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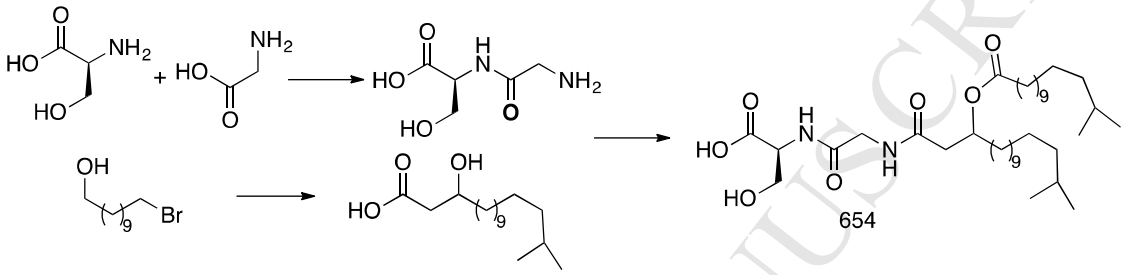
Structural Verification Via Convergent Total Synthesis of Dipeptide–Lipids Isolated from *Porphyromonas gingivalis*

Christopher Dietz[a], Theresa K. Hart[a], Reza Nemati[a], Xudong Yao[a], Frank C. Nichols,[b] and Michael B. Smith[a]*

Abstract

We have proven the structure of lipid 654, isolated from the dental pathogen *Porphyromonas gingivalis*. The lipid was prepared by a convergent synthetic route that is significantly shorter than other published routes, producing the targeted compound in higher yield. Further, we have shown that this dipeptide-lipid is selectivity hydrolyzed by lipase, consistent with results from lipid 654 obtained from *P. gingivalis*.



Structural Verification Via Convergent Total Synthesis of Dipeptide–Lipids Isolated from *Porphyromonas gingivalis*

Christopher Dietz[a], Theresa K. Hart[a], Reza Nemati[a], Xudong Yao[a], Frank C. Nichols,[b] and Michael B. Smith[a]*

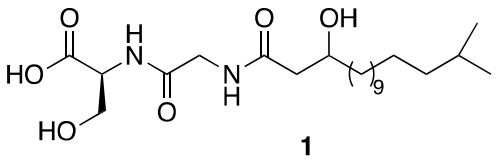
[a] Department of Chemistry, The University of Connecticut, Storrs, CT
[b] Division of Periodontology, The University of Connecticut Health Center, Farmington, CT

Abstract

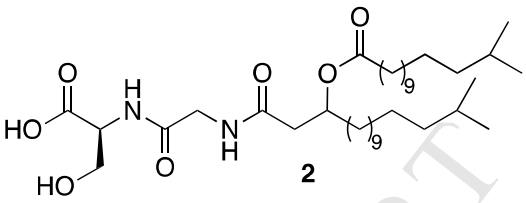
A periodontal pathogen, *Porphyromonas gingivalis,* produces two serine dipeptide lipid classes that we labeled lipid 654 and lipid 430, and both contain L-serine as the terminal amino acid. The lipid 654 and lipid 430 classes are each comprised of three species with differing fatty acid substitutions, but the most abundant species demonstrate unit masses of either 654 or 430, respectively. Recently we observed that the lipid 654 can be hydrolyzed by specific lipases to lipid 430. However, a substantial percentage of the naturally occurring lipid 654 cannot be enzymatically hydrolyzed to lipid 430. The observed partial hydrolysis could be due to the presence of a mixture of stereoisomers. Testing this theory requires structural verification of our labeled 654 and 430 by total synthesis. We present herein details of the convergent synthesis of lipids 430 and 654, which confirm the proposed structure of *P. gingivalis* lipid 654 to be (3*R* and 3*S*)-L-serine-**2**. The bis(fatty acid) (3*R*)-L-serine-**2** was prepared as well as the synthetic precursor, serine dipeptide mono-fatty acid (3*R*)-L-serine-**1**, which is the structure of lipid 430. We also synthesized the (3*S*)-L-serine-**2** diastereomer as well as (3*S*)-L-serine-**1.** Using these synthetic standards, we confirmed that PLA2-mediated hydrolysis of lipid 654 is enantioselective in that only the (3*R*)-L-serine-**2**, but not (3*S*)-L-serine **2** is enzymatically hydrolyzed.

Introduction

Inflammatory periodontal disease in adults is initiated with the accumulation of specific bacteria in the sulcus around the teeth, followed by a chronic inflammatory reaction by the host against the colonizing microorganisms. The anaerobic Gram-negative organism, *Porphyromonas gingivalis*, is thought to be a major periodontal pathogen[1] associated with destructive periodontal disease in adults. *P. gingivalis* and other phylogenetically related organisms produce a variety of novel lipids,[2] including phosphorylated dihydroceramide lipids.[3] More recent work in our laboratories identified serine dipeptide lipid classes in *P. gingivalis* that comprise a new class of ligands for Toll-like receptor 2 (TLR2).[4] These agonists are also produced by common oral and intestinal *Bacteroidetes*,[5] 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 a serine-glycine dipeptide in amide linkage to a 3-OH isobranched (iso) $C_{17:0}$ fatty acid. We labeled this compound lipid 430 (see **1**). The 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 **2**).[6] We labeled this isolate lipid 654. A search of the literature indicated that this structure was originally proposed for a serine lipid product isolated from a limited number of *Flavobacteria* and was termed flavolipin.[7-9] We have also observed that lipid 430 was produced by enzymatic hydrolysis of lipid 654 and was, therefore, a logical synthetic precursor. Other fatty acids can substitute into these lipid classes, but they are less abundant relative to branched $C_{15:0}$ and 3-OH iso $C_{17:0}$.[6] Both of the serine dipeptide lipids, lipid 430 and lipid 654, are recovered in lipid extracts of *P. gingivalis*, but lipid 430 is recovered in low levels compared with lipid 654.



1



2

We have previously observed catalytic deesterification of *P. gingivalis* lipid 654 to lipid 430 using commercially available preparations of phospholipase A2 (PLA2).[6] PLA2 is an important enzyme in the release of arachidonic acid from glycerol-based phospholipids and levels of this enzyme are increased in chronically inflamed tissues. However, it was noted that a portion of lipid 654 isolated from *P. gingivalis* was not hydrolyzed by PLA2. Chiral fractionations show about 10-15% of one isoform and 85-90% of the other isoform of 654 in the *P. gingivalis* 654. Recent HPLC analysis with direct infusion into the mass spectrometer suggest that the S isoform is less than 10%. These results suggest that enzymatic hydrolysis is stereospecific.

The lipid 654 isolated from *P. gingivalis* contains only L-serine as determined by chiral GC-MS analysis.[6] The absolute configuration of the β carbon of 3-OH iso $C_{17:0}$ is unknown in the bacterial lipid 654 isolated from *P. gingivalis*. Our initial structural identification of **2** and of **1** was by ^{1}H NMR and mass spectral analysis. Identification of the stereochemistry of **1** and **2**, as well as confirmation of which isoform is selectively hydrolyzed, required verification by total synthesis for enzymatic hydrolysis studies.

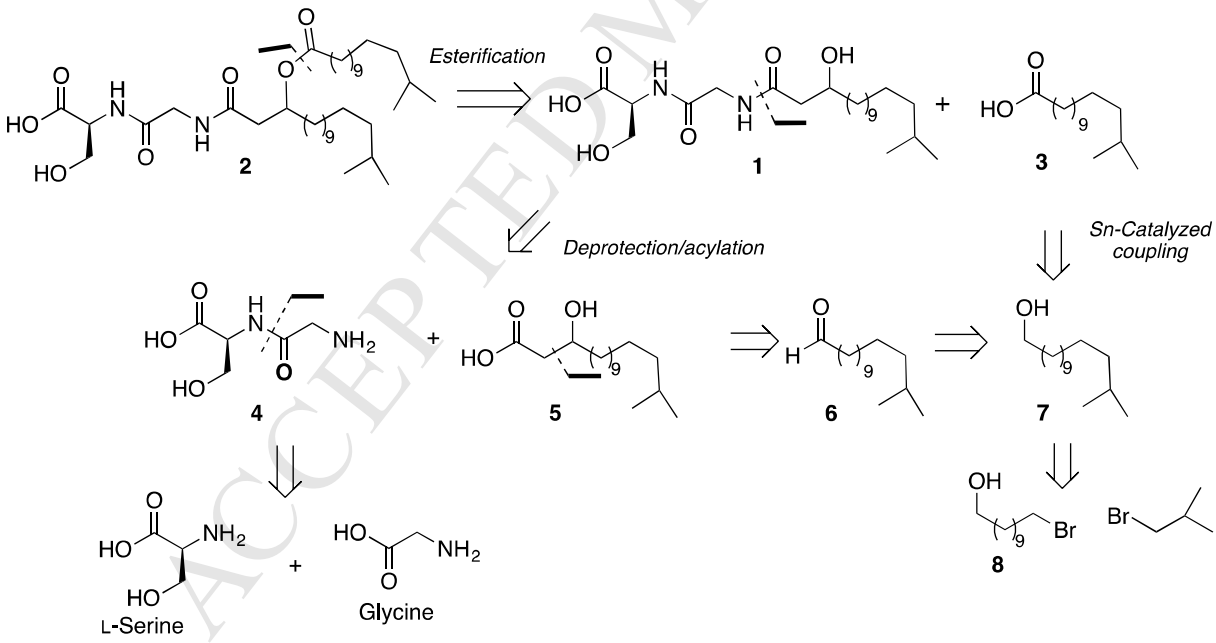
This investigation prepared both (3*R*) and (3*S*)-L-serine-lipid 430 and thereby both (3*R*) and (3*S*)-L-serine-lipid 654 to ascertain if only one diastereomeric form of synthetic lipid 654 was susceptible to PLA2 enzymatic hydrolysis. If selective hydrolysis is observed, the

diastereospecificity of the partial hydrolysis of *P. gingivalis* lipid 654 by PLA2 would be confirmed. The biological activity associated with lipid 654 and lipid 430[2,6] is sufficiently important that synthesis is a priority for structural verification, the accumulation of synthetic standards, and the preparation of each lipid in sufficient quantity for further biological evaluation.

Structure **2** has been reported as an isolate from several natural products, and it has been synthesized several times. Uchida and coworkers isolated dipeptide lipids from *Flavobacterium* sp. No. 3559, and the compound labeled WB-3559 D was identified as **2**.[7] They reported a linear synthesis of **2** from D-glucose, via a β-hydroxy aldehyde that was oxidized to the acid. Glycine and L-serine were attached sequentially in subsequent steps. In this work, WD-3559 D was shown to be a platelet aggregation inhibitor, and stimulated mouse plasma euglobulin clot lysis time.[7b] Kawai and coworkers identified a serine-containing lipid along with ornithine-containing lipids from extractable cellular lipids in *Flavabacterium meningosepticum* and *F. indologes*, which are opportunistic pathogens.[8] The serine-containing lipid was identified as **2**[8a] and shown to be an immunoactivator that exhibited immuomodulator activity,[8b] and it was also identified as a blocking agent against endotoxemia.8c Finally, Andoh and coworkers[9] isolated a dipeptide lipid from strain B-572 of *Flexibacter topostinus* sp. nov. and it was named topostin D640, with structure **2**, L-serine, (3*R*). Shioiri and coworkers synthesized topostin D640 in 13 steps in a linear synthesis that utilized asymmetric hydrogenation of a β-keto ester intermediate.[10] Topostins are inhibitors of mammalian DNA atopoisomerase I (topo I).

Uchida and coworkers reported a synthesis of WB-3559 D[7c] in which (3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxole-5-carbaldehyde[11] was prepared in three steps from D-glucose. Separate preparation of the triphenylphosponium ylid from 1-chloro-11-

methyldodecane allowed Wittig olefination and then hydrogenation to attach the isobranched lipid chain. Deprotection and $NaIO_4$ cleavage of the diol led to (3*R*)-hydroxy-15-methylhexadecanal. Protection of the alcohol, oxidation of the aldehyde with Ag_2O and deprotection gave (3*R*)-hydroxy-15-methylhexadecanoic acid, **5**. The fatty acid side chain was prepared from isobutyraldehyde and 10-carboxydecyltriphenylphosphorane, and coupled to **5**. Sequential coupling with protected glycine and then a protected serine gave (3*R*)-**1** after deprotection.[7c] Uchida and coworkers also prepared a derivative of **5** via a Reformatsky reaction of 3-hydroxy-15-methylhexadecanal, added the fatty ester moiety and final coupling with a glycine-serine dipeptide gave **1** in about 13 linear steps from the aldehyde (16 steps from D-glucose).[7b]



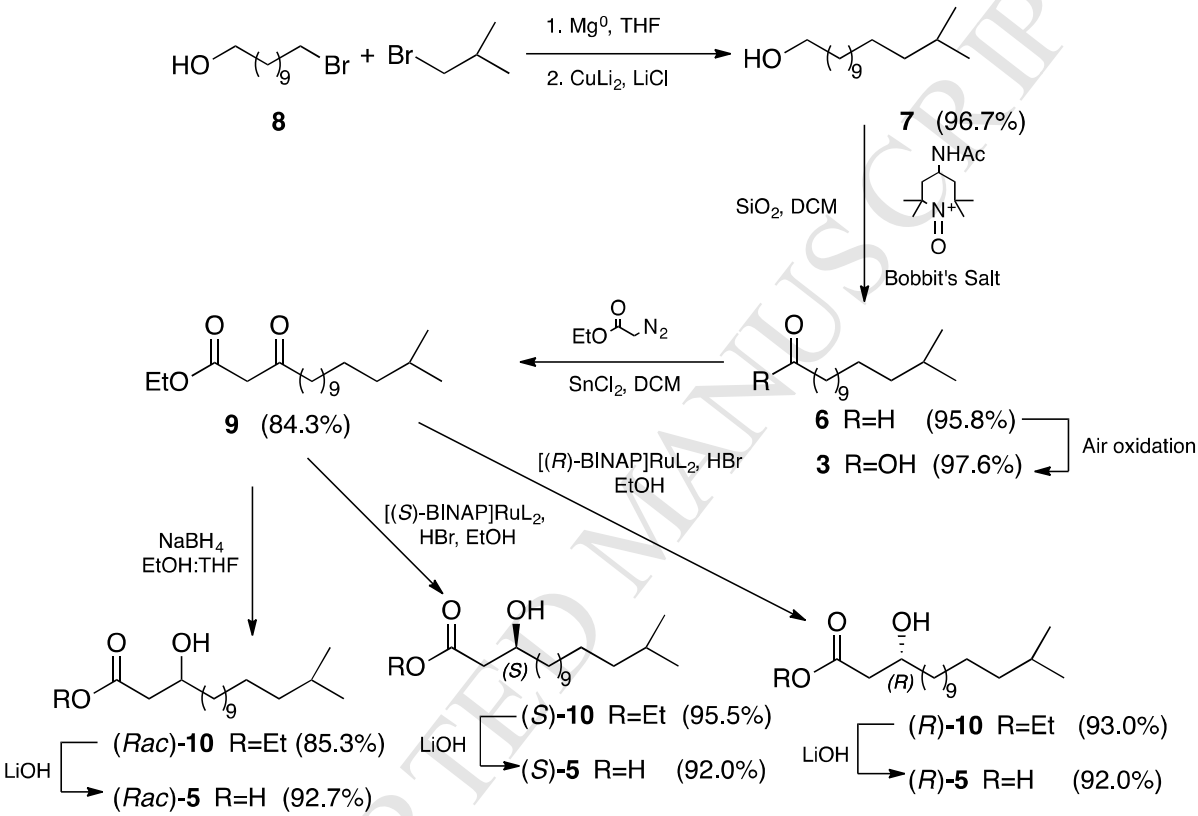
Scheme 1. Convergent Retrosynthetic Analysis of **1** and **2**

Shiozaki and coworkers prepared (3*R*)-**5** in 7 steps from (3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxole-5-carbaldehyde (10 steps from D-glucose) and coupling using both L-serine and D-serine gave (3*R*, L -serine)-**1** and (3*R*, D-serine)-**1**.[12] Subsequently, the structure of flavolipin was revised and (3*R*)-**5** and (3*S*)-**5** were prepared by this route and each was coupled with glycine and then serine. In this work, it was shown that flavolipin was (3*R*,L-serine)-**2**, which had the same macrophage stimulation activity as natural flavolipin, but the (3*S*,D-serine) diastereomer was nearly inactive.[12b]

Shioiri and coworkers published a 13-step synthesis of topostin D654 from decane-1,10-diol.[10] Initial conversion to **7** was followed by oxidation to **3** allowed coupling with the magnesium enolate anion of ethyl hydrogen malonate to give **9**. Asymmetric hydrogenation using a Ru-BINAP catalyst system gave (3*R*)-**10**, which was saponified to give **5** and coupled sequentially with a projected glycine followed by a protected serine. Deprotection gave topostin D654, which is (3*R*)-**2**.[10]

The synthetic goal was to verify the structural identity of lipid 654 as **2**, which would correlate with the previously identified and synthesized flavolipin. The previous syntheses produced hydroxy acid **5**, which could be converted to serine-glycine derivative **1**, which we believed to be lipid 430. While the stereochemistry of our lipid 430 and lipid 654 were unknown, our previous work indicated that the serine dipeptide lipids of *P. gingivalis* are primarily constituted with L-serine.[6] This assumption is also consistent with previous reports of **2** by Uchida, Shiozaki, and Shioiri. In our previous synthesis of lipids from *P. gingivalis*, we prepared **7** by a $LiCuCl_4$-Grignard coupling that was readily oxidized to aldehyde **6**. This aldehyde could be oxidized to **3** or coupled to ethyl diazoacetate to give **9**.[13] We therefore envisioned a convergent strategy that utilized elements of our previous work that would take

advantage of the previously published syntheses to prepare **1** and then **2** using a protected L-serine-glycine dipeptide. We believed that this approach would allow us to generate both diastereomers of L-serine **1** and of L-serine **2**, using L-serine, and in a shorter linear synthesis than previously reported.



Scheme 2. Synthesis of **5**

Our retrosynthesis began with cleavage of the labile ester linkage from the putative structure for lipid 654 (**2**), which generated the putative structure of lipid 430 (**1)** and 13-methyltetradecanoic acid (**3)**. Disconnection of the peptide linkage to the β-hydroxy fatty acid moiety in **2** gave the L-serine-glycine dipeptide **4** and β-hydroxy acid derivative **5**. Disconnection of **5** led to an acetate surrogate and 13-methyltetradecanal, **6**. Disconnection of **4**

led to serine and glycine, to be prepared by coupling a serine derivative with carboxyl and alcohol protection with a nitrogen-protected glycine. Aldehyde **6** can be prepared from 13-methyltetradecan-1-ol (**7**) using our previously published coupling of the Grignard reagent prepared from 1-bromo-2-methylpropane with 11-bromoundecan-1-ol, **8**.[14] We planned a coupling reaction of ethyl diazoacetate with **6** to give a β-keto ester.[13,14] Subsequent reduction of the ketone, followed by saponification, would generate the racemic β-hydroxy acid, **5**. One approach could use the asymmetric hydrogenation procedure reported by Shioiri.[10] Alternatively, a classical resolution of this β-hydroxy fatty acid would yield each enantiopure diastereomer.

Results and Discussion

The initial steps of the synthesis focused on the fatty acid portion of the targeted lipids and the preparation of **7**, based on our published synthesis of dihydroceramides isolated from *P. gingivalis*.[14] The Grignard reagent prepared from 1-bromo-2-methylpropane was coupled to **8**, in the presence of a lithium tetrachlorocuprate complex with *N*-methylpyrrolidone as an additive.[14] A large excess of Grignard reagent was used to convert the alcohol to an alkoxide *in situ*, thus avoiding the use of a protecting group. The use of excess reagent can be avoided if 11-bromoundecanol is protected prior to the coupling reaction. In the latter case, however, there was a decrease in overall yield for the three steps. The fact that 2-methylbromopropane is much cheaper than the other reagents therefore led us to use the unprotected alcohol in the coupling step. Oxidation of alcohol **7** with an oxoammonium reagent "Bobbitt's salt"[15a] (4-acetamido-2,2,6,6-tetramethylpiperidine 1-oxyl)[15b,c] yielded aldehyde **6** with no over-oxidation to the acid. Upon exposure to air at ambient temperature, this aldehyde proved to be rather unstable,

however, with rapid oxidation to the carboxylic acid **3**. If aldehyde **6** was maintained in wet THF solution until needed, the yield was near quantitative for the oxidation step and 91% from **8**.

Racemic β–hydroxy ester **9** was prepared by the reaction of **6** with ethyl diazoacetate in the presence of catalytic tin (II) chloride, using the protocol developed by Roskamp et al.[13,14] Ester **9** was obtained in 84% yield under very mild conditions, although small amounts of carboxylic acid **3** were also observed. Reduction of the ketone moiety in β-keto ester **9** with sodium borohydride generated racemic β–hydroxy ester **10**. Saponification of **10** using lithium hydroxide yielded racemic β-hydroxy acid **5** in an overall yield of 62% from **8**.

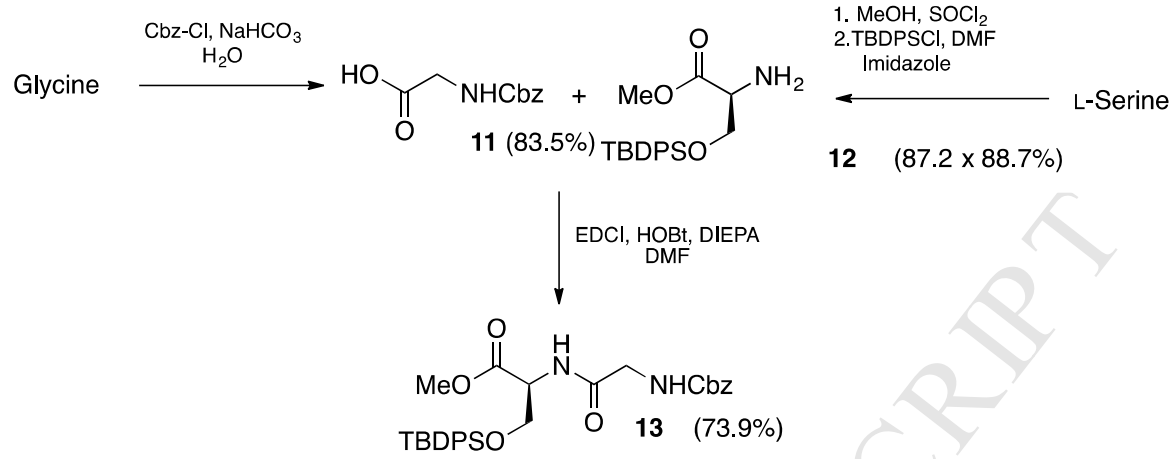
A traditional resolution of racemic **5** using brucine was also attempted. Racemic β-hydroxy acid **5** was heated at reflux with brucine in different polar solvents (methanol, ethanol, acetone, and chloroform). We obtained crystals from the reaction in chloroform, but all attempts to separate the diastereomeric salts failed because the resolving agent co-crystallized with both diastereomers under all conditions examined. We therefore abandoned this approach.

The best route to enantioenriched **5** was the asymmetric hydrogenation of β–keto ester **9**, as reported by Shioiri.[10] Catalytic hydrogenation of the ketone moiety in **9** using ruthenium catalyst complexed with (*S*)-BINAP or (*R*)-BINAP as chiral ligands led to β-hydroxy esters (3*S*)-**10** and (3*R*)-**10** respectively. Shioiri reported the specific rotation of (3*R*)-**10** to be $[\alpha]_D^{23} = -12.5°$ (c 1.02, $CHCl_3$).[10] Our synthetic (3*R*)-**10** to be $[\alpha]_D^{23} = -9.2°$ (c 1.02, $CHCl_3$) and synthetic (3*S*)-**10** to be $[\alpha]_D^{23} = +11.8°$ (c 1.02, $CHCl_3$).

To confirm the stereoselectivity of each ester, we converted each ester to the alcohol, **5**, by LiOH saponification conditions to yield (3*R*)-**5** in 68 % yield and (3*S*)-**5** in 70 % yield, respectively, from **8**. The specific rotation of our synthetic β-hydroxy fatty acid (**5**) was

determined to be $[\alpha]_D^{23}$ +14.5° (c 1.0, $CHCl_3$) for (3*S*-**5**) and $[\alpha]_D^{23}$ –10.5° (c 1.0, $CHCl_3$) for (3*R*-**5**). There is a small variation in the specific rotation reported in the literature for (3*R*-**5**): $[\alpha]_D^{21}$ 10° (c 0.05, $CHCl_3$);[9] $[\alpha]_D^{23}$ –12.0° (c 1.0, $CHCl_3$)[7b,c] To confirm our identification, the corresponding Mosher's ester was prepared for both (3*R*)-**5** and (3*S*)-**5**. Based on Mosher ester analysis, the enantiopurity of (3*R*)-**5** was 97% ee and (3*S*)-**5** was 98% ee. Based on analysis of the hydroxy acids **5**, and (3*R*)-**10** was formed in at least 97 %ee and (3*S*)-**10** was formed in at least 98 %ee.

We also attempted to generate enantiopure or enantioenriched **5** via enzymatic resolution of racemic **5**. Sugai and Ohta reported the use of a *Pseudomonas* lipase with vinyl acetate for the enzymatic transesterification of 2-hydroxytetracosanoic acid.[16a,b] Mori and coworkers used lipase PS and vinyl acetate to resolve racemic **5**, and used the resulting (3*R*)-hydroxy-15-methylhexadecanoic acid in a synthesis of sulfobactin.[16c] We applied this enzymatic resolution technique to compound **5**, but we used the commercially available Amano lipase PS. Our measured specific rotation data for (3*R*+*3S*)-**5** obtained by the enzymatic hydrolysis was $[\alpha]_D^{23}$ = +6.4° (c 1.02, $CHCl_3$). Saponification of the acetate product gave (3*R*+*3S*)-**5** and $[\alpha]_D^{23}$ = –8.7° (c 1.02, $CHCl_3$). After several attempts, our best result provided (3*R*)-**5** in about 67% ee and (3*S*)-**5** in about 79% ee, based on conversion to the respective Mosher's ester followed by ^1H NMR analysis that showed a 84*R*:15*S* mixture for (3*R*)-**5** and a 11*R*:89*S* mixture for (3*S*)-**5**. We modified the exposure time of racemic **5** to the lipase, but we observed no improvement in enantiomeric enrichment.



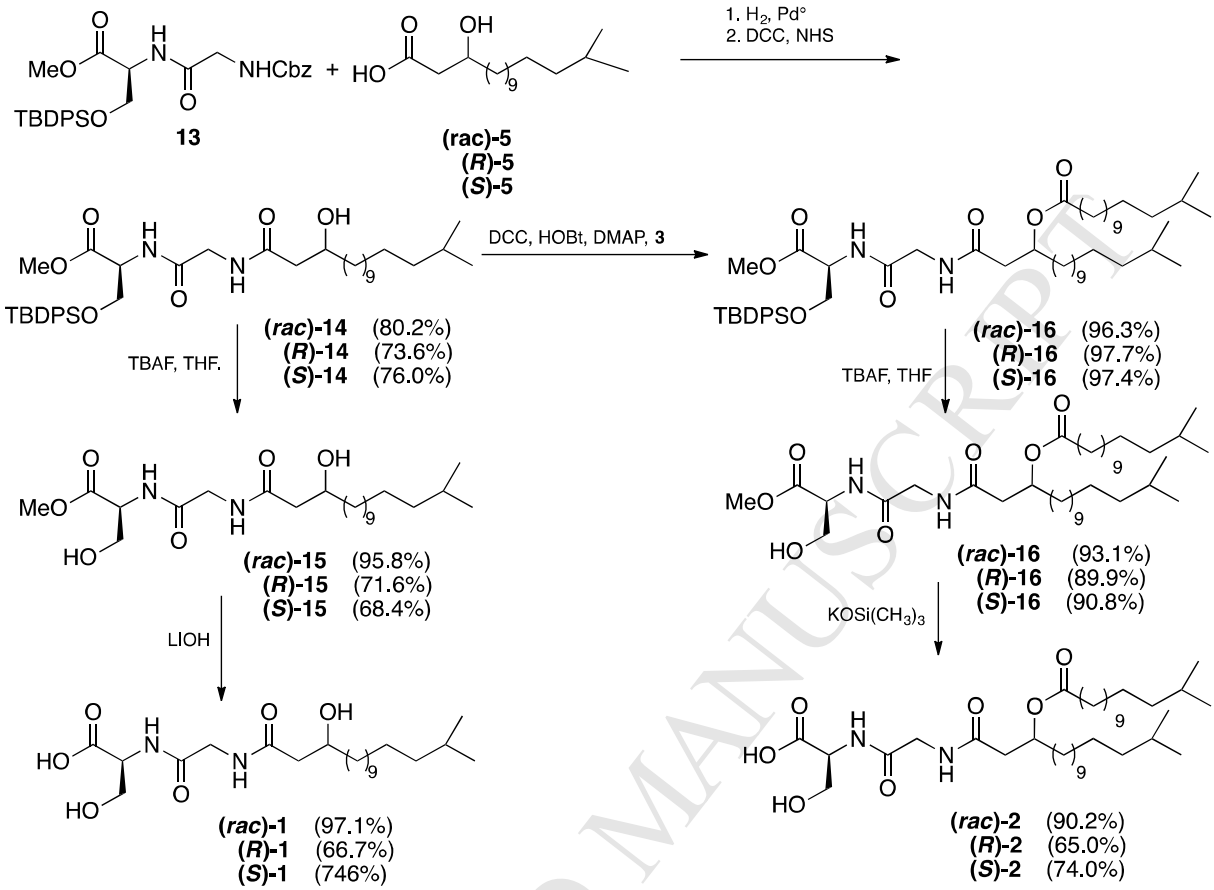
Scheme 3. Synthesis Protected Dipeptide **13**

The glycine-serine dipeptide portion of lipid 654 was prepared using standard methodology.[17] The amino group of glycine was protected as the benzyl carbamate to produce amino acid derivative **11**. Both the carboxylic acid and alcohol moieties of L-serine required protection to avoid undesired byproducts in the planned couplings. The reaction of L-serine with thionyl chloride in methanol gave the L-serine methyl ester,[17] which reacted with *tert*-butyldimethylsilyl chloride to give the Boc-protected derivative **12**. Dipeptide **13** was prepared by coupling the amino portion of **12** to the carboxyl group of **11** using DCC, where HOBt was used to generate the needed activated ester.

The coupling reaction of deprotected **14** and **5** proved challenging. Peptides with free amines, especially ones containing glycine, are known to undergo cyclisation to form a diketopiperazine derivative.[18] Dipeptides substituted with an amine at one terminus and an ester at the other can spontaneously cyclize to form a 2,5-DKP at a range of pHs.[18] Deprotection of the amino moiety by hydrogenation of dipeptide **13** using a palladium catalyst gave almost exclusively the DKP product and little coupling to give **14**. Formation of this unwanted product could be partially suppressed with the addition of methanolic HCl to protonate the amine, but we

were unable to isolate **14** free of DKP. Attempts to couple the HCl salt of **14** with β–hydroxy acid **5** failed and coupling was only observed upon neutralization of the amine salt. The addition of a stoichiometric amount of base led to the DKP and the yield our coupling product was only 30-40%.

Methodology developed by Shute and Rich for the coupling of dipeptides to tetrapeptides provided a solution to the coupling problem.[19] Activation of acid **5** with *N*-hydroxysuccinimide[7a] formed the reactive ester and this crude product was added to the hydrogenolysis product of dipeptide **13.** The presence of the activated ester facilitated the production of (3*R*+3*S*)-**14**. Subsequent deprotection with TBAF followed by LiOH saponification gave (3*R*+3*S*)-**2** (75 % yield). Using an identical procedure beginning with (3*R*)-**5**, we obtained (3*R*)-**2** in 68 % yield), and using (3*S*)-**5** in this sequence gave (3*S*)-**2** in 71 % yield. Spectroscopic evaluation demonstrated that (3*R*)-**2** correlates with the proposed structure of lipid 430 and the biological evaluation (see below) confirmed this conclusion.



Scheme 4. Synthesis of **1** and **2** from **5** and **13**

The final synthetic step was esterification of the alcohol moiety in (3*R*+3*S*)-**14** with fatty acid **3**. A DCC mediated coupling gave product (3*R*+3*S*)-**15** in > 90% yield. The sensitivity of the ester linkage in the lipid 654 derivatives led to deprotection of **14** and **16** using two different methods. Lipid derivative (3*R*+3*S*)-**14** was deprotected in a straightforward manner in two steps. First, the silyl ether was removed using tetrabutylammonium fluoride to liberate the hydroxyl derivative of the serine moiety. After purification, this product was subjected to saponification with lithium hydroxide to yield (3*R*+3*S*)-**1** in 87% yield over the two steps.

The desilylation of lipid derivative (3*R*+3*S*)-**16** was done in the same manner as before but a different hydrolysis of the methyl ester was used because saponification of the fatty acid

occurred under basic conditions. After treatment with TBAF, reaction with potassium trimethylsilanolate led to (3*R*+3*S*)-**2**. This S_N2 type of deprotection was successful, but the yield was lower over the two steps, giving 66% of (3*R*+3*S*)-L-serine-**2**. In an identical manner, (3*R*)-**16** led to (3*R*)-L-serine-**2** (65% overall yield) and (3S)-**16** led to (3*S*)-**2** (78% overall yield). Spectroscopic analysis of (3*R*)-L-serine-**2** showed that it correlates structurally with lipid 654. The specific rotation of **2** from various literature syntheses is quite varied: $[\alpha]_D^{23}$ = +19.75° (c 0.8, $CHCl_3$),[7a] $[\alpha]_D^{23}$ = +19.5° (c 0.3, $CHCl_3$),[7c] $[\alpha]_D^{23}$ = +17.1° (c 2.0, $CHCl_3$),[9] $[\alpha]_D^{23}$ = +12.8 and +13.3° (c 0.13; 0.14, $CHCl_3$; prepared from different precursors,[12a] $[\alpha]_D^{23}$ = +18.9° (c 0.39, $CHCl_3$).[12b] and $[\alpha]_D^{23}$ = +18.4° (c 0.4, $CHCl_3$).[10] The specific rotation of our synthetic (3*R*)-L-serine-**2** was $[\alpha]_D^{23}$ = +12.8$_5$° (c 1.02, $CHCl_3$) and the specific rotation of our (3*S*)-L-serine-**2** was $[\alpha]_D^{23}$ = +6.3° (c 1.02, $CHCl_3$). Our specific rotation data was consistent with some literature reports but showed a discrepancy relative to others. However, the enantiopurity of **10** and **5** is clear based on our Mosher's ester analysis, and there is no evidence of racemization in the subsequent conversion to **1** and to **2**.

Enzymatic Hydrolysis of Synthetic Lipid 654 ((2*R*)-L-serine-2)

HPLC purified synthetic (3*R*)-L-serine-**2** and (3*S*)-L-serine-**2**[6,4] were subjected to enzymatic hydrolysis using a commercially available PLA2 preparation. Approximately 250 ng of each synthetic lipid was sonicated in Tris buffer (10 mM, pH 7.5, 1 mL) containing 150 mM NaCl and 2.5 mM $CaCl_2$. Control samples received no enzyme. Approximately 100 U of porcine pancreatic PLA2 (Sigma) was added and the samples were stirred for 5 days at 25°C. At

5 days, the samples were acidified with glacial acetic acid and extracted with $CHCl_3$ (1 mL x 3 extractions). The pooled extracts were dried and suspended in HPLC solvent (hexane:isopropanol:water, 6:8;0.75, v/v/v). The samples were evaluated using MRM-MS as previously described[6,4] and the ratio of lipid 430 to lipid 654 was determined for both control and enzyme treated samples. The results in Figure 1 show that the (3*R*) diastereomer of synthetic lipid 654, (3*R*)-L-serine-**2**, is hydrolyzed by PLA2 but the (3*S*)-L-serine-**2** is not. Of note, we recently reported that the lipid 430 recovered following porcine pancreatic PLA2 hydrolysis of *P. gingivalis* lipid 654 is a potent inhibitor of osteoblast function both *in vivo* and *in vitro*.[6]

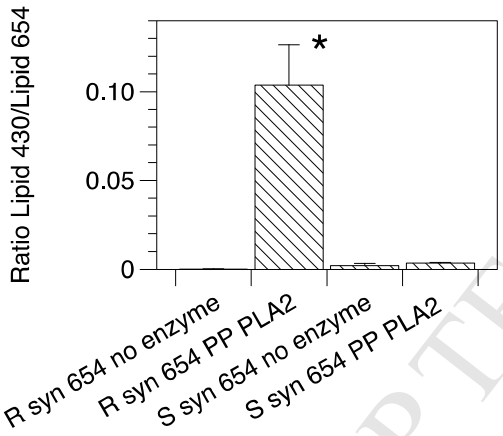


Figure 1. Hydrolysis of (3*R*)-L-serine-**2** and (3*S*)-L-serine **-2** by porcine pancreatic (PP, secretory) PLA2. Each lipid preparation was aliquoted (250 ng/sample) and the samples were dried. Samples were processed as described in the text and each trial was run in triplicate. Multiple Reaction Monitoring (MRM) was performed using a Sciex QTrap 4000. The 654.3-381.3 or 430.3- 382.3 *m/z* transitions were used to calculate the lipid 430/lipid 654 ratios. Hydrolysis of (3*R*)-L-serine-**2** (R syn 654) was significantly different (denoted by *) from all other trials by one factor ANOVA with Scheffe contrasts comparisons. No other pairwise comparisons were significantly different.

Conclusions

We have prepared (3*R*)-L-serine-**2** and (3*R*)-L-serine **1**, as well as (3*S*)-L-serine-**2** and (3*S*)-L-serine **1** by a convergent synthetic route that is significantly shorter than other published routes, producing the targeted compounds in higher yield. Based on sour total synthesis and biological comparison with natural bacterial lipid we have demonstrated that lipid 654 is (3*R*)-L-serine-**2** and lipid 430 is (3*R*)-L-serine-**1**. In summary, the convergent synthesis described herein confirms the proposed structures of lipid 654 produced from *P. gingivalis*. Our convergent synthesis gave lipid 430, (3*R*)-L-serine-**1**, in 8 linear steps and 24.7% overall yield, using the protected dipeptide **13** that was prepared separately. We similarly prepared (3*S*)-L-serine-**1** in 8 linear steps and 26.8% overall yield. Lipid 654, (3*R*)-L-serine-**2**, was prepared in 9 linear steps and 34% overall yield. We similarly prepared (3*S*)-L-serine-**2** in 9-linear steps and 31.1% overall yield. Using the same approaches, (3*R*+3*S*)-L-serine-**1** was prepare in 8 linear steps and 49.9% overall yield and (3*R*+3*S*)-L-serine-**2** was prepared in 9 linear steps ad 46.3% overall yield.

Demonstration that there is a difference in enzymatic reactivity with the different diastereomers allows us to use the (*R*) and (*S*) synthetic preparations of lipid 654 to evaluate biological properties of enantioenriched or enantiopure lipids to engage the innate immune system, including the engagement of Toll-like receptor 2. Furthermore, it is important to determine the levels of (*R*) and (*S*) lipid 654 present in other members of the *Bacteroidetes* phylum as well as the levels of these isoforms in diseased tissues where lipid 654 is known to accumulate. These studies have begun, and the results will be the subject of a future communication.

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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[15] 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. 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 of synthetic **2** and **1** 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 **2** and **1**. Lipid samples were injected into a triple quadrupole mass spectrometer, 4000 QTrap from Sciex (Foster City, CA). IR spectra were taken on FT/IR-410/C031560585 JASCO and 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 Uni-melt capillary melting point apparatus or Digimelt MPA160. Perfect. For products described as waxy solid or semi-solids, melting points could not be obtained.

Methyl L-serinate

Thionyl chloride (2.75 mL, 37.8 mmol) was added, dropwise, to a suspension of L-serine (4.015 g, 38.2 mmol) in 50 mL of dry methanol. The solution was heated at reflux with stirring for 5 h and then cooled to ambient temperature. The solvent was removed *in vacuo* and the crude white solid was redissolved in a minimal amount of methanol. This solution was treated with an equal volume of toluene and the flask was swirled periodically to induce crystallization. Filtration yielded methyl L-serinate as a crystalline solid (3.9675 g, 33.3 mmol, 87.2%).[20] MP, 161-162 °C. ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.14 (t, J=3.9 Hz, 1H), 4.01 (dd, J=11.8, 4.5 Hz, 1H), 3.93 (dd, J=11.8, 3.1 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 169.4, 60.7, 56.1, 53.71.

Methyl *O*-(*tert*-butyldiphenylsilyl)-L-serinate 12

A stirring solution of methyl L-serinate (2.003 g, 16.8 mmol) in 15 mL of DMF was treated with imidazole (3.435 g, 50.4 mmol).[17] (*tert*-Butyl)diphenylsilyl chloride (4.80 mL, 18.5 mmol) was added, dropwise, and the reaction was stirred overnight at ambient temperature. At this time, the solvent was removed *in vacuo* and the resulting oil was dissolved in 100 mL of diethyl ether. This solution was washed with water (5 X 400 mL), 10 % LiCl (2 X 100), and brine (50 mL). Drying with $MgSO_4$, filtering, and concentration under reduced pressure yielded methyl *O*-(*tert*-butyldiphenylsilyl)-L-serinate, **12**, as a yellow oil (5.331 g, 14.9 mmol, 88.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (td, *J* = 7.7, 1.6 Hz, 4H), 7.50-7.35 (m, 6H), 3.98 (dd, *J* = 9.9, 4.3 Hz, 1H), 3.89 (dd, *J* = 9.9, 3.8 Hz, 1H), 3.71 (s, 3H), 3.58 (t, *J* = 4.1 Hz, 1H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.6, 133.1, 133.0, 129.9, 127.8, 127.7, 66.1, 56.5, 52.1, 26.8, 19.3. HRMS (AccuTOF): [MH]+ Calc'd for $C_{20}H_{28}NO_3Si$ *m/z* 358.1838. Found: *m/z* 358.1819.

((Benzyloxy)carbonyl)glycine [*N*-Cbz-Glycine], 11

Glycine (3.000 g, 40.0 mmol) and sodium bicarbonate (6.710 g, 79.9 mmol) were dissolved in 100 mL of H_2O at ambient temperature and this solution was cooled to 0 °C using an ice bath. Benzyl chloroformate (8.56 mL, 59.9 mmol) in 25 mL of dioxane was added, dropwise. This solution was slowly warmed to ambient temperature and stirred overnight. The reaction mixture was transferred to a separatory funnel and washed with EtOAc (2 X 50 mL). The aqueous layer was acidified with 10 % HCl to a pH=1 (to pH paper) and extracted with EtOAc (3 X 50 mL). The later organic layers were dried, filtered, and solvents evaporated at reduced pressure to yield ((benzyloxy)carbonyl)glycine, **11**,[21] as a white solid (6.982 g, 33.4 mmol, 83.5 %), MP, 112-114 °C. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.40–7.24 (m, 5H), 5.10

(s, 2H), 3.84 (s, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.6, 159.1, 138.1, 129.4, 129.0, 128.8, 67.7, 43.1.

Methyl *N*-(((benzyloxy)carbonyl)glycyl)-*O*-(*tert*-butyldiphenylsilyl)-L-serinate, 13

A solution of ((benzyloxy)carbonyl)glycine (1.748 g, 8.36 mmol) in 10 mL of DMF was cooled to 0°C using an ice bath. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 1.603 g, 8.36 mmol), HOBt (1.280 g, 8.36 mmol) and then diisopropylethylamine (DIPEA, 1.46 mL, 8.36 mmol) were added in that order. The solution was stirred for 15 min before the addition of methyl *O*-(*tert*-butyldiphenylsilyl)-L-serinate, **12** (2.988 g, 8.36 mmol). The reaction was warmed to ambient temperature and stirred for 48 h. At that time, the DMF was removed *in vacuo* and the resulting oil was dissolved in 100 mL of diethyl ether. The ether was washed with water (5 X 400 mL), saturated $NaHCO_3$ (3 X 100) mL, and brine (50 mL). The organic layer was dried, filtered, and solvents evaporated at reduced pressure. Purification by column chromatography (1 % MeOH:DCM) yielded (((benzyloxy)carbonyl)glycyl)-*O*-(*tert*-butyldiphenylsilyl)-L-serinate**, 13**, as a clear oil (3.390 g, 6.18 mmol, 73.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (t, *J* = 7.6 Hz, 4H), 7.49–7.34 (m, 6H), 6.77 (d, *J* = 6.9 Hz, 1H), 5.36 (s, 1H), 5.12 (q, *J*= 12.3, 7.6 Hz, 2H) 4.68 (dt, *J* = 8.3, 2.9 Hz, 1H), 4.13 (dd, *J* = 10.3, 2.8 Hz, 1H), 3.93–3.75 (m, 3H), 3.74 (s, 3H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 168.6, 156.4, 136.1, 135.5, 132.8, 132.6, 130.1, 128.6, 128.2, 128.1, 127.9, 127.8, 67.3, 64.2, 54.1, 52.5, 44.4, 26.7, 19.3. HRMS (AccuTOF): [MH]+ Calc'd for $C_{30}H_{37}N_2O_6Si$ *m/z* 549.2421. Found: *m/z* 549.2423.

13-Methyltetradecan-1-ol, 7

Magnesium turnings (2.90 g, 0.119 mol) were added to a 500 mL round bottom flask with a stirbar and flame-dried under a nitrogen atmosphere. After cooling, the Mg turnings were suspended in dry THF (50 mL) and 1-bromo-2-methylpropane (8.00 mL, 0.0736 mol) was rapidly added with vigorously stirring. Upon initiation, dry THF (50 mL) was added and the flask was immersed in an ice bath to modulate the exothermic reaction. The reaction was stirred until the flask cooled to ambient temperature and the temperature was lowered to -78 °C using dry ice acetone bath. 11-Bromoundecan-1-ol (**8**, 4.005 g, 0.0172 mol) dissolved in THF (20 mL) and LiCl (1.53 g, 0.036 mol) and copper (II) chloride (2.54 g, 0.019 mol), dissolved in THF (30 mL), were added. The mixture was slowly warmed to ambient temperature and stirred overnight. The reaction was quenched with satd NH_4Cl (100 mL) before being transferred to a separatory funnel with diethyl ether (200 mL) and H_2O (100 mL). The layers were separated and the organic layer was washed with satd $NaHCO_3$ (50 mL X 3) and brine (50 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 (10% EtOAc:hexane) to yield 13-methyltetradecan-1-ol, **7**,[10,22] as a white solid (3.793 g, 0.017 mol, 96.7%), MP, 26-27 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (t, 2H), 1.63–1.44 (m, 3H), 1.27 (m, 18H), 1.17 (m, 3H), 0.86 (d, J = 6.6 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 63.3, 39.2, 33.0, 30.1, 29.9, 29.8, 29.8, 29.6, 28.1, 27.6, 25.9, 22.8.

13-Methyltetradecanal, 6

4-(Acetyamino)-2,2,6,6-tetramethyl-1-oxo-piperidinium tetrafluoroborate[15] (Bobbit's salt, 5.285 g, 0.0176 mol) was added to 13-ethyltetradecanol (**7**, 3.498 g, 0.0153 mol) dissolved in 250 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 13-methyltetradecanal, **6**,[23] as a clear solid, (3.319 g, 0.0147 mol, 95.8%), MP, ~25 °C. ^{1}H NMR(400 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.9 Hz, 1H), 2.41 (td, *J* = 7.4, 1.9 Hz, 2H), 1.63 (quin, *J* = 7.2 Hz, 2H), 1.51 (sep, 1H), 1.36–1.22 (m, 16H), 1.15 (m, 2H), 0.86 (d, *J* = 6.6 Hz, 6H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 13C NMR (101 MHz, $CDCl_3$) δ 203.0, 44.0, 39.1, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 28.0, 27.5, 22.7, 22.2.

13-Methyltetradecanoic acid, 3

13-Methyltetradecanal (**6**, 1.251 g, 5.53 mmol) was dissolved in 50 mL of THF, and 5 mL of H_2O and 0.5 mL of 1M HCl were added. The reaction mixture was stirred for 72 h at ambient temperature. The resulting solution was concentrated under reduced pressure, the residue dissolved in 15 mL of H_2O, and extracted with DCM (15 mL X 3). The resulting solution was filtered through a small plug of silica gel and rinsed with DCM before being concentrated *in vacuo* to yield 13-methyltetradecanoic acid[10,24] (**3**) as a white solid (1.307 g, 5.39 mmol, 97.6%), MP, 48-49 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (t, *J* = 7.5 Hz, 2H), 1.63 (quin, *J* = 7.4 Hz, 2H), 1.51 (sep, *J* = 6.6 Hz, 1H), 1.39–1.18 (m, 16H), 1.15 (q, *J* = 6.7 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 13C NMR (101 MHz, $CDCl_3$) δ 179.6, 39.1, 34.0, 30.0, 29.8, 29.7, 29.7, 29.5, 29.3, 29.1, 28.0, 27.5, 24.8, 22.7.

Ethyl 15-methyl-3-oxohexadecanoate, 9

Tin (II) chloride dehydrate (0.417 g, 1.85 mmol) was suspended in 15 mL of dry DCM. Ethyl diazoacetate (2.60 g, 19.4 mmol) was added, dropwise via syringe, and the reaction was stirred for 15 min. 13-Methylpentadecanal (**6**, 4.186 g, 18.5 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 satd 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 15-methyl-3-oxohexadecanoate,[10,25] **9**, as a clear solid (4.871 g, 0.0156 mol, 84.3%), MP, ~25 °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* = 7.3 Hz, 2H), 1.60 (quin, *J* = 7.2 Hz, 2H), 1.50 (sep, *J* = 6.5 Hz, 1H), 1.30-1.20 (m, 20H), 1.15 (q, *J* = 7.3, 6.8 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.0, 167.3, 61.4, 49.4, 43.1, 39.1, 30.0, 29.8, 29.7, 29.7, 29.5, 29.4, 29.1, 28.0, 27.5, 23.5, 22.7, 14.2.

Ethyl 3-hydroxy-15-methylhexadecanoate, 10

A solution of ethyl 15-methyl-3-oxohexadecanoate (**9**, 3.114 g, 0.001 mol) in 2.5 mL of ethanol and 22.5 mL of THF was cooled to 0 °C on an ice bath. Sodium borohydride (0.301 g, 0.008 mol) was added and the resulting slurry was stirred vigorously for 1 h. The reaction was quenched by the slow addition of 50 mL of a 10 % aq citric acid solution. Subsequent neutralization with satd K_2CO_3 solution was followed by extraction with EtOAc (3 X 50 mL). The organic layers were washed with 25 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-15-methylhexadecanoate,[10] **10**, as a clear oil (2.674 g, 0.0085 mol, 85.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (q, *J* = 7.1 Hz, 2H), 4.06–3.93 (m, 1H), 2.91 (d, *J* = 3.9 Hz, 1H), 2.50 (dd, *J* = 16.4, 3.1 Hz, 1H), 2.39 (dd, *J* = 16.4, 9.0 Hz, 1H), 1.60–1.20 (m, 23H), 1.15 (q, *J* = 6.6 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 68.1, 60.7, 41.4, 39.1, 36.6, 30.0, 29.8, 29.7, 29.6, 29.6, 28.0, 27.5, 25.5, 22.7, 14.2.

Ethyl (3*R*)-hydroxy-15-methylhexadecanoate, (3*R*)-10

In a nitrogen atmosphere glove box, (*R*)-BINAP (45 mg, 0.072 mmol) and bis(2-methylallyl)(1,5-cyclooctadiene)ruthenium(II) (20 mg, 0.063 mmol) were dissolved in 9 mL of freshly distilled acetone. Methanolic HBr (0.22 mL, 0.73 M, 0.16 mmol) was added, and the solution was stirred for 1 h. The reaction was concentrated to dryness *in vacuo* before being redissolved in 15 mL of ethanol and *β*-keto ester **9** (0.962 g, 3.08 mmol) was added. The system was flushed with hydrogen and heated at reflux for 48 h. The resulting solution was filtered and the solvents removed at reduced pressure. Subsequent purification via column chromatography (10% EtOAc:hexanes) yielded ethyl (3*R*)-hydroxy-15-methylhexadecanoate, (3*R*)-**10**[10] $[\alpha]_D^{23}$ – 9.2° (c 1.0, $CHCl_3$), as a clear solid (0.901 g, 2.86 mol, 93.0 %), MP, ~25 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (q, *J* = 7.2 Hz, 2H), 4.05–3.94 (m, 1H), 2.91 (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), 1.60–1.20 (m, 23H), 1.15 (q, *J* = 6.6 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 68.1, 60.7, 41.4, 39.1, 36.6, 30.0, 29.8, 29.7, 29.6, 29.6, 28.0, 27.5, 25.5, 22.7, 14.2. A ^{1}H NMR analysis that showed a 97*R*:03*S* mixture for (3*R*)-**5**.

Ethyl (3*S*)-hydroxy-15-methylhexadecanoate, (3*S*)-10

In a nitrogen atmosphere glove box, (*S*)-BINAP (45 mg, 0.072 mmol) and bis(2-methylallyl)(1,5-cyclooctadiene)ruthenium(II) (20 mg, 0.063 mmol) were dissolved in 9 mL of freshly distilled acetone. Methanolic HBr (0.22 mL, 0.73 M, 0.16 mmol) was added, and the solution stirred for 1 h. The reaction was concentrated to dryness *in vacuo* before being redissolved in 15 mL of ethanol and *β*-keto ester **9** (0.954 g, 3.05 mmol) was added. The system was flushed with hydrogen gas and heated at reflux for 48 h. The resulting solution was filtered and the solvents removed at reduced pressure. Subsequent purification via column chromatography (10% EtOAc:hexanes) yielded ethyl (3*S*)-hydroxy-15-methylhexadecanoate, (3*S*)-**10** as a clear solid (0.917 g, 2.92 mol, 95.5 %), MP, ~25 °C. $[\alpha]_D^{23}$ +11.8° (c 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (q, *J* = 7.1 Hz, 2H), 4.06–3.93 (m, 1H), 2.91 (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), 1.55–1.39 (m, 3H), 1.38–1.21 (m, 20H), 1.15 (q, *J* = 7.1, 6.6 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 68.1, 60.7, 41.4, 39.1, 36.6, 30.0, 29.8, 29.7, 29.6, 29.6, 28.0, 27.5, 25.5, 22.7, 14.2. A ^{1}H NMR analysis that showed a 02*R*:98*S* mixture for (3*S*)-**5**.

Enzymatic Resolution

Lipase PS (.275 g) was added to a stirred solution of (±)-**5** (5.50 g, 1.92 mmol) and 2,6-di-tert-butyl-4-methylphenol (5 mg) in vinyl acetate (8.8 mL), and stirred at 52-58 ˚C for 168 h. The resulting mixture was cooled to ambient temperature and filtered through Celite and concentrated under reduced pressure. Column chromatography (MeOH: DCM), yielded (3*R*)-5 (128 mg) as a white solid; $[\alpha]_{\mathrm{D}}^{23}$ +6.4° (c 1.02, $CHCl_3$). After saponification of the acetate

product, (3*S*)-**5** (177 mg) was purified to give a white solid; $[\alpha]_D^{23}$ –8.7° (c 1.02, $CHCl_3$). ^1H NMR spectra were identical with those of (±)-5.[16c] Conversion to the corresponding Mosher's ester as described above was followed by ^1H NMR analysis that showed a 84*R*:15*S* mixture for (3*R*)-**5** and a 11*R*:89*S* mixture for (3*S*)-**5**.

Ethyl (*R*)-15-methyl-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl) oxy)-hexadecanoate

Under a nitrogen atmosphere, (*S*)-(–)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (Mosher's acid,[26] 23 mg, 0.098 mmol) was dissolved in 2 mL of dry DCM and cooled to 0 °C in an ice bath. Subsequent addition of DCC (24 mg, 0.12 mol) was followed by stirring for 15 min, at which time ethyl (3*R*)-hydroxy-15-methylhexadecanoate, (3*R*)-**10** (29 mg, 0.092 mmol) was added, along with a catalytic amount of DMAP. The reaction was equilibrated to ambient temperature slowly and stirred overnight. Evaporation of solvents *in vacuo* was followed by addition of 15 mL of EtOAc and washing with H_2O 3 X 10 mL and 10 mL of satd brine. The resulting organic layer was dried over $MgSO_4$, filtered, and the solvents removed at reduced pressure. Subsequent purification via column chromatography (1-10% EtOAc:hexanes) yielded ethyl (*R*)-15-methyl-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)hexadecanoate as a clear oil (38 mg, 0.072 mmol, 78 %). ^1H NMR (400 MHz, $CDCl_3$) δ 7.55-7.50 (m, 2H), 7.42-7.37 (m, 3H), 5.52-5.45 (p, *J* = 6.4 Hz, 1H), 4.06 (q, *J* = 7.1 Hz, 2H), 3.53 (s, 3H), 2.64 (dd, *J* = 16.0, 7.9 Hz, 1H), 2.55 (dd, *J*= 16.0, 5.0 Hz, 1H) 1.80-1.60 (m, 2H), 1.55-1.47 (sep, *J* = 6.6 Hz, 1H), 1.36-1.12 (m, 23H), 0.86 (d, *J* = 6.6 Hz, 6H).

Ethyl (*S*)-15-methyl-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)-hexadecanoate

Under a nitrogen atmosphere, (*S*)-(–)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (Mosher's acid,[26] 23 mg, 0.098 mmol) was dissolved in 2 mL of dry DCM and cooled to 0 °C in an ice bath. Subsequent addition of DCC (23 mg, 0.11 mmol) was followed by stirring for 15 min, at which time ethyl (3*S*)-hydroxy-15-methylhexadecanoate, (3*S*)-**10** ((28 mg, 0.089 mmol) was added, along with a catalytic amount of DMAP. The reaction was equilibrated to ambient temperature slowly and stirred overnight. Evaporation of solvents *in vacuo* was followed by addition of 15 mL of EtOAc and washing with H_2O 3 X 10 mL and 10 mL of satd brine. The resulting organic layer was dried over $MgSO_4$, filtered, and the solvents removed at reduced pressure. Subsequent purification via column chromatography (1-10% EtOAc:hexanes) yielded ethyl (*R*)-15-methyl-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)hexadecanoate as a clear oil (45 mg, 0.084 mmol, 94 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50-7.55 (m, 2H), 7.37-7.42 (m, 3H), 5.48 (quin, *J* = 6.3 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.55 (s, 3H), 2.69 (dd, *J* = 16.0, 8.0 Hz, 1H), 2.59 (dd, *J*= 16.0, 4.8 Hz, 1H), 1.70-1.57 (m, 2H), 1.52 (sep, *J* = 6.6 Hz, 1H), 1.36-1.12 (m, 23H), 0.86 (d, *J* = 6.6 Hz, 6H).

3-Hydroxy-15-methylhexadecanoic acid, (3*R*+3*S*)-5

Hydroxy ester **10** (2.674 g, 0.0085 mol) was dissolved in 8 mL of MeOH and 16 mL of THF and 1 M aqueous LiOH (9.40 mL, 0.0094 mol) 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 50 mL). The organic layers were washed with 25 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-15-methylhexadecanoic acid,[10] (3*R*+3*S*)-**5,** as a white solid (2.257 g, 0.0079 mol, 92.7 %), MP, 54-56 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09–

3.98 (m, 1H), 2.58 (dd, *J* = 16.6, 3.2 Hz, 1H), 2.48 (dd, *J* = 16.6, 8.9 Hz, 1H), 1.59–1.43 (m, 3H), 1.35-1.20 (m, 18H), 1.15 (q, *J* = 6.7 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.4, 68.1, 41.2, 39.1, 36.6, 30.0, 29.8, 29.7, 29.6, 29.6, 29.5, 28.0, 27.5, 25.5, 22.7.

3(*R*)-Hydroxy-15-methylhexadecanoic acid, (3*R*)-5

Hydroxy ester **(3*R*)-10** (0.818 g, 0.0026 mol) was dissolved in 8 mL of methanol and 16 mL of THF and 1 M aqueous LiOH (2.9 mL, 0.0029 mol) 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 50 mL). The organic layers were washed with 25 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*R*)-3-hydroxy-15-methylhexadecanoic acid,[10] (3*R*)-**5,** as a white solid (0.708 g, 0.0024 mol, 92 %), $[\alpha]_D^{23}$ –10.5° (c 1.0, $CHCl_3$), MP, 54-55 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09–3.98 (m, 1H), 2.58 (dd, *J* = 16.6, 3.2 Hz, 1H), 2.48 (dd, *J* = 16.6, 8.9 Hz, 1H), 1.59–1.43 (m, 4H), 1.35-1.20 (m, 18H), 1.15 (q, *J* = 6.7 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.8, 68.1, 41.1, 39.1, 36.6, 30.0, 29.8, 29.7, 29.6, 29.5, 28.0, 27.5, 25.5, 22.7.

3(*S*)-Hydroxy-15-methylhexadecanoic acid, (3*S*)-5

Hydroxy ester **(3*S*)-10** (0.815 g, 0.0026 mol) was dissolved in 8 mL of methanol and 16 mL of THF and 1 M aqueous LiOH (2.9 mL, 0.0029 mol) 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 50 mL). The organic layers were washed with 25 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*S*)-3-hydroxy-15-methylhexadecanoic acid,[10] (3*S*)-**5,** as a white solid (0.679 g, 0.0024 mol, 92 %), $[\alpha]_D^{23}$ +14.5° (c 1.0, $CHCl_3$), MP, 54-55 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09–3.98 (m, 1H), 2.58 (dd, *J* = 16.6, 3.2 Hz, 1H), 2.48 (dd, *J* = 16.6, 8.9 Hz, 1H), 1.59–1.43 (m, 4H), 1.35-1.20 (m, 18H), 1.15 (q, *J* = 6.7 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.3, 68.1, 41.0, 39.1, 36.6, 30.0, 29.8, 29.7, 29.6, 29.5, 28.0, 27.5, 25.5, 22.7.

Methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate, (3*R*+3*S*)-L-serine-14

N-Hydroxysuccinimide (116 mg, 1.00 mmol) and dicyclohexylcarbodiimide (DCC, 216 mg, 1.05 mmol) were added to a stirring solution of 3-hydroxy-15-methylhexadecanoic acid (**5**, 250 mg, 0.873 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 5 mL of dioxane and this solution was added via cannula to a solution of methyl *N*-(((benzyloxy)carbonyl)glycyl)-*O*-(*tert*-butyldiphenylsilyl)-L-serinate (**13**, 598 mg, 1.09 mmol) and Pd/C (176 mg) in 10 mL of dioxane. 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-15-methylhexadecanoyl)glycyl)-L-serinate, (3*R*+3*S*)-L-serine-**14**, as a clear oil (0.478 g, 0.700 mmol, 80.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62-7.55 (m, 4H), 7.49–7.34 (m, 6H), two signals [6.86 and 6.99 (d, *J*= 7.0 and 8.1 Hz, 1H)], two signals [6.34 and 6.56 (d, *J*= 5.4 and 5.6 Hz, 1H), 4.67 (dt, *J* = 8.2, 3.0 Hz, 1H), 4.17–4.06 (m, 2H), 4.04–3.82 (m, 3H), 3.82–3.71 (m, 4H), 2.40 (dt, *J* = 14.7, 2.2 Hz, 1H), 2.26 (dd, *J* = 14.5,

9.4 Hz, 1H), 1.57-1.35 (m, 4H), 1.35-1.18 (m, 18H), 1.16 (dd, *J* = 13.6, 7.8 Hz, 2H), 1.03 (s, 9H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 172.8, 170.8, 168.9, 168.7, 135.5, 135.5, 132.9, 132.9, 132.7, 132.6, 130.0, 127.9, 127.9, 68.9, 68.9, 64.2, 54.3, 52.6, 43.1, 43.0, 43.0, 42.8, 39.1, 37.1, 30.0, 29.8, 29.7, 29.6, 29.6, 28.0, 27.5, 26.8, 25.5, 22.7, 19.3. HRMS (AccuTOF): [MH]+ Calc'd for $C_{39}H_{63}N_2O_6Si$ *m/z* 683.4455. Found: *m/z* 683.4470.

Methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3R-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate, (3*R*)-L-serine-14

N-Hydroxysuccinimide (96 mg, 0.830 mmol) and dicyclohexylcarbodiimide (DCC, 188 mg, 0.913 mmol) were added to a stirring solution of 3-hydroxy-15-methylhexadecanoic acid (3*R*)-**5**, 159 mg, 0.554 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 5 mL of dioxane and this solution was added via cannula to a solution of methyl *N*-(((benzyloxy)carbonyl)glycyl)-*O*-(*tert*-butyldiphenylsilyl)-L-serinate (**13**, 349 mg, 0.637 mmol) and Pd/C (122 mg) in 10 mL of dioxane. 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 *O*-(*tert*-butyldiphenylsilyl)-*N*-((3R-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate, (3*R*)-L-serine-14 as a clear oil (0.279 g, 0.405 mmol, 73.6%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.54 (m, 4H), 7.49 – 7.35 (m, 6H), 6.89 (d, *J* = 8.1 Hz, 1H), 6.39 (t, *J* = 5.6 Hz, 1H), 4.67 (dt, *J* = 8.2, 2.9 Hz, 1H), 4.18 – 4.06 (m, 2H), 3.97 (s, 1H), 3.94 – 3.77 (m, 2H), 3.75 (s, 3H), 3.36 (d, *J* = 3.6 Hz, 1H), 2.41 (dd, *J* = 14.6, 2.6 Hz, 1H), 2.27 (dd, *J* = 14.6, 9.4 Hz, 1H), 1.56 – 1.34 (m, 4H), 1.34 – 1.20 (m, 18H), 1.19 – 1.13 (m, 2H), 1.04 (s, 9H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 170.7, 168.6, 135.6, 135.5, 132.9, 132.6, 130.1, 128.0, 127.9, 77.4, 77.1, 76.8,

68.9, 64.3, 54.3, 52.6, 43.1, 42.9, 39.1, 37.2, 30.0, 29.8, 29.7, 29.6, 29.6, 28.0, 27.5, 26.8, 25.5, 22.7, 19.3. HRMS (AccuTOF): [MH]+ Calc'd for $C_{39}H_{63}N_2O_6Si$ *m/z* 683.4455. Found: *m/z* 683.4434.

Methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3S-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate, (3*S*)-L-serine-14

N-Hydroxysuccinimide (87 mg, 0.752 mmol) and dicyclohexylcarbodiimide (DCC, 171 mg, 0.827 mmol) were added to a stirring solution of 3-hydroxy-15-methylhexadecanoic acid ((3*S*)-**5**, 144 mg, 0.501 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 5 mL of dioxane and this solution was added via cannula to a solution of methyl *N*-(((benzyloxy)carbonyl)glycyl)-*O*-(*tert*-butyldiphenylsilyl)-L-serinate (**13**, 316 mg, 0.576 mmol) and Pd/C (111 mg) in 10 mL of dioxane. 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 *O*-(*tert*-butyldiphenylsilyl)-*N*-((3R-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate, (3*S*)-L-serine-**14** as a clear oil (0.261 g, 0.382 mmol, 76.0%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.54 (m, 4H), 7.49 – 7.34 (m, 6H), 6.86 (d, *J* = 7.0 Hz, 1H), 6.34 (d, *J* = 5.4 Hz, 1H), 4.67 (dt, *J* = 8.2, 3.0 Hz, 1H), 4.13 (dd, *J* = 10.4, 2.9 Hz, 1H), 4.03 – 3.81 (m, 4H), 3.74 (s, 3H), 3.53 – 3.44 (m, 1H), 2.40 (dd, *J* = 14.6, 2.6 Hz, 1H), 2.26 (dd, *J* = 14.7, 9.5 Hz, 1H), 1.57 – 1.36 (m, 4H), 1.35 – 1.20 (m, 18H), 1.20 – 1.11 (m, 2H), 1.04 (s, 9H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 170.7, 168.5, 135.6, 133.0, 132.7, 130.1, 127.9, 127.9, 77.4, 77.1, 76.7, 68.9, 64.3, 54.3, 52.6, 43.0, 42.8, 39.1, 37.2, 30.0, 29.8, 29.7, 29.7, 29.6, 28.0, 27.5, 26.8, 25.5, 22.7, 19.3. HRMS (AccuTOF): [MH]+ Calc'd for $C_{39}H_{63}N_2O_6Si$ *m/z* 683.4455. Found: *m/z* 683.4435.

Methyl (3-hydroxy-15-methylhexadecanoyl)glycyl-L-serinate, (3*R*+3*S*)-15

Methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate ((3*R*+3*S*)-L-serine-**14**, 162 mg, 0.237 mmol) was dissolved in 5 mL of dry THF under nitrogen. The solution was cooled to 0°C and was treated with 1 M tetrabutylammonium fluoride (TBAF, 0.26 mL, 0.261 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-15-methylhexadecanoyl)glycyl-L-serinate, (3*R*+3*S*)-**15**, as a white solid, (101 mg, 0.227 mmol, 95.8 %), MP, 105-107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.6 Hz, 1H), 7.20 – 7.11 (m, 1H), 4.67 – 4.58 (m, 1H), 4.12 (dd, *J* = 16.9, 6.2 Hz, 1H), 4.03 – 3.83 (m, 5H), 3.76 (s, 3H), 2.44 (dd, *J* = 14.0, 2.4 Hz, 1H), 2.30 (dd, *J* = 14.1, 9.7 Hz, 1H), 1.60 – 1.05 (m, 23H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 173.3, 171.2, 171.0, 169.6, 77.4, 77.1, 76.7, 69.6, 62.6, 54.9, 52.8, 43.7, 43.2, 39.1, 37.5, 30.0, 29.8, 29.8, 29.7, 29.6, 28.0, 27.5, 25.7, 22.7. HRMS (AccuTOF): [MH]+ Calc'd for $C_{23}H_{45}N_2O_6$ *m/z* 445.3278. Found: *m/z* 445.3307.

Methyl (3(*R*)-hydroxy-15-methylhexadecanoyl)glycyl-L-serinate, (3*R*)-L-serine-15

Methyl O-(tert-butyldiphenylsilyl)-N-((3R-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate ((3*R*)-L-serine-**14**, 276 mg, 0.403 mmol) was dissolved in 5 mL of dry THF under nitrogen. The solution was cooled to 0 °C and was treated with 1 M tetrabutylammonium fluoride (TBAF, 0.44 mL, 0.444 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*R*-

hydroxy-15-methylhexadecanoyl)glycyl-L-serinate, (3*R*)-L-serine-**15**, as a white solid, (129 mg, 0.289 mmol, 71.6 %), MP, 100-104 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, *J* = 7.8 Hz, 1H), 7.00 (t, *J* = 5.9 Hz, 1H), 4.73 – 4.65 (m, 1H), 4.19 (dd, *J* = 16.9, 6.3, 2.1 Hz, 1H), 4.12-4.04 (m, 1H), 4.04 – 3.86 (m, 3H), 3.82 (s, 3H), 2.50 (dt, *J* = 14.1, 2.6 Hz, 1H), 2.40 – 2.30 (m, 1H), 1.63 – 1.44 (m, 4H), 1.44 – 1.23 (m, 18H), 1.19 (q, *J* = 6.8 Hz, 2H), 0.91 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.31, 171.10, 169.40, 77.27, 77.02, 76.76, 69.56, 62.71, 54.85, 52.80, 43.67, 43.30, 39.08, 37.43, 29.97, 29.75, 29.70, 29.63, 29.54, 27.98, 27.44, 25.62, 25.56, 22.67. HRMS (AccuTOF): [MH]+ Calc'd for $C_{23}H_{45}N_2O_6$ *m/z* 445.3278. Found: *m/z* 445.3285.

Methyl (3(*S*)-hydroxy-15-methylhexadecanoyl)glycyl-L-serinate, (3*S*)-L-serine-15

Methyl O-(tert-butyldiphenylsilyl)-N-((3S-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate ((3*S*)-L-serine-**14**, 216 mg, 0.316 mmol) was dissolved in 5 mL of dry THF under nitrogen. The solution was cooled to 0°C and was treated with 1 M tetrabutylammonium fluoride (TBAF, 0.35 mL, 0.347 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*S*-hydroxy-15-methylhexadecanoyl)glycyl-L-serinate, (3*S*)-L-serine-**15**, as a white solid, (96 mg, 0.216 mmol, 68.4 %), MP, 109-111 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, *J*= 7.4 Hz, 1H), 6.88-6.78 (m, 1H), 4.65 (dt, *J* = 7.3, 3.4 Hz, 1H), 4.21 – 4.10 (m, 1H), 4.04 (s, 1H), 3.98 – 3.92 (m, 2H), 3.92 – 3.80 (m, 2H), 3.78 (s, 3H), 2.45 (dt, *J* = 13.9, 2.5 Hz, 1H), 2.35 – 2.26 (m, 1H), 1.56 – 1.45 (m, 3H), 1.29 – 1.23 (m, 18H), 1.15 (q, *J* = 6.8 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.16, 170.90, 169.43, 77.28, 77.02, 76.77, 69.27, 62.83, 62.80, 54.86, 54.81, 52.84, 43.51, 43.26, 39.09, 37.25, 29.97, 29.75, 29.70, 29.63, 29.53, 28.00, 27.45,

25.60, 22.68. HRMS (AccuTOF): [MH]+ Calc'd for $C_{23}H_{45}N_2O_6$ *m/z* 445.3278. Found: *m/z* 445.3242.

(3-Hydroxy-15-methylhexadecanoyl)glycyl-L-serine, (3*R*+3*S*)-L-serine-1

A solution of 0.96 M LiOH (0.49 mL, 0.466 mmol) was added to a solution of methyl (3-hydroxy-15-methylhexadecanoyl)glycyl-L-serinate ((3*R*+3*S*)-L-serine-**15**, 189 mg, 0.424 mmol) in 2 mL of methanol at 0 °C, and 4 mL of tetrahydrofuran 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_2O. 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-15-methylhexadecanoyl)glycyl-L-serine, (3*R*+3*S*)-L-serine-**1**, as a crystalline solid (177 mL, 0.412 mmol, 97.1 %), MP, 117-120 °C. ^{1}H NMR ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.49 and 4.51 (two s, 1H), 4.11 (d, *J*= 17.3 Hz, 1H), 4.04 – 3.86 (m, 2H), 3.85 – 3.74 (m, 2H), 3.66 (d, *J*= 17.0 Hz, 1H), 3.37 – 3.29 (m, 1H), 2.41 – 2.33 (m, 1H), 2.27 – 2.19 (m, 1H), 1.53 – 1.34 (m, 4H), 1.34 – 1.14 (m, 18H), 1.09 (q, *J* = 7.1, 6.7 Hz, 2H), 0.80 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD+$CDCl_3$) δ 173.79, 173.58, 172.23, 172.09, 169.65, 169.56, 77.38, 77.06, 76.74, 69.24, 68.90, 62.14, 62.04, 54.69, 54.56, 49.83, 49.62, 49.40, 49.19, 48.97, 48.76, 48.55, 43.48, 43.29, 42.87, 42.77, 38.99, 37.44, 37.17, 29.87, 29.65, 29.60, 29.54, 29.47, 27.89, 27.34, 25.48, 25.43, 22.54. HRMS (AccuTOF): [MH]+ Calc'd for $C_{22}H_{43}N_2O_6$ *m/z* 431.3121. Found: *m/z* 431.3131.

(3(*R*)-Hydroxy-15-methylhexadecanoyl)glycyl-L-serine, (3*R*)-L-serine-1

A solution of 0.51 M LiOH (0..33 mL, 0.168 mmol) was added to a solution of methyl (3*R*)-hydroxy-15-methylhexadecanoyl)glycyl-L-serinate ((3*R*)-L-serine-**15**, 68 mg, 0.153 mmol) in 2 mL of methanol at 0 °C, and 4 mL of tetrahydrofuran 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_2O. 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(*R*)-hydroxy-15-methylhexadecanoyl)glycyl-L-serine, (3*R*)-L-serine-**1**, as a crystalline solid (44 mg, 0.102 mmol, 66.7 %), MP, 122-126 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.53 (s, 1H), 4.15 (d, *J* = 16.8 Hz, 1H), 3.95 (s, 1H), 3.68 (d, *J* = 16.9 Hz, 1H), 3.31 (d, 25.0 Hz, 1H), 2.38 (d, *J* = 13.7 Hz, 1H), 2.24 (t, *J* = 12.1 Hz, 1H), 1.60 – 1.42 (m, 3H), 1.40 – 1.20 (m, 18H), 1.13 – 1.09 (m, 2H), 0.82 (d, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.74, 172.42, 169.59, 77.31, 77.06, 77.06, 76.80, 69.32, 62.23, 55.93, 43.54, 42.96, 39.04, 37.47, 29.92, 29.69, 29.65, 29.58, 29.51, 27.94, 27.39, 25.48, 22.65, 22.60. HRMS (AccuTOF): [MH]+ Calc'd for $C_{22}H_{43}N_2O_6$ *m/z* 431.3121. Found: *m/z* 431.3131.

(3(*S*)-Hydroxy-15-methylhexadecanoyl)glycyl-L-serine, (3*S*)-L-serine-1

A solution of 0.51 M LiOH (0.40 mL, 0.203 mmol) was added to a solution of methyl (3(S)-hydroxy-15-methylhexadecanoyl)glycyl-L-serinate (3*S*)-L-serine-**15**, 82 mg, 0.184 mmol) in 2 mL of methanol at 0 °C, and 4 mL of tetrahydrofuran 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_2O. 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(*S*)-hydroxy-15-

methylhexadecanoyl)glycyl-L-serine, (3*S*)-L-serine-**1**, as a crystalline solid (59 mg, 0.137 mmol, 74.6 %), MP, 109-111 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.53 (s, 1H), 4.13 (d, *J* = 16.9 Hz, 1H), 3.94 (d, *J* = 11.6 Hz, 2H), 3.82 (d, *J* = 11.6 Hz, 1H), 3.76 – 3.64 (m, 2H), 2.38 (d, *J* = 13.8 Hz, 1H), 2.24 (t, *J* = 12.0 Hz, 1H), 1.21 (s, 17H), 1.11 (s, 2H), 0.82 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.77, 172.35, 169.54, 77.37, 77.06, 76.74, 69.31, 62.20, 54.59, 43.53, 42.91, 39.02, 37.47, 29.90, 29.68, 29.63, 29.56, 29.49, 27.92, 27.38, 25.46, 22.59. HRMS (AccuTOF): [MH]+ Calc'd for $C_{22}H_{43}N_2O_6$ *m/z* 431.3121. Found: *m/z* 431.3131.

6-(Methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate, (3*R*+3*S*)-L-serine-16

13-Methyltetradecanoic acid (35 mg, 0.156 mmol) was dissolved in 5 mL of dry DCM and stirred at 0 °C. To the solution was then treated with DCC (32 mg, 0.156 mmol), HOBt (21 mg, 0.156 mmol), and a catalytic amount of DMAP. After stirring for 15 min, methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate, ((3*R*+3*S*)-**14**, 98 mg, 0.142 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-(methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate, (3*R*+3*S*)-L-serine-**16**, as a clear oil (124 mg, 0.137 mmol, 96.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (tt, *J* = 6.8, 1.6 Hz, 4H), 7.49–7.34 (m, 6H), 6.54 (dd, *J* = 8.1, 5.2 Hz, 1H), 6.36 (t, *J*= 4.6 Hz, 1H), 5.16 (quin, *J* = 6.3 Hz, 1H), 4.69-4.62 (m, 1H), 4.12 (dd, *J*

= 10.3, 2.9 Hz, 1H), 4.02–3.81 (m, 3H), 3.74 (s, 3H), 2.55–2.42 (m, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 1.70-1.59 (m, 4H), 1.50 (sep, *J* = 6.6 Hz, 2H), 1.37-1.20 (m, 36H), 1.15 (q, *J* = 7.1, 6.7 Hz, 4H), 1.03 (s, 9H), 0.86 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.41, 170.44, 170.40, 169.95, 168.24, 135.55, 135.51, 132.83, 132.80, 132.64, 130.10, 130.05, 127.96, 127.87, 77.38, 77.06, 76.74, 71.10, 64.15, 54.25, 52.56, 42.88, 41.42, 39.11, 34.55, 34.17, 30.00, 29.77, 29.73, 29.61, 29.56, 29.41, 29.35, 29.20, 28.02, 27.47, 26.77, 25.31, 25.05, 22.71, 19.30. HRMS (AccuTOF): [MH]+ Calc'd for $C_{54}H_{91}N_2O_7Si$ *m/z* 907.6596. Found: *m/z* 907.6557.

(6*S*,13*R*)-6-(Methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate, (3*R*)-L-serine-16

13-Methyltetradecanoic acid (77 mg, 0.316 mmol) was dissolved in 5 mL of dry DCM and stirred at 0 °C. To the solution was then treated with DCC (78 mg, 0.380 mmol), HOBt (58 mg, 0.379 mmol), and a catalytic amount of DMAP. After stirring for 15 min, methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3*R*-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate, (3*R*)-L-serine-**14** (178 mg, 0.261 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*,13*R*)-6-(methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate, (3*R*)-L-serine-**16** as a clear oil (231 mg, 0.255 mmol, 97.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (t, *J* = 6.6 Hz, 4H), 7.49 – 7.34 (m, 6H), 6.53 (d, *J* = 8.1 Hz, 1H), 6.36 (t, *J*= 4.6 Hz, 1H), 5.16 (d, *J* = 24.6 Hz, 1H), 4.69-4.62 (m, 1H), 4.12 (dd, *J* = 10.3, 2.9

Hz, 1H), 4.00 – 3.82 (m, 3H), 3.74 (s, 3H), 2.55 – 2.43 (m, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 1.70 – 1.55 (m, 4H), 1.50 (sep, *J* = 6.6 Hz, 2H), 1.25 (s, 36H), 1.15 (q, *J* = 7.1, 6.7 Hz, 4H), 1.03 (s, 9H), 0.86 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.43, 170.42, 169.96, 168.23, 135.56, 135.52, 132.83, 132.65, 130.10, 130.05, 127.96, 127.87, 77.38, 77.06, 76.74, 71.10, 64.15, 54.28, 52.57, 42.87, 41.43, 39.12, 34.55, 34.17, 30.00, 29.78, 29.73, 29.62, 29.57, 29.42, 29.35, 29.21, 28.02, 27.48, 26.78, 25.32, 25.05, 22.71, 19.31, 1.07. HRMS (AccuTOF): [MH]+ Calc'd for $C_{54}H_{91}N_2O_7Si$ *m/z* 907.6596. Found: *m/z* 907.6586.

(6*S*,13*S*)-6-(Methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate, (3*S*)-L-serine-16

13-Methyltetradecanoic acid (84 mg, 0.346 mmol) was dissolved in 5 mL of dry DCM and stirred at 0 °C. The solution was then treated with DCC (85 mg, 0.415 mmol), HOBt (64 mg, 0.418 mmol), and a catalytic amount of DMAP. After stirring for 15 min, methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-((3S-hydroxy-15-methylhexadecanoyl)glycyl)-L-serinate, (3*S*)-L-serine-**14** (189 mg, 0.277 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*,13*S*)-6-(methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate, (3*S*)-L-serine-**16** as a clear oil (245 mg, 0.269 mmol, 97.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.54 (m, 4H), 7.48 – 7.34 (m, 6H), 6.53 (d, *J* = 8.1 Hz, 1H), 6.36 (t, *J*= 4.6 Hz, 1H), 5.16 (d, *J* = 24.6 Hz, 1H), 4.69-4.62 (m, 1H), 4.12 (dd, *J* = 10.3, 2.9 Hz,

1H), 4.04 – 3.79 (m, 2H), 3.73 (s, 3H), 2.57 – 2.41 (m, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 1.70-1.40 (m, 6H), 1.35-1.10 (m, 40H), 1.03 (s, 9H), 0.86 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.40, 170.46, 169.98, 168.31, 135.53, 135.49, 132.78, 132.62, 130.08, 130.02, 127.93, 127.85, 77.38, 77.06, 76.74, 71.08, 64.13, 54.24, 52.52, 42.89, 41.37, 39.09, 34.53, 34.15, 29.98, 29.76, 29.71, 29.60, 29.55, 29.39, 29.33, 29.19, 28.00, 27.45, 26.75, 26.69, 25.29, 25.03, 22.69, 22.63, 19.27, 1.05. HRMS (AccuTOF): [MH]+ Calc'd for $C_{54}H_{91}N_2O_7Si$ *m/z* 907.6596. Found: *m/z* 907.6583.

1-((2-(((*S*)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate, (3*R*+3*S*)-L-serine-17

6-(Methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate ((3*R*+3*S*)-L-serine-**16**, 197 mg, 0.217 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 (0.24 mL, 0.24 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% MeOH:DCM) to yield 1-((2-(((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate, (3*R*+3*S*)-L-serine-**17**, as a clear oil (132 mg, 0.202 mmol, 93.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ two signals [7.05 and 7.12 (d, *J*= 7.6 and 7.8 Hz, 1H), 6.74 – 6.64 (m, 1H), 5.26 – 5.11 (m, 1H), 4.65 (h, *J* = 7.7, 3.8 Hz, 1H), 4.08 – 3.87 (m, 4H), 3.78 (d, *J* = 1.9 Hz, 3H), 2.52 – 2.45 (m, 2H), 2.30 (td, *J* = 7.6, 1.7 Hz, 2H), 1.68 – 1.43 (m, 6H), 1.40 – 1.20 (m, 36H), 1.15 (t, *J* = 6.6 Hz, 4H), 0.86 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.45, 174.28, 170.92, 170.80, 170.70, 169.05, 169.01, 77.38, 77.06, 76.74, 71.46, 62.82, 55.05, 52.72,

43.32, 41.91, 41.81, 39.12, 39.08, 34.60, 34.56, 34.50, 30.01, 29.78, 29.74, 29.70, 29.62, 29.57, 29.40, 29.33, 29.22, 29.19, 28.02, 27.48, 25.31, 25.06, 25.01, 22.71. HRMS (AccuTOF): [MH]+ Calc'd for $C_{38}H_{73}N_2O_7$ *m/z* 669.5418. Found: *m/z* 669.5425.

(*R*)-1-((2-(((*S*)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate, (3*R*)-L-serine-17

(6*S*,13*R*)-6-(Methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate, ((3*R*)-L-serine-**16**, 231 mg, 0.255 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 (0.25 mL, 0.25 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% MeOH:DCM) to yield ((*R*)-1-((2-(((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate, (3*R*)-L-serine-**17**, as a clear oil (153 mg, 0.229 mmol, 89.9 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, *J*= 7.7 Hz, 1H), 6.45 (t, *J* = 5.5 Hz, 1H), 5.25-5.10 (m, 1H), 4.67 (dq, *J* = 7.1, 3.5 Hz, 1H), 4.13-3.87 (m, 4H), 3.79 (s, 3H), 2.49 (d, *J* = 6.1 Hz, 2H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.69 – 1.44 (m, 6H), 1.38-1.20 (m, 36H), 1.15 (q, *J* = 6.7 Hz, 4H), 0.86 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.56, 170.70, 170.65, 168.95, 77.38, 77.06, 76.74, 71.54, 62.99, 55.01, 52.77, 43.43, 42.06, 39.12, 34.62, 30.01, 29.78, 29.74, 29.62, 29.56, 29.38, 29.33, 29.19, 28.03, 27.48, 25.32, 25.06, 25.01, 22.72. HRMS (AccuTOF): [MH]+ Calc'd for $C_{38}H_{73}N_2O_7$ *m/z* 669.5418. Found: *m/z* 669.5389.

(*S*)-1-((2-(((*S*)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate, , (3*S*)-L-serine-17

(6*S*,13*S*)-6-(Methoxycarbonyl)-2,2,25-trimethyl-8,11-dioxo-3,3-diphenyl-4-oxa-7,10-diaza-3-silahexacosan-13-yl 13-methyltetradecanoate, ((3*S*)-L-serine-**16**, 245 mg, 0.269 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 (0.27 mL, 0.27 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% MeOH:DCM) to yield ((*S*)-1-((2-(((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate, (3*S*)-L-serine--**17,** as a clear oil (164 mg, 0.245 mmol, 90.8 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, *J*= 7.6 Hz, 1H), 6.49 (t, *J* = 5.3 Hz, 1H), 5.26-5.10 (m, 1H), 4.70 – 4.61 (m, 1H), 4.13 – 3.88 (m, 4H), 3.79 (s, 3H), 2.49 (d, *J* = 5.9 Hz, 2H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.72 – 1.44 (m, 6H), 1.43 – 1.19 (m, 36H), 1.15 (q, *J* = 6.7 Hz, 4H), 0.86 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.38, 170.79, 170.61, 168.94, 77.38, 77.06, 76.74, 71.54, 62.92, 55.02, 52.76, 43.43, 41.94, 39.12, 34.63, 34.55, 30.01, 29.79, 29.74, 29.62, 29.57, 29.38, 29.34, 29.22, 28.03, 27.48, 25.32, 25.06, 22.72. HRMS (AccuTOF): [MH]+ Calc'd for $C_{38}H_{73}N_2O_7$ *m/z* 669.5418. Found: *m/z* 669.5395.

(15-Methyl-3-((13-methyltetradecanoyl)oxy)hexadecanoyl)glycyl-L-serine, (3*R*+3*S*)-L-serine-2

A stirring solution of 1-((2-(3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate ((3*R*+3*S*)-L-serine-**17**, 119 mg, 0.178 mmol), in 10 mL of dry DCM, was cooled to 0 °C. Potassium trimethylsilonoate (25 mg, 0.196 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 hrs. The solvents were evaporated under reduced pressure, and the resulting oil was dissolved in 10 mL of

H_2O and acidified with 10% HCl. The resulting precipitate was filtered to yield 15-methyl-3-((13-methyltetradecanoyl)oxy)hexadecanoyl)glycyl-L-serine, (3*R*+3*S*)-L-serine-**2**, as a white sticky solid (105 mg, 0.161 mmol, 90.2%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.17 (bs, 1H), 4.27 (bs, 1H), 4.12-3.50 (s, 5H), 1.67 – 1.41 (m, 6H), 1.40-1.20 (m, 36H), 1.12 (t, *J* = 6.6 Hz, 4H), 0.83 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.04, 171.67, 169.92, 77.32, 77.06, 76.81, 71.21, 62.26, 55.95, 42.78, 41.09, 39.07, 34.58, 34.33, 31.91, 29.98, 29.76, 29.70, 29.67, 29.60, 29.52, 29.38, 29.22, 27.96, 27.44, 25.28, 25.07, 22.63. HRMS (+TOF MS): [MNa]+ Calc'd for $C_{37}H_{70}N_2NaO_7$ *m/z* 677.5075. Found: *m/z* 677.5081.

((*R*)-15-methyl-3-((13-methyltetradecanoyl)oxy)hexadecanoyl)glycyl-*L*-serine, (3*R*)-L-serine-2

A stirring solution of 1(*R*)-1-((2-(((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate, ((3*R*)-L-serine-**17,** 25 mg, 0.037 mmol) in 10 mL of dry DCM was cooled to 0 °C. Potassium trimethylsilonoate (5.3 mg, 0.051 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 hrs. The solvents were evaporated under reduced pressure, and the resulting oil was dissolved in 10 mL of H_2O and acidified with 10% HCl. The resulting precipitate was filtered to yield ((*R*)-15-methyl-3-((13-methyltetradecanoyl)oxy)hexadecanoyl)glycyl-L-serine, (3*R*)-L-serine-**2**, as a white sticky solid (18 mg, 0.028 mmol, 74%). $[\alpha]_D^{23}$ = +12.8$_5$° (c 1.02, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15 (bs, 1H), 4.31 (bs, 1H), 4.11 – 3.44 (m, 4H), 2.47 (s, 2H), 2.24 (t, *J* = 7.5 Hz, 2H), 1.68 – 1.35 (m, 6H), 1.35 – 0.99 (m, 36H), 0.81 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, CD_3OD+ $CDCl_3$) δ 174.08, 173.94, 171.48, 169.54, 77.38, 77.06, 76.74, 71.08, 62.05, 49.39, 49.17, 48.96, 48.74, 48.53, 48.32, 48.10, 42.63, 40.87, 38.89, 34.37, 34.09, 29.78, 29.56, 29.52, 29.48, 29.43,

29.37, 29.26, 29.16, 29.00, 27.78, 27.25, 25.06, 24.87, 22.39. HRMS (+TOF MS): [MNa]+ Calc'd for $C_{37}H_{70}N_2NaO_7$ *m/z* 677.5075. Found: *m/z* 677.5081.

((*S*)-15-methyl-3-((13-methyltetradecanoyl)oxy)hexadecanoyl)glycyl-*L*-serine, (3*S*)-L-serine-2

A stirring solution of 1(*S*)-1-((2-(((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-15-methyl-1-oxohexadecan-3-yl 13-methyltetradecanoate, ((3*S*)-L-serine-**17,** 25 mg, 0.037 mmol) in 10 mL of dry DCM was cooled to 0 °C. Potassium trimethylsilonoate (5.3 mg, 0.051 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 hrs. The solvents were evaporated under reduced pressure, and the resulting oil was dissolved in 10 mL of H_2O and acidified with 10% HCl. The resulting precipitate was filtered to yield ((*S*)-15-methyl-3-((13-methyltetradecanoyl)oxy)hexadecanoyl)glycyl-L-serine, (3*S*)-L-serine-**2**, as a white sticky solid (16 mg, 0.024 mmol, 65%). $[\alpha]_D^{23}$ = +6.3° (c 1.02, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 (t, *J*= 5.6 Hz, 1H), 4.29 (s, 1H), 4.10 – 3.60 (m, 4H), 2.56 – 2.35 (m, 2H), 2.23 (t, *J* = 7.5 Hz, 2H), 1.65 – 1.40 (m, 6H), 1.40 – 0.99 (m, 40H), 0.81 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.03, 173.75, 171.50, 169.73, 77.38, 77.06, 76.74, 71.20, 62.24, 55.60, 50.01, 49.80, 49.58, 49.37, 49.16, 48.94, 48.73, 42.74, 41.11, 39.04, 34.53, 34.33, 29.95, 29.73, 29.70, 29.66, 29.62, 29.55, 29.46, 29.40, 29.33, 29.18, 27.93, 27.41, 25.24, 25.02, 22.60. HRMS (+TOF MS): [MNa]+ Calc'd for $C_{37}H_{70}N_2NaO_7$ *m/z* 677.5075. Found: *m/z* 677.5081.

Supplementary Material

Both ^{1}H and ^{13}C NMR data are provided for relevant compounds in the Supplementary Material. Data is provided for • Methyl L-serinate, **1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16,**

17, and the Mosher's ester ^{1}H NMR data for **5**, for both the enzymatic resolution and the asymmetric hydrogenation routes.

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