.44 (m, 2H), 2.29 (t, J = 7.5 Hz, 2H), 1.51 (hept, J = 13.2, 6.6 Hz, 1H), 1.39–1.16 (m, 38H), 1.15 (q, J = 6.7 Hz, 2H), 1.03 (s, 9H), 0.86 (d, J = 6.6 Hz, 6H). ¹³C NMR (101 MHz, $CDCl_3$) δ 173.42, 170.44, 170.40, 169.95, 168.21, 135.56, 135.52, 132.83, 132.65, 130.11, 130.06, 127.97, 127.88, 71.10, 64.15, 54.28, 54.26, 52.57, 42.88, 41.43, 39.12, 34.56, 34.18, 30.01, 29.72, 29.62, 29.57, 29.47, 29.42, 29.36, 29.21, 28.03, 27.48, 26.78, 25.32, 25.06, 22.72, 19.31. HRMS (AccuTOF): [MH]+ Calc'd for $C_{54}H_{81}D_9N_2O_7Si$ *m/z* 916.7160. Found: *m/z* 916.7154.

1-((2-((((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxoheptadecan-3-yl 13-methyltetradecanoate, 15A

(6*S*)-6-(Methoxycarbonyl)-2,2-dimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silaheptacosan-13-yl 13-methyltetradecanoate (**14A**, 123 mg, 0.136 mmol) was dissolved in 5 mL of dry THF under nitrogen. The solution was cooled to 0 °C and was treated with 1 M TBAF (0.15 mL, 0.15 mmol). The reaction was slowly warmed to ambient temperature and stirred for 1 h. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (5% MeOH:DCM) to yield 1-((2-((((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxoheptadecan-3-yl 13-methyltetradecanoate, **15A**, as a white solid (69 mg, 0.103 mmol, 76.1 % brsm) MP 84-92 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.06 (dd, J = 46.7, 7.5 Hz, 1H), 6.44 (s, 1H), 5.24–5.12 (m, 1H), 4.66 (t, J = 3.4 Hz, 1H), 4.11–3.89 (m, 4H), 3.79 (s, 3H), 3.20 (d, J = 68.5 Hz, 1H), 2.49 (dd, J = 6.0, 3.2 Hz, 2H), 2.31 (td, J = 7.6, 2.1 Hz, 2H), 1.51 (hept, J = 6.6 Hz, 1H), 1.38–1.20 (m, 40H), 1.15 (q, J = 6.8 Hz, 2H), 0.93–0.79 (m, 9H). 13C NMR (101 MHz, CDCl$_3$) δ 174.55, 174.39, 170.78, 170.71, 170.66, 170.60, 168.97, 168.93, 71.53, 62.96, 55.02, 52.76, 43.43, 42.04, 41.95, 39.12, 34.62, 34.55, 31.99, 30.01, 29.76, 29.71, 29.62, 29.57, 29.42, 29.38, 29.33, 29.21, 28.03, 27.48, 25.32, 25.06, 25.00, 22.76, 22.72, 14.17. HRMS (-TOF MS): [M-H]- Calc'd for C$_{38}$H$_{72}$N$_2$O$_7$ *m/z* 667.5261. Found: *m/z* 667.5243.

1-((2-((((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxo-[14,14,15,15,16,16,17,17,17-²H₉]-heptadecan-3-yl- 13-methyltetradecanoate, 15B

(6*S*)-6-(Methoxycarbonyl)-2,2-dimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-sila-[24,24,25,25,26,26,27,27,27-²H₉]-heptacosan-13-yl 13-methyltetradecanoate (**14B**, 192 mg, 0.277 mmol) was dissolved in 5 mL of dry THF under nitrogen. The solution was cooled to 0 °C and was treated with 1 M TBAF (0.30 mL, 0.15 mmol). The reaction was slowly warmed to ambient temperature and stirred for 1 h. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (5% MeOH:DCM) to yield 1-((2-((((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxo-[14,14,15,15,16,16,17,17,17-²H₉]-heptadecan-3-yl 13-methyltetradecanoate, **15B**, as a white solid (107 mg, 0.236 mmol, 85.1 % brsm) MP 82-88 °C. ^{1}H NMR (400 MHz, CDCl₃) δ 7.10 (dd, J = 43.2, 7.8 Hz, 1H), 6.48 (d, J = 4.2 Hz, 1H), 5.18 (dt, J = 19.0, 6.4 Hz, 1H), 4.66 (t, J = 3.4 Hz, 1H), 4.14–3.89 (m, 4H), 3.79 (s, 3H), 3.27 (d, J = 65.9 Hz, 1H), 2.49 (dd, J = 6.1, 3.1 Hz, 2H), 2.31 (td, J = 7.6, 2.3 Hz, 2H), 1.50 (t, J = 7.0 Hz, 1H), 1.41–1.18 (m, 34H), 1.15 (q, J = 6.4 Hz, 2H), 0.86 (d, J = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, CDCl₃) δ 174.56, 174.39, 170.79, 170.73, 170.67, 170.61, 168.98, 168.93, 71.53, 62.96, 62.92, 55.02, 52.76, 43.44, 43.40, 42.05, 41.94, 39.12, 34.62, 34.55, 30.01, 29.78, 29.73, 29.62, 29.57, 29.48, 29.38, 29.33, 29.22, 29.19, 28.03, 27.48, 25.32, 25.06, 25.00, 22.71. HRMS (AccuTOF): [MH]+ Calc'd for $C_{38}H_{63}D_9N_2O_7$ *m/z* 678.5983. Found: *m/z* 678.5992.

(3-((13-Methyltetradecanoyl)oxy)heptadecanoyl)glycyl-L-serine 2A

A stirring solution of 1-((2-((((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxoheptadecan-3-yl 13-methyltetradecanoate (**15A**, 48 mg, 0.078 mmol), in 3 mL of dry DMF, was cooled to 0 °C. Potassium trimethylsilanoate (11 mg, 0.083 mmol) was added, and the reaction was stirred on the ice bath for 1.5 h before being warmed to ambient temperature and stirred for an additional 1.5 h. The solution was diluted with 10 mL of 10 mL of H_2O and acidified with 10% HCl. The resulting cloudy precipitate was extracted with ethyl acetate (3 X 15mL). The organic layer was dried, filtered, and solvents evaporated under reduced pressure. The resulting solid was purified by column chromatography (10% MeOH:DCM) to yield (3-((13-methyltetradecanoyl)oxy)heptadecanoyl)glycyl-L-serine, **2A**, as a white sticky solid (33 mg, 0.050 mmol, 65 % brsm). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.07 (d, J = 8.4 Hz,0.5H), 6.77 (d, J = 8.2 Hz,0.5H), 5.17–5.07 (m, 1H), 4.26 (s, 1H), 4.11–3.60 (m, 4H), 3.36–3.30 (m, 1H), 2.47 (s, 2H), 2.23 (s, 2H), 1.67–1.41 (m, 6H), 1.21 (s, 41H), 0.82 (t, J = 7.0 Hz, 9H). ^{13}C NMR spectrum could not be obtained due to liposome formation. HRMS (-TOF MS): [M-H]- Calc'd for $C_{37}H_{70}N_2O_7$ m/z 653.5105. Found: m/z 653.5077.

(3-((13-Methyltetradecanoyl)oxy)-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl-L-serine, 2B

A stirring solution of 1-((2-((((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxo-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]heptadecan-3-yl 13-methyltetradecanoate (**15A**, 98 mg, 0.144 mmol), in 3 mL of dry DMF, was cooled to 0 °C. Potassium trimethylsilonoate (18 mg, 0.144 mmol) was added, and the reaction was stirred on the ice bath for 1.5 h before being warmed to ambient temperature and stirred for an additional 1.5 h. The solution was diluted with 10 mL of 10 mL of H_2O and acidified with 10% HCl. The resulting cloudy precipitate was extracted with ethyl acetate (3 X 15mL). The

organic layer was dried, filtered, and solvents evaporated under reduced pressure. The resulting solid was purified by column chromatography (10% MeOH:DCM) to yield (3-((13-methyltetradecanoyl)oxy)-[14,14,15,15,16,16,17,17,17-^{2}H$_9$]-heptadecanoyl)glycyl-L-serine, **2B**, as a white sticky solid (55 mg, 0.083 mmol, 58% brsm). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.09 (d, J = 8.1 Hz,0.5H), 6.78 (d, J = 8.4 Hz,0.5H), 5.24–5.08 (m, 1H), 4.30 (s, 1H), 4.14–3.55 (m, 4H), 3.43–3.28 (m, 1H), 2.52 (s, 2H), 2.25 (t, J = 7.4 Hz, 2H), 1.57 (d, J = 13.1 Hz, 4H), 1.51–1.44 (m, 1H), 1.42–1.15 (m, 30H), 1.12 (q, J = 6.8 Hz, 2H), 0.83 (d, J = 6.6 Hz, 6H). ^{13}C NMR spectrum could not be obtained due to liposome formation. HRMS (-TOF MS): [M-H]- Calc'd for C$_{37}$H$_{61}$D$_9$N$_2$O$_7$ *m/z* 662.5670. Found: *m/z* 662.5640.

References

1. (a) Dietz, C.; Hart, T. K.; Nemati, R.; Yao, X.; Nichols, F. C.; Smith, M. B. *Tetrahedron*, **2016**, 72, 7557-7569. Also see (b) Nichols, F.C.; Riep, B.; Mun, J.; Morton, M.D.; Kawai, T.; Dewhirst, F.E.; Smith, M.B. *J Lipid Res.* **2006**, 47, 844-853; (c) Nichols, F.C.; Riep, B.; Mun, J.; Morton, M.D.; Bojarski, M.T.; Dewhirst, F.E.; Smith, M.B. *J Lipid Res.* **2004** 45, 2317-2330; (d) Clark, R.B.; Cervantes, J.L.; Maciejewski, M.W.; Farrokhi, V.; Nemati, R.; Yao, X.; Anstadt, E.; Fujiwara, M.; Wright, K.T.; Riddle, C.; La Vake, C.J.; Salazar, J.C.; Finegold, S.; Nichols, F.C. *Infect Immun.* **2013**, 81, 3479-3489.

2. Holt, S.C.; L. Kesavalu, L.; Walker, S.; Genco. C.A. *Periodontol. 2000,* **1999***, 20, 168-238.

3. Nichols, F.C.; Yao, X.; Bajrami, B.; Downes, J.; Finegold, S.M.; Knee, E.; Gallagher, J.J.; Housley, W.J.; Clark, R.B. *PLoS One* **2011**, 6, e16771. doi: 10.1371/journal.pone.0016771.

4. Wang, Y.H.; Nemati, R.; Anstadt, E.; Liu, Y.; Son, Y.; Zhu, Q.; Yao, X.; Clark, R.B.; Rowe, D.W.; Nichols, FC. *Bone* **2015**, 81,654-661.

5. (a) Smith, M.B. *March's Advanced Organic Chemistry, 7th Ed*, Wiley, NJ, **2013**, p.1449; (b) Bobbitt, J.M.; Flores, M.C.L. *Heterocycles* **1988***, 27, 509-533; (c) Ma, Z.; Bobbitt, J.M. *J. Org. Chem.* **1991***, 56, 6110-6114.

6. (a) Holmquist. C. R.; Roskamp, E. J. *J. Org. Chem.* **1989**. 54, 3258-260; (b) Brockwell, J.C.; Holmquist, C.R. *J. Chem. Ed.* **1992***, 69, 68; (c) Mun, J.; Onorato, A.; Nichols, F.C.; Morton, M.D.; Saleh, A.I.; Welzel, M.; Smith, M.B. *Org. Biomol. Chem.* **2007**, 5, 3826-3833.

7. (a) Bergbreiter, D. E.; Whitesides, G. M. *J. Org. Chem*. **1975**, 40, 779-782. (b) Cahiez, G.; Chaboche, C.; Jezequel, M. *Tetrahedron* **2000**, 56, 2733-2737.

8. Shute, R. E.; Rich, D. H. *J. Chem. Soc*. **1987**, 15, 1155-1156.

9. Lovric, M.; Cepanec, I.; Litvic, M.; Bartolincic, A.; Vinkovic, V. *Croatica Chemica Acta CCACAA* **2007**, 80, 109-115.

10. Bowler, J.; Lilley, T. J.; Pittam, J. D.; Wakeling, A. L. *Steroids* **1989**, 54, 71–99.

11. Savile, C. K.; Reed, D. W.; Meesapyodsuk, D.; Covello, P. S.; Buist, P. H. *J. Chem. Soc., Perkin Trans*. **2001**, 1, 1116-1121.

12. Veken, B. J. van der; Odeurs, R.O.; Brown, A.; Mckean, D. C.; Morrisson, A. R. *J. Mol. Str.* **1986**, 147, 57-66.

13. (a) Jorapur, Y. R.; Chi, D. Y. *J. Org. Chem*. **2005**, 70, 10774-10777. (b) Chandrasekhar, S.; Shyamsunder, T.; Chandrashekar, G.; Narsihmulu, C. *Synlett* **2004**, 3, 522- 524.

14. Paul, B.; Das, D.; Ellington, B.; Marsh, E. N. G. *J. Am. Chem. Soc*. **2013**, 135, 5234-5237.

15. Mai, A.; Rotili, D.; Tarantino, D.; Ornaghi, P.; Tosi, F.; Vicidomini, C.; Sbardella, G.; Nebbioso, A.; Miceli, M.; Altucci, L.; Filetici, P. *J. Med. Chem*. **2006**, 49, 6897-6907.
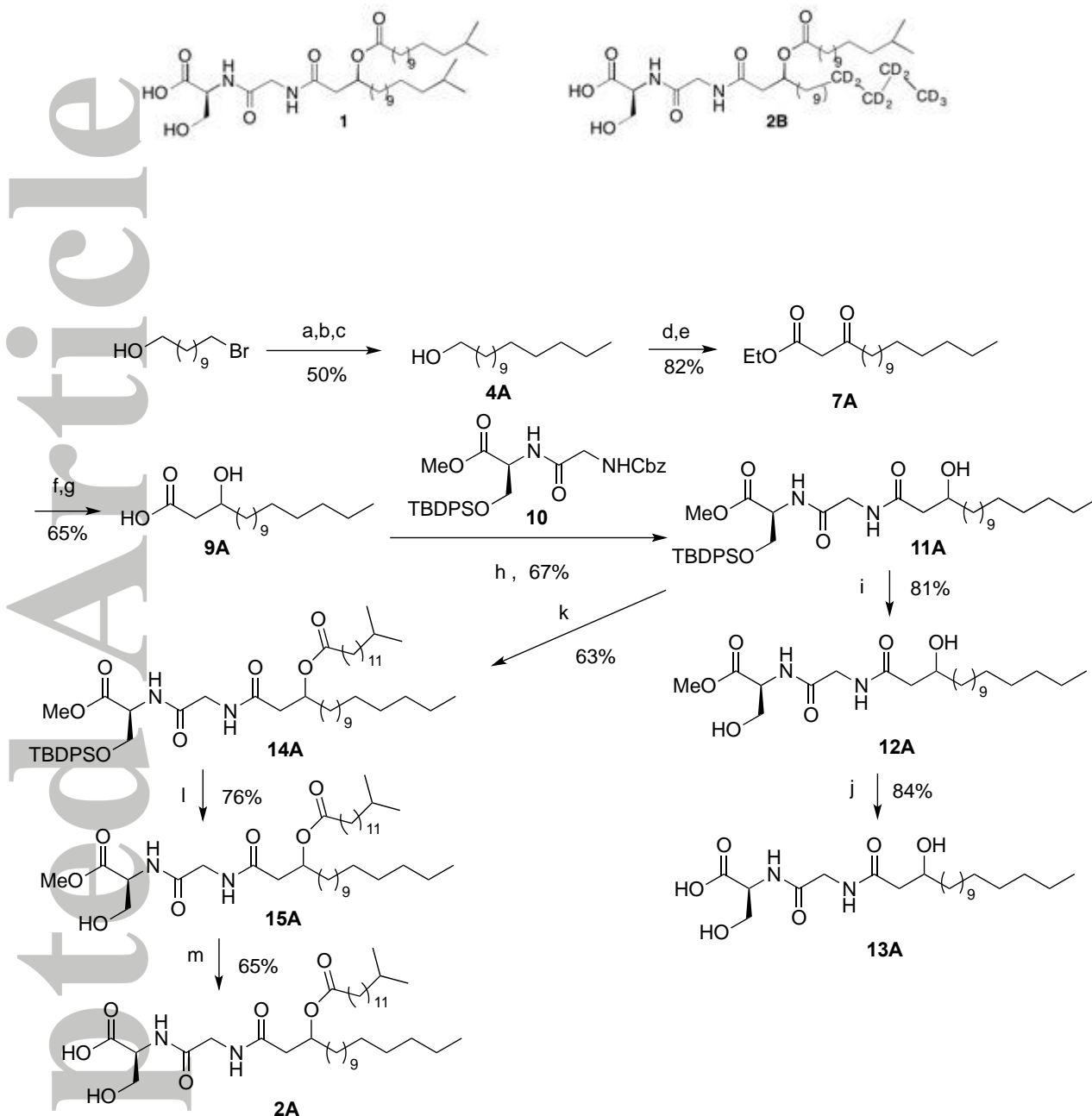
Scheme 1. Synthesis of non-deuterated **2A**

Reagents: (a) TBDMSCl, imidazole, 61%; (b) (i) 1-bromobutane (**3A**), Mg⁰, THF (ii) CuCl₂, LiCl, NMP, 93%; (c) TBAF, 87.4%; (d) Bobbitt's salt, SiO₂, 90.8%; (e) SnCl₂, ethyl diazoacetate, 89.6%; (f) NaBH₄, 77.6%; (g) LiOH, 83.6%; (h) (i) NHS, EDC, DMAP (ii) Pd/C, H₂, 67%; (i) TBAF, 80.5% (j) LiOH, 84%; (k) 13-methylpentadecanoic acid, EDC, DMAP, 63.4%; (l) TBAF, 76.1% (m) KOSi(CH)₃, 65%.
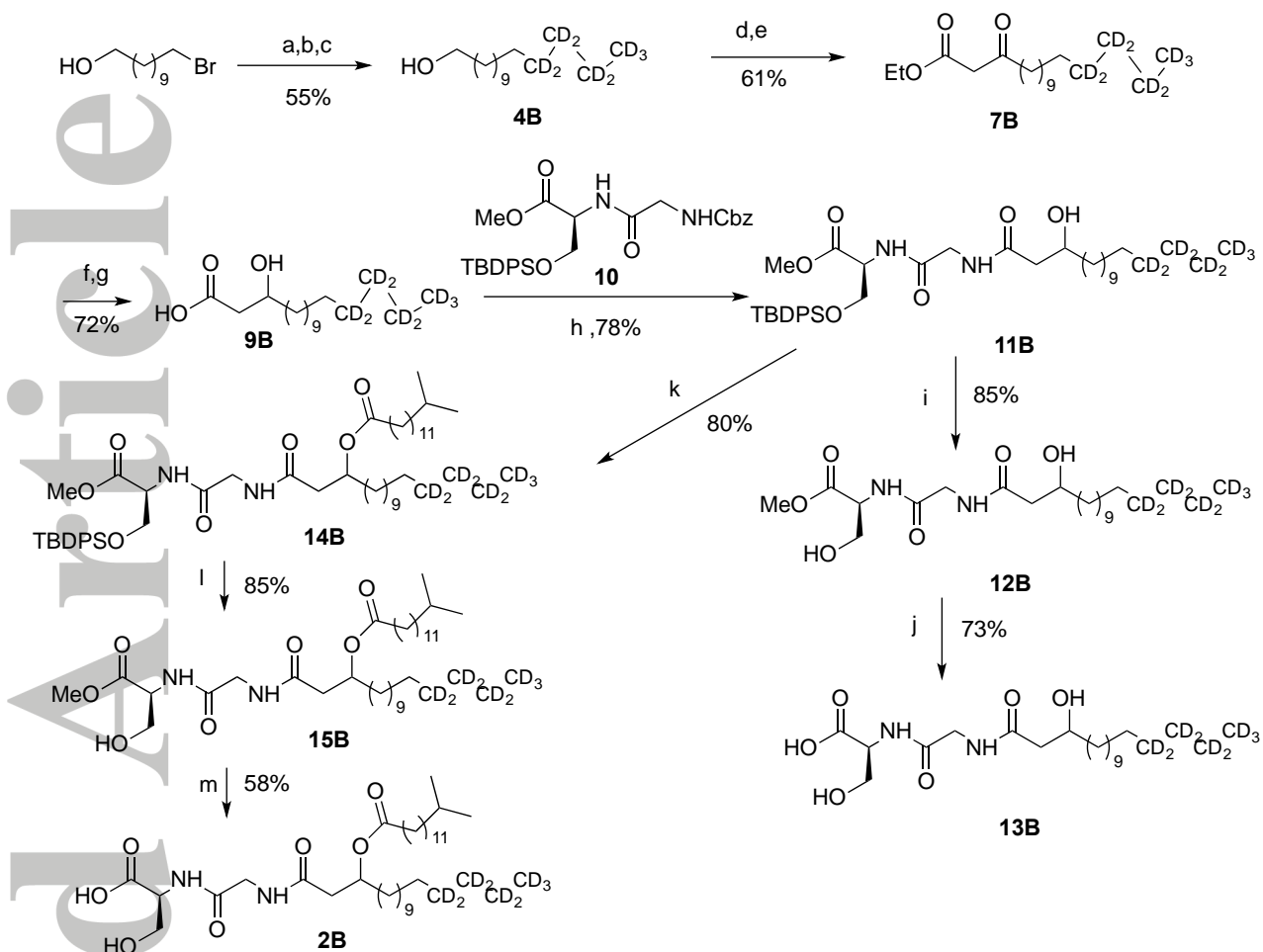
Scheme 2. Synthesis of Deuterated **2B**

Reagents: (a) TBDMSCl, imidazole, 56.4%; (b) (i) 1-bromo-[²H₉]-butane (**3B**), Mg⁰, THF (ii) CuCl₂, LiCl, NMP, 98.3%; (c) TBAF, 99.1%; (d) Bobbitt's salt, SiO₂, 75.6%; (e) SnCl₂, ethyl diazoacetate, 80.6%; (f) NaBH₄, 79.7%; (g) LiOH, 89.3% (h) (i) **10**, NHS, EDC, DMAP (ii) Pd/C, H₂, 78.2%; (i) TBAF, 85.1% (j) LiOH, 73%; (k) 13-methylpentadecanoic acid, EDC, DMAP, 79.6%; (l) TBAF, 85.1% (m) KOSi(CH)₃, 58%.