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Structure−Activity Relationships in Toll-like Receptor-2 Agonistic Diacylthioglycerol Lipopeptides

Wenyan Wu, Rongti Li, Subbalakshmi S. Malladi, Hemamali J. Warshakoon, Matthew R. Kimbrell, Michael W. Amolins, Rehman Ukani, Apurba Datta, and Sunil A. David*

Department of Medicinal Chemistry, University of Kansas, 2030 Becker Drive, Lawrence, Kansas 66047

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The N-termini of bacterial lipoproteins are acylated with a (*S*)-(2,3-bisacyloxypropyl)cysteinyl residue. Lipopeptides derived from lipoproteins activate innate immune responses by engaging Toll-like receptor 2 (TLR2) and are highly immunostimulatory and yet without apparent toxicity in animal models. The lipopeptides may therefore be useful as potential immunotherapeutic agents. Previous structure−activity relationships in such lipopeptides have largely been obtained using murine cells, and it is now clear that significant species-specific differences exist between human and murine TLR responses. We have examined in detail the role of the highly conserved Cys residue as well as the geometry and stereochemistry of the Cys-Ser dipeptide unit. (*R*)-Diacylthioglycerol analogues are maximally active in reporter gene assays using human TLR2. The Cys-Ser dipeptide unit represents the minimal part-structure, but its stereochemistry was found not to be a critical determinant of activity. The thioether bridge between the diacyl and dipeptide units is crucial, and replacement by an oxoether bridge results in a dramatic decrease in activity.

Introduction

The link between immune stimulation resulting from acute infection and the resolution of tumors has long been recognized,[1] and the phenomenon of infection-related "spontaneous regression" of cancer has been documented throughout history, beginning as early as 2600 B.C.[2,3] The foundations of modern immunotherapy for cancer were laid in 1891 by William B. Coley, who, in 1891, injected live streptococcal organisms into a long-bone sarcoma lesion and observed shrinkage of the tumor.[4] More than a century has elapsed since Coley's empirical findings were first reported, but the active principle(s) in Coley's toxin remain undetermined. This long lag period is perhaps not altogether surprising because the recognition of innate immune system is recent,[5−7] and our understanding of the structure and function of the family of Toll-like receptors (TLRs[a])[8,9] that recognize "pathogen-associated molecular patterns (PAMPs)"[10] and of the effector mechanisms that mediate innate immune responses[11−13] is yet nascent. Nonetheless, there exists considerable potential in harnessing innate immune responses in a wide range of disease states, notably cancer, as evidenced by the number of TLR agonists already in clinical trials[14] and the resurgence of interest in the immunotherapy of neoplastic states.[15−17]

The exoskeleton of the Gram-positive organism, similar to that of Gram-negative bacteria, comprises underlying peptidoglycan (PGN), a polymer of β-1→4-linked *N*-acetylglucosamine-*N*-acetylmuramic acid glycan strands that are cross-linked by short peptides.[18−20] Unlike Gram-negative bacteria that bear lipopolysaccharide on the outer leaflet of the outer membrane, the external surface of the peptidoglycan layer is decorated with lipoteichoic acids (LTA).[21,22] LTA are anchored in the peptidoglycan substratum via a diacylglycerol moiety and bear a surface-exposed, polyanionic, 1−3-linked polyglycerophosphate appendage[23,24] that varies in its subunit composition in LTAs from various Gram-positive bacteria; in *S. aureus*, the repeating subunit contains D-alanine and α-D-*N*-acetylglucosamine.[25] Lipoproteins are found in the bacterial cytoplasmic membrane and are also common constituents of the cell wall of both Gram-negative and Gram-positive bacteria.[25−27] The free amine of the N-terminus of lipoproteins is acylated with a (*S*)-(2,3-bis-acyloxypropyl)cysteinyl residue which has previously been demonstrated to be immunostimulatory,[28,29] as shown by studies on synthetic peptides containing the bis-acylthioglycerol unit,[25,30,31] in contradistinction to enterobacterial LPS which is recognized by Toll-like receptor-4 (TLR4), PGN,[32,33] LTA,[34,35] lipopeptides,[36,37] and some nonenterobacterial LPS[38,39] signal via TLR2.[40,41]

In comparing the various constituents of the Gram-positive cell wall, we have found lipopeptides to be extremely potent TLR2 agonists and yet without apparent toxicity in animal models.[42] In murine cells, PAM_2CSK_4 (*S*-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-*R*-cysteinyl-*S*-seryl-*S*-lysyl-*S*-lysyl-*S*-lysyl-*S*-lysyl-*S*-lysine), a commercially available, synthetic model lipopeptide, was equipotent in its NF-κB inducing activity relative to LPS but elicited much lower proinflammatory cytokines while both LPS and the lipopeptide potently induced the secretion of a similar pattern of chemokines suggestive of the engagement of downstream TRIF pathways.[42] Furthermore, we

*To whom correspondence should be addressed. Phone: 785-864-1610. Fax: 785-864-1961. E-mail: sdavid@ku.edu.

[a] Abbreviations: EDIPA, *N*,*N*-diisopropylethylamine; IC_{50}, concentration inducing half-maximal response; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-12, interleukin-12; LPS, lipopolysaccharide; LTA, lipoteichoic acid; PAM_2CSK_4, *S*-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-*R*-cysteinyl-*S*-seryl-*S*-lysyl-*S*-lysyl-*S*-lysyl-*S*-lysyl-*S*-lysine; PAMP, pathogen-associated molecular pattern; PE, phycoerythrin; PGN, peptidoglycan; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-α.

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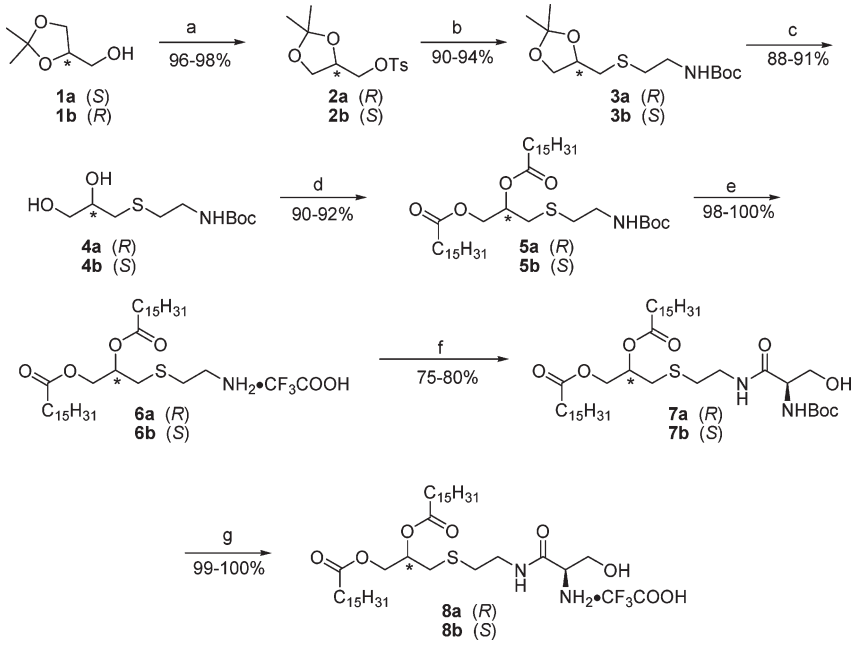
Scheme 1[a]



[a] Reagents and conditions: (a) *p*-TsCl, pyr, DCM, 0 °C to room temp, 8 h; (b) *N*-Boc-2-aminoethanethiol, NaH, DMF, 0 °C to room temp, 8 h; (c) 70% AcOH, room temp, 12 h; (d) $C_{15}H_{31}COCl$, pyr, DMAP, DCM, 0 °C to room temp, 10 h; (e) TFA, room temp, 30 min; (f) Boc-L-serine-OH, EDCI, EDIPA, DMAP, DCM, 0 °C to room temp, 8 h; (g) TFA, room temp, 30 min.

have found that while LPS elicited a robust fever response in rabbits at a dose of 100 ng/kg, a 200 μg/kg dose (2000×) of the lipopeptide was without any discernible pyrogenic or other apparent acute toxic effect (manuscript in preparation). These observations suggest that the lipopeptides may be remarkably potent, yet nontoxic immunotherapeutic agents. Indeed, in a phase I/phase II clinical trial[43] on a single, intraoperative 20−30 μg intratumoral injection of MALP-2[28] (a diacyl lipopeptide similar in its core structure to PAM_2CSK_4) in patients with inoperable carcinoma of the pancreas, the lipopeptide was well-tolerated and proved efficacious in prolonging survival.[43]

Considerable structure−activity relationships dictating TLR2 binding and subsequent stimulation of innate immune responses have been documented for a range of synthetic lipopeptides. However, the majority of earlier SAR studies had been performed with murine cells, many even before the TLRs had been discovered, and it is now clear that significant species-specific differences exist between human and murine TLR responses[36,44] as a consequence of subtle structural differences in the ligand binding pocket within TLR2.[45]

The minimal structure for biological activity is the Cys-Ser lipodipeptide[46] with the lipoaminoacid Cys-OH derivative being almost inactive;[47] the remainder of the peptidic unit appears, in large part, not to be critical for activity, since a variety of analogues of different lengths with different amino acid sequences were found to be equipotent.[46,48−50] The (*R*)-configuration at the asymmetric glyceryl carbon confers maximum activity,[30,51] and the threshold of the acyl chain length of the two ester-bound fatty acids is eight carbons, with shorter acyl analogues being inactive.[36] Furthermore, the recent high-resolution crystal structure of lipopeptides complexed to human- and murine-derived TLR1/TLR2 heterodimers[45] raises additional questions, since the binding site in TLR2 is highly unusual, being located in the convex region formed at the border of the central and C-terminal domains with the peptidic unit interacting with interfacial residues at the neck region of the binding pocket.[45] It was of interest to examine whether the chirality of the highly conserved Cys residue (L-Cys versus D-Cys) and the thioether bridge connecting the diacylglycerol unit to the peptide chain are critical determinants of activity and if the thioether could be bioisosterically substituted by an oxoether linkage. The geometry of the Cys-Ser dipeptide unit also appears to be crucial in that there are key interactions with conserved Tyr316 and Pro315 residues at the neck of the binding pocket in the crystal structure.[45] We note that the carboxylic acid of Cys is in amide linkage with the amine of serine, and we wished to test whether inversion of the orientation of Ser, achieved by the coupling the carboxyl group of serine to the amino group of cysteamine, could yield analogues that were TLR2 receptor antagonists.

Results and Discussion

Much of the previous work in the literature concerning the structure−activity relationships in lipopeptides has utilized murine TLR2, and in an effort to confirm the minimal partstructure of the lipopeptide that was TLR-agonistic on human TLR2, we first targeted the stereoselective syntheses of 1,2-dipalmitoyl-(*R*)-3-(2-aminoethylthio)propanediol and 1,2-dipalmitoyl-(*S*)-3-(2-aminoethylthio)propanediol (**6a** and **6b**, Scheme 1) starting from the corresponding enantiopure 1,2-isopropylideneglycerol. The (*R*)-cysteamine lipoamino acid **6a** was weakly active (IC_{50} = 1 μM, Figure 1) relative to the commercially available reference lipopeptide PAM_2CSK_4 (IC_{50} = 67 pM), while the (*S*)-analogue **6b** was completely inactive. Coupling L-serine to the free amine of the cysteamine resulted in the lipodipeptides **8a** and **8b**; similar to the cysteamine analogues, only **8a** was active with an IC_{50} of 1.2 μM; it is noted that in these latter compounds, the dipeptide unit is inverted, since, unlike in the naturally occurring and in synthetic PAM_2CSK_4, it is the carboxyl group of the serine

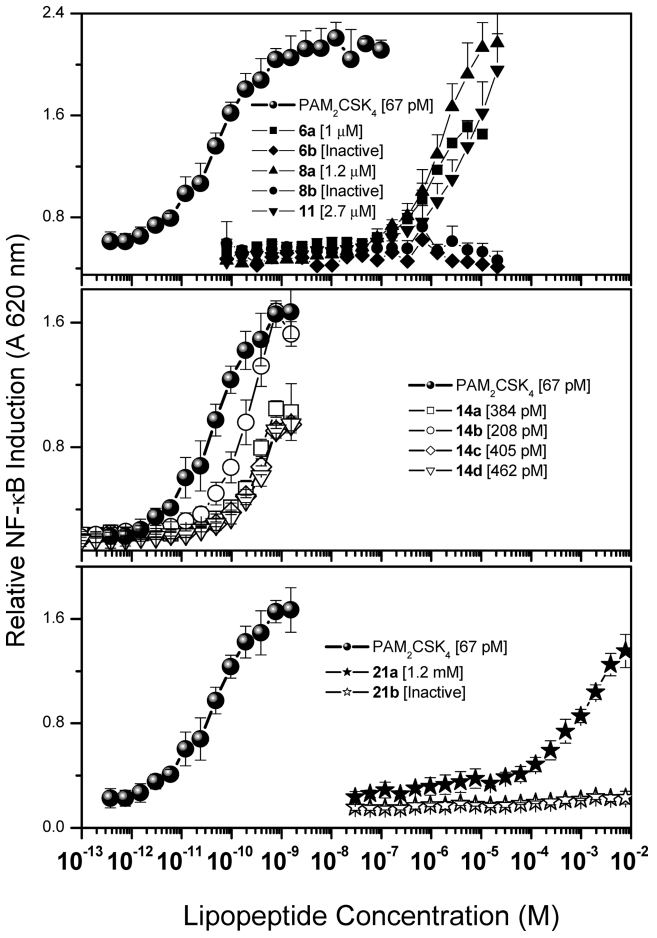


Figure 1. TLR2 agonistic activities of the lipopeptide analogues. HEK293 cells stably transfected with a TLR2-NF-κB-SEAP reporter gene was used in a 384 well format. Data points represent the mean and SD determined on triplicate samples.

that is coupled to the lipopeptide. The L-cysteine-containing lipoamino acid, compound **11** (Scheme 2), was also found to be weakly TLR2-agonistic, which is in contradistinction to the virtually absent activity on murine cells described earlier.[47] The results emphasize differences in ligand specificity between murine and human TLR2. The difference in activity between the (*R*)- and (*S*)-isomers, which was consistent with previous SAR results,[30,51] served to focus our subsequent SAR in which we elected to examine only the (*R*)-diacylthioglycerol analogues.

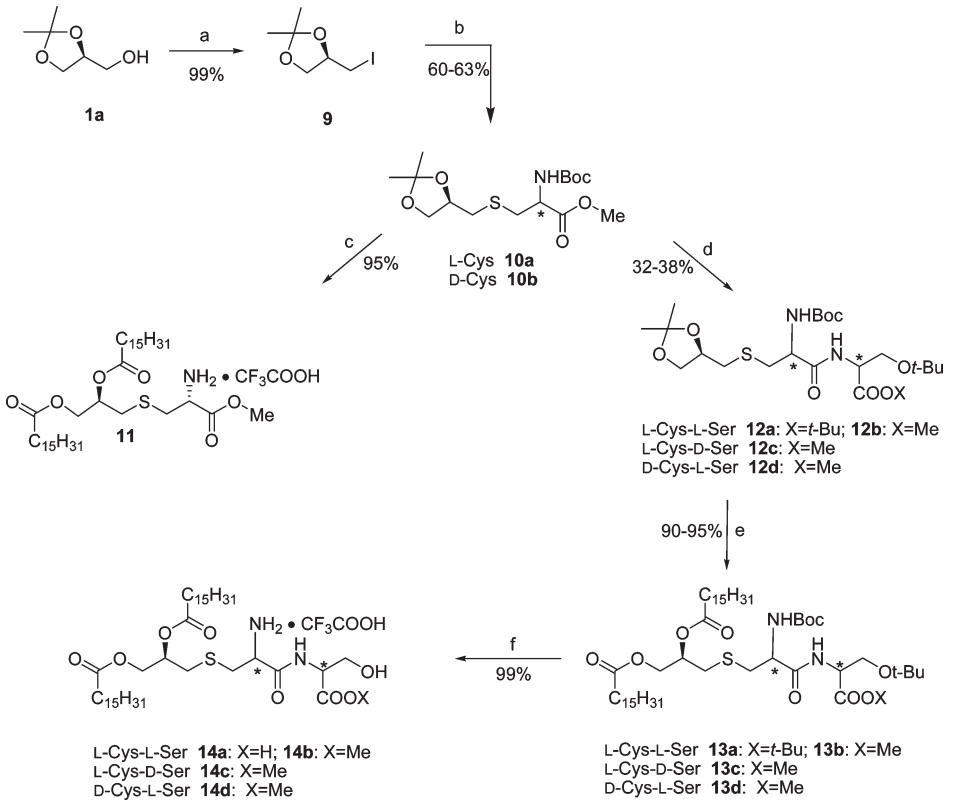
The PAM_2Cys-Ser compounds of the **14** series comprising a combination of L- or D- amino acids at either position and with the carboxyl terminus as either the carboxylic acid or the methyl ester (Scheme 2) were all highly active with the IC_{50} values in the midpicomolar range (Figure 1), signifying that the stereochemistry of the dipeptide unit is noncritical as long as the orientation of the amide bond is in the correct configuration as discussed earlier. These results are consonant with the crystal structure of PAM_2CSK_4 complexed with TLR2, in which the carbonyl Cys-Ser amide bond forms H-bonding with Pro315 and the NH of the amide interacts with Y326, but the side chains of the dipeptide unit show no significant van der Waals interactions within the binding pocket.[45] The relative unimportance of the side chains of the dipeptide core is also in agreement with observations that in naturally occurring lipopeptides, the second amino acid is far less conserved and can be replaced by other residues with nonbulky side chains such as Gly,[46,48−50] presumably as long as steric interactions within the narrow neck region of the binding pocket[45] are not compromised. While the PAM_2CS methyl ester compounds are indeed highly active, they are about a fifth as potent as the reference PAM_2CSK_4 lipopeptide. We have found that, as with other amphipathic compounds that we have recently characterized,[52] the apparent differences in potencies are related, at least in part, to the substantially higher binding of the more hydrophobic PAM_2CS analogues to albumin present in cell culture media, details of which will be published elsewhere.

It was of particular interest to examine the role of the thioether bridge between the diacyl and dipeptide units. O-Alkylation of (*R*)-1,2-isopropylideneglycerol with racemic ethyl 2,3-ethoxypropanoate yielded the key intermediate **15**, from which the oxoether-bridged compounds were obtained (**21a** and **21b**, Scheme 3) in which two of the three stereocenters were held fixed. The activity of **21a** (lipopeptide terminating with L-Ser) was about 8 orders of magnitude lower (IC_{50} = 1.2 mM) than that of PAM_2CSK_4, while **21b** (D-Ser) was inactive at the highest concentration tested (10 mM, Figure 1). While these results are indicative of the absolute requirement of the thioether linkage, we do not yet understand why the stereochemistry of the terminal Ser residue is manifested in differential activity only in the ether-linked analogues (**21a**, **21b**) but not in the thioether-linked analogues (**14a**−**d**).

We next tested whether the ester-linked palmitoyl groups could be replaced with potentially hydrolytically more stable ether and amide linkages without compromising activity. Synthesis of analogue **28** with the ether-linked C_{16} hydrocarbon appendages could not be obtained by direct O-alkylation of an advanced intermediate such as the deprotected species from series **12** under a variety of conditions. This necessitated the installation of the ether-linked C_{16} groups on the glycerol backbone (**23**) first, followed by transformation of the free hydroxyl to the iodo intermediate (**25**) and subsequent S-alkylation with cysteine (Scheme 4). We found that the ether analogue **28** was entirely inactive (Figure 2).

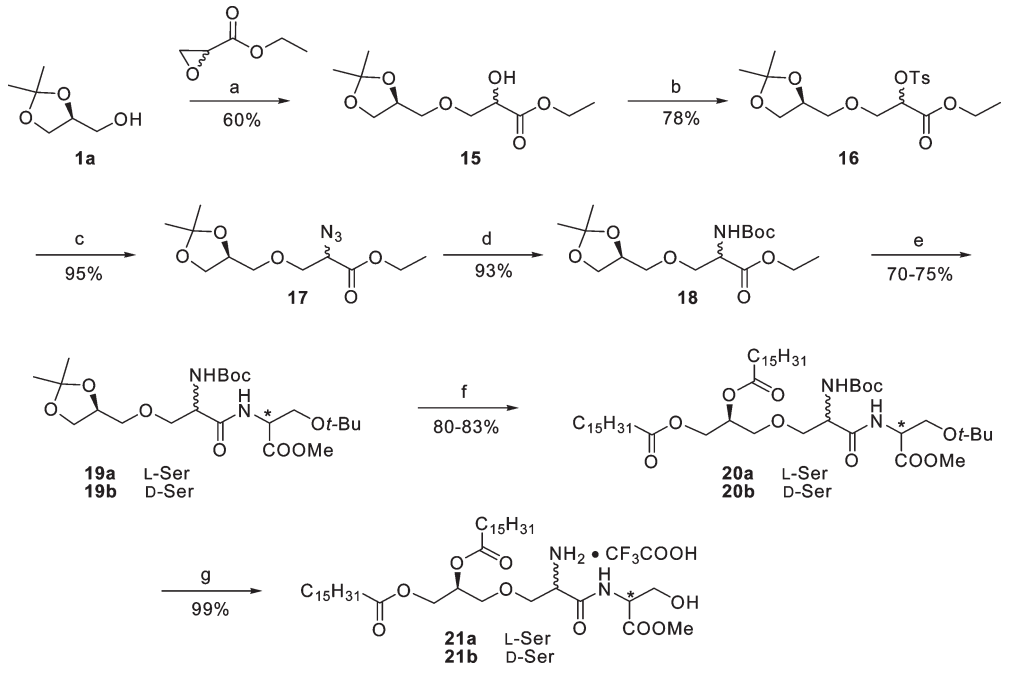
We next investigated the bioisoteric replacement of one (internal, secondary alcohol-derived; Scheme 5) ester as well as both of the esters (Scheme 6) with amide-linked hydrocarbon chains. Synthesis of the monoamide analogue **37** proceeded smoothly starting from D-Ser-OMe (**29**). The diamide analogue, **45**, however, was more problematic to synthesize. Acetonide deprotection of the monoamide precursor **35**, followed by attempts at converting the free hydroxyl group to the amine via an azide intermediate, was unsuccessful. The strategy of first N-acylating (*R*)-methyl 2,3-diaminopropanoate with palmitoyl chloride and then converting the ester to the iodo via the alcohol as described in Scheme 5 was also not fruitful because of unexpected difficulty in the conversion of the alcohol to the iodo group. This is probably due either to steric bulk of the long-chain amides or to an internal H-bond between the free alcohol and one of the amide groups. We therefore resorted to first installing the iodo on a *N*,*N*′-di-Boc-protected 2,3-diaminopropane-1-ol, followed by S-alkylation with *N*-Troc-L-Cys-OMe (Scheme 6), affording the required orthogonality of the protecting groups. The amines on the diaminopropanethiol fragment of compound **41** were then acylated with hexadecanoic acid. Next, the base-labile *N*-Troc protecting group on the cysteine unit had to be converted to the *N*-Boc derivative in order to carry out a subsequent

Scheme 2[a]



[a] Reagents and conditions: (a) PPh_3, I_2, imidazole, toluene, 90 °C, 2 h; (b) Boc-L- or -D-Cys-OMe, TEA, DMF, 85 °C, 4 h; (c) (i) 70% AcOH, room temp, 24 h; (ii) $C_{15}H_{31}COCl$, pyr, DMAP, DCM, 0 °C to room temp, 8 h; (iii) TFA, room temp, 30 min; (d) (i) LiOH, THF/H_2O, room temp, 10 h; (ii) H-L-Ser(O*t*-Bu)-O*t*Bu·HCl or H-L-Ser(O*t*-Bu)-OMe·HCl or H-D-Ser(O*t*-Bu)-OMe·HCl, EDCI, EDIPA, DMAP, DCM, 0 °C to room temp, 8 h; (e) (i) 70% AcOH, room temp,12 h; (ii) $C_{15}H_{31}COCl$, pyr, DMAP, DCM, 0 °C to room temp, 10 h; (f) TFA, room temp, 30 min.

Scheme 3[a]



[a] Reagents and conditions: (a) ethyl 2,3-epoxypropanoate, $BF_3 \cdot OEt_2$, DCM, room temp, 5 h; (b) *p*-TsCl, TEA, DCM, 0 °C to room temp, 8 h; (c) NaN_3, DMF, room temp, 12 h; (d) (i) PPh_3, H_2O, THF, reflux, 6 h; (ii) Boc_2O, DCM, room temp, 8 h; (e) (i) LiOH, THF/H_2O, room temp, 10 h; (ii) H-L-Ser(*t*-Bu)-OMe·HCl or H-D-Ser(*t*-Bu)-OMe·HCl, EDCI, EDIPA, DMAP, DCM, 0 °C to room temp, 8 h; (f) (i) 70% AcOH, room temp, 12 h; (ii) $C_{15}H_{31}COCl$, pyr, DMAP, DCM, 0 °C to room temp, 10 h; (g) TFA, room temp, 30 min.

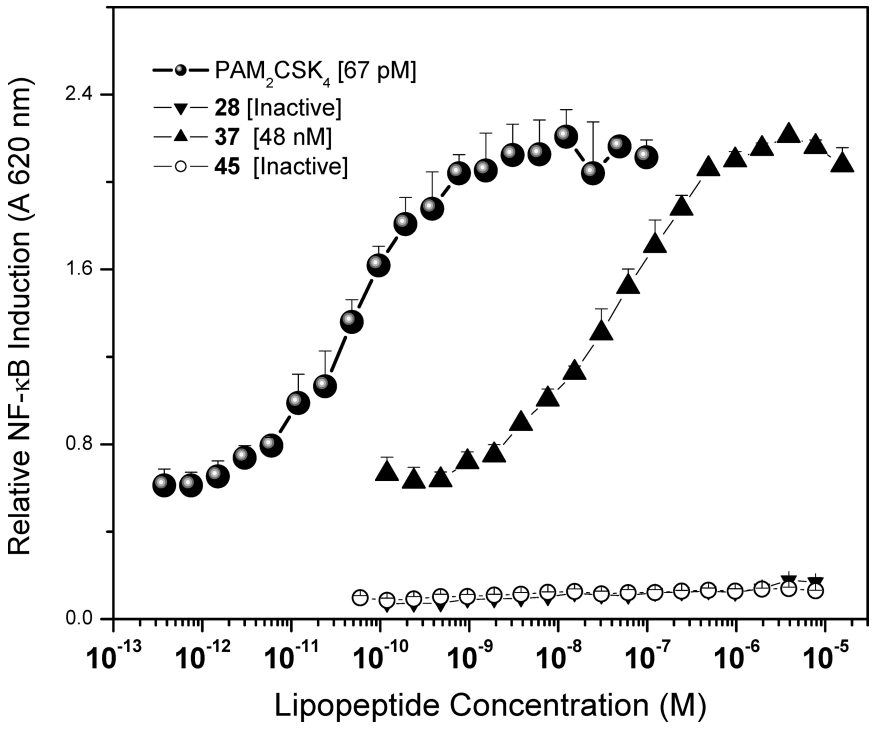
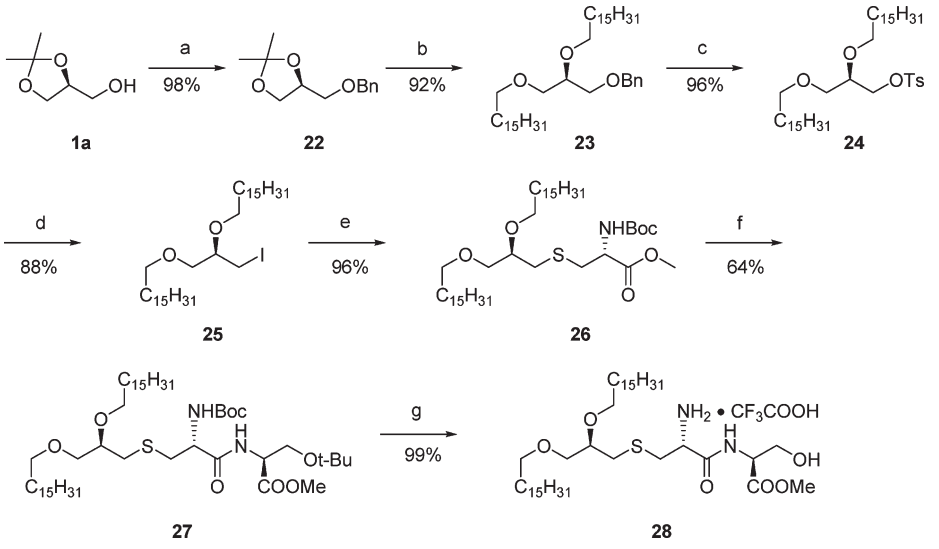


Figure 2. TLR2 agonistic activities of the ether, monoamide, and diamide lipopeptide analogues. HEK293 cells stably transfected with a TLR2-NF-κB-SEAP reporter gene was used in a 384-well format. Data points represent the mean and SD determined on triplicate samples.

Scheme 4[a]



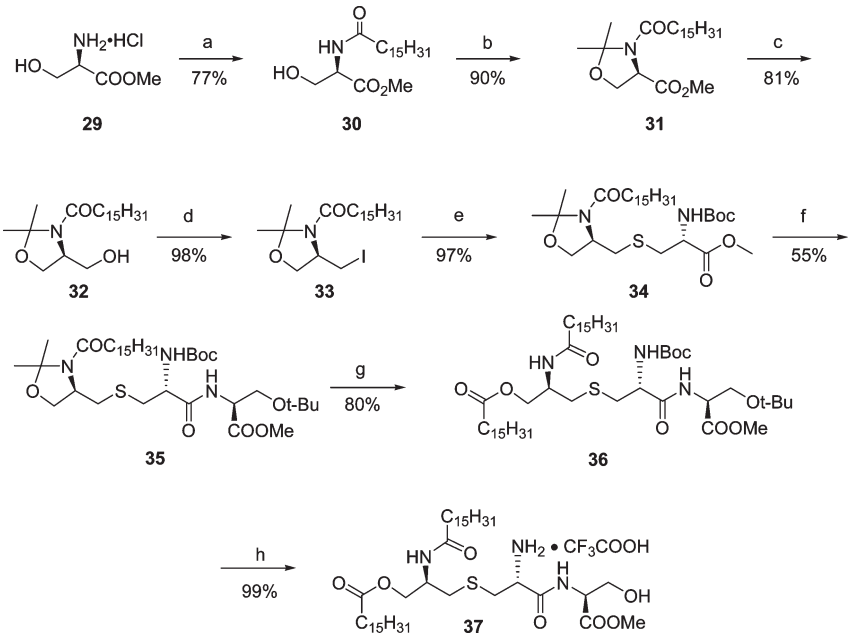
[a] Reagents and conditions: (a) BnBr, NaH, DMF, 0 °C to room temp, 8 h; (b) (i) 70% AcOH, room temp, 12 h; (ii) $C_{16}H_{33}I$, NaH, DMF, 0 °C to room temp, 8 h; (c) (i) Pd/C, H_2, 8 h; (ii) TsCl, pyr, DMAP, CH_3CN, 70 °C, 12 h; (d) I_2, KI, DMF, 80 °C, 24 h; (e) Boc-L-Cys-OMe, TEA, DMF, 85 °C, 2 h; (f) (i) LiOH, THF/H_2O, room temp, 10 h; (ii) H-L-Ser(tBu)-OMe·HCl, EDCI, EDIPA, DMAP, DCM, 0 °C to room temp, 8 h; (g) TFA, room temp, 30 min.

base-catalyzed deesterfication step. Coupling of the terminal Ser residue proceeded smoothly.

The monoamide derivative **37** was found to be partially active (IC_{50} = 48 nM, Figure 2) although weaker by about 3 orders of magnitude compared to the reference lipopeptide PAM_2CSK_4, while the diamide derivative **45** was completely inactive. The progressive loss of activity from the monoamide to the diamide analogue suggests that both ester groups are important for maximal TLR2-stimulatory activity, although this is yet to be formally tested by synthesizing the regioisomer of **37** with the amide group replacing the external (primary alcohol-derived) ester group.

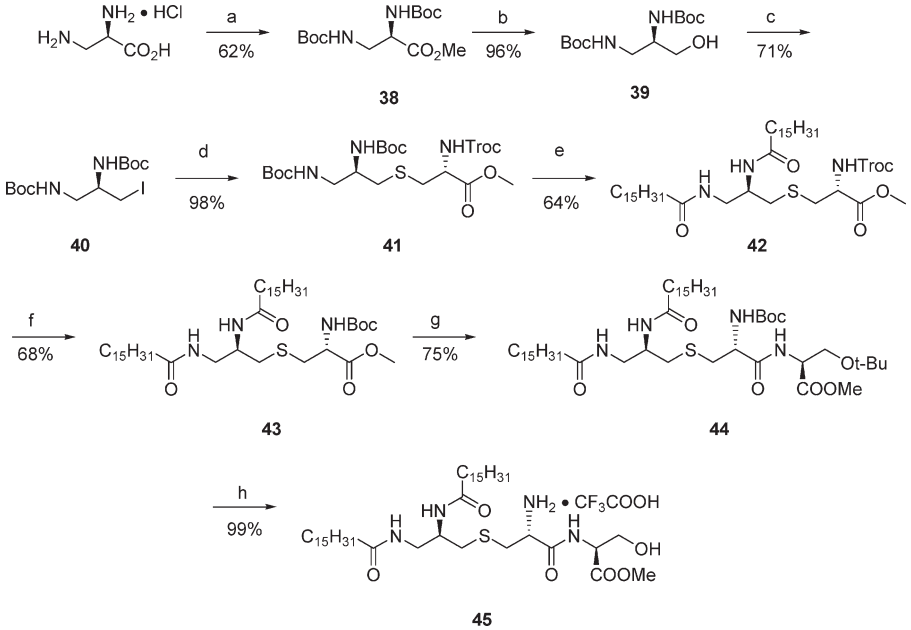
We were also keen to test the possibility that some of the inactive compounds could perhaps behave as TLR2 antagonists, particularly with **8a** and **8b**, since we had designed these compounds with inverted cysteamine−serine amide bonds in the hope that this would be the case. In this context that TLR2-mediated immunopathology has been implicated in a number of inflammatory bowel diseases,[53−56] and the only reported TLR2 receptor antagonists are rather weak lipolanthionine derivatives.[57] Although we were disappointed that **6a**, **6b**, **21a**, **b**, **28**, **37**, and **45** were all inactive as TLR2 receptor antagonists, the negative results add to the SAR data in that they demonstrate that these compounds do not engage TLR2.

Scheme 5[a]



[a] Reagents and conditions: (a) $C_{15}H_{31}COCl$, aqueous $NaHCO_3$, EtOAc, room temp, 1 h; (b) 2,2-DMP, PPTS, toluene, 90 °C, 22 h; (c) $LiBH_4$, THF, 0 °C (3 h) to room temp (6 h); (d) PPh_3, I_2, toluene, 90 °C, 2 h; (e) Boc-L-Cys-OMe, TEA, DMF, 85 °C, 2 h; (f) (i) $Ba(OH)_2$, CH_3CN/H_2O, 60 °C, 1 h; (ii) H-L-Ser(*t*-Bu)-OMe·HCl, EDCI, EDIPA, DMAP, DCM, 0 °C to room temp, 8 h; (g) (i) 70% AcOH, room temp, 12 h; (ii) $C_{15}H_{31}COCl$, pyr, DMAP, DCM, 0 °C to room temp, 10 h; (h) TFA, room temp, 30 min.

Scheme 6[a]



[a] Reagents and conditions: (a) (i) $(Boc)_2O$, TEA, DCM, room temp, 2 h; (ii) MeOH, EDCI, HOBt, TEA, DMAP, DCM, 10 h; (b) $NaBH_4$, MeOH, THF, reflux, 4 h; (c) I_2, PPh_3, imidazole, DCM, 0 °C to room temp, 2 h; (d) Troc-L-Cys-OMe, TEA, DMF, 85 °C, 2 h; (e) (i) TFA, room temp, 30 min; (ii) $C_{15}H_{31}COOH$, EDCI, HOBt, TEA, DMAP, DMF, 60 °C, 10 h; (f) (i) Zn dust, AcOH, H_2O, THF, room temp, 1 h; (ii) $(Boc)_2O$, TEA, DCM, room temp, 1 h; (g) (i) $Ba(OH)_2$, THF/H_2O, 60 °C, 1 h; (ii) H-L-Ser(tBu)-OMe·HCl, EDCI, HOBt, EDIPA, DMAP, DMF, 60 °C, 10 h; (h) TFA, room temp, 30 min.

In addition to the lipopeptides being potentially useful as immunotherapeutic agents for cancer, they also display potent adjuvant properties. Lipopeptides greatly enhance MHC class I restricted CTL proliferation to an immunodominant influenza peptide Mx_{58-66} in a human autologous DC/$CD8^+$ T cell coculture model.[58] Lipopeptide-primed dendritic cells also stimulate the proliferation of allogeneic naive $CD8^+$ CTLs,[58] and immunization of mice with influenza-specific peptide results in greatly enhanced numbers of interferon-γ-secreting $CD8^+$ CTLs when lipopeptides were used as adjuvants.[59] Similarly, MALP-2 induces robust, long-lived CTL responses against HIV-1 Tat protein.[60,61] The synthetic methods that we have developed are easily scalable, and the availability of a free amino group in PAM_2CS should allow the facile introduction of electrophilic labels such as isothiocyanate for covalent coupling to vaccine antigens. Differential protein

binding should also allow considerable control of pharmacokinetic properties. These are currently being investigated.

Experimental Section

Chemistry. All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. Moisture- or air-sensitive reactions were conducted under argon atmosphere in oven-dried (120 °C) glass apparatus. Solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using silica gel 635 (60−100 mesh), while thin-layer chromatography was carried out on silica gel CCM precoated aluminum sheets. All yields reported refer to isolated material characterized by ^{1}H, ^{13}C NMR, and mass spectrometry. Purity for all final compounds was confirmed to be at least 97% by LC−MS using a Zorbax Eclipse Plus 4.6 mm × 150 mm, 5 μm analytical reverse phase C_{18} column with H_2O−isopropanol and H_2O−CH_3CN gradients and an Agilent ESI-TOF mass spectrometer (mass accuracy of 3 ppm) operating in the positive ion acquisition mode.

Syntheses of Compounds 2a ((*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-Methylbenzenesulfonate) and 2b ((*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-Methylbenzenesulfonate). O-Tosylation of (*S*)-(+)- [1a] and (*R*)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol [1b]. To enantiopure (*S*)-(+)- or (*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol (compounds **1a** or **1b**, respectively, 1.0 g, 7.57 mmol, Sigma-Aldrich, Inc., St. Louis, MO) dissolved in anhydrous DCM (30 mL) and cooled to 0 °C was added pyridine (1.22 mL, 15.14 mmol), followed by *p*-toluenesulfonyl chloride (*p*-TsCl; 2.89 g, 22.70 mmol). The reaction solution was brought to room temperature and stirred for 8 h. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 49:1) to afford the literature compounds **2a** (*R*) and **2b** (*S*) in 96−98% yields.[62,63]

Syntheses of Compounds 3a ((*R*)-*tert*-Butyl 2-((2,2-Dimethyl-1,3-dioxolan-4-yl)methylthio)ethylcarbamate) and 3b ((*S*)-*tert*-Butyl 2-((2,2-Dimethyl-1,3-dioxolan-4-yl)methylthio)ethylcarbamate). To a solution of **2a** or **2b** (800 mg, 2.80 mmol) in anhydrous DMF (15 mL) cooled to 0 °C was added sodium hydride (NaH; 101 mg, 4.19 mmol). After the mixture was stirred for 30 min at 0 °C, *N*-Boc-cysteamine (*N*-Boc-2-aminoethanethiol; 595 mg, 3.36 mmol) was added dropwise. The reaction solution was then brought to room temperature and stirred for 8 h. After unreacted NaH was quenched with 1 M HCl, the solvent was removed under reduced pressure, and the residue was dissolved in EtOAc and washed with water. The water layer was extracted thrice with EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4. After removal of solvent under reduced pressure, the residue was purified by flash column chromatography (hexane/EtOAc = 5:1) to afford the title compounds **3a** and **3b** as colorless oils (90−94%).

3a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.98 (d, J = 27.4, 1H), 4.22 (p, J = 6.2, 1H), 4.07 (dd, J = 6.1, 8.3, 1H), 3.67 (dd, J = 6.4, 8.3, 1H), 3.35 (m, 2H), 2.78−2.58 (m, 4H), 1.41 (s, 9H), 1.40 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.78, 109.67, 79.48, 75.58, 68.82, 39.79, 33.01, 28.40, 26.90, 25.55. MS (ESI) calculated for $C_{13}H_{25}NO_4S$, m/z 291.15, found 292.16 $(M + H)^+$ and 314.14 $(M + Na)^+$.

3b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.92 (s, 1H), 4.23 (p, J = 6.2, 1H), 4.12−4.04 (m, 1H), 3.68 (dd, J = 6.4, 8.3, 1H), 3.31 (m, 2H), 2.79−2.58 (m, 4H), 1.43 (s, 9H), 1.41 (d, J = 0.4, 3H), 1.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.78, 109.65, 79.45, 75.57, 68.81, 39.80, 24.95, 32.98, 28.40, 26.89, 25.55. MS (ESI) calculated for $C_{13}H_{25}NO_4S$, m/z 291.15, found 292.15 $(M + H)^+$ and 314.14 $(M + Na^+)$.

Syntheses of Compounds 4a ((*R*)-*tert*-Butyl 2-(2,3-Dihydroxypropylthio)ethylcarbamate) and 4b ((*S*)-*tert*-Butyl 2-(2,3-Dihydroxypropylthio)ethylcarbamate). Acetonide deprotection of compounds **3a** and **3b** (900 mg, 3.09 mmol) was achieved by adding 70% acetic acid ($AcOH/H_2O$ = 7:3) and stirring at room temperature for 12 h. After removal of solvent under vacuum, the reaction residue was dissolved in EtOAc and washed with saturated $NaHCO_3$ solution. The aqueous layer was extracted thrice with EtOAc. The combined EtOAc layers were washed with brine and dried over Na_2SO_4. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 3:1) to afford the title compounds **4a** and **4b** (88−91%).

4a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 8.3, 1H), 7.34 (d, J = 8.0, 1H), 4.93 (s, 1H), 3.85−3.75 (m, 1H), 3.70 (dt, J = 5.2, 10.3, 1H), 3.55 (dd, J = 5.9, 11.3, 1H), 3.31 (d, J = 5.3, 2H), 2.77−2.57 (m, 4H), 1.42 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.20, 79.80, 70.40, 65.26, 39.85, 35.53, 32.96, 28.40. MS (ESI) calculated for $C_{10}H_{21}NO_4S$, m/z 251.12, found 252.13 $(M + H)^+$ and 274.11 $(M + Na)^+$.

4b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 6.98 (s, 1H), 4.87 (s, 1H), 3.79 (ddd, J = 4.2, 6.9, 12.0, 1H), 3.75−3.68 (m, 1H), 3.55 (dt, J = 5.6, 11.3, 1H), 3.32 (d, J = 3.9, 2H), 2.78−2.55 (m, 4H), 1.43 (s, 9H). MS (ESI) calculated for $C_{10}H_{21}NO_4S$, m/z 251.34, found 252.13 $(M + H)^+$ and 274.12 $(M + Na)^+$.

Syntheses of Compounds 5a ((*R*)-3-(2-(*tert*-Butoxycarbonylamino)ethylthio)propane-1,2-diyl Dipalmitate) and 5b ((*S*)-3-(2-(*tert*-Butoxycarbonylamino)ethylthio)propane-1,2-diyl Dipalmitate). O-Palmitoylation of Compounds 5a and 5b. To a solution of compounds **4a** and **4b** (200 mg, 0.80 mmol) in anhydrous DCM (8 mL) at 0 °C were added pyridine (385 μL, 4.78 mmol) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP). Palmitoyl chloride (876 mg, 3.19 mmol) was then added dropwise, and the mixture was brought to room temperature, after which the reaction solution was stirred for 10 h. The reaction mixture was sequentially washed with water, saturated $NaHCO_3$, brine and then dried over Na_2SO_4. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 8:1) to afford the title compounds **5a** and **5b** as white solids (90−92%).

5a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (m, 1H), 4.88 (s, 1H), 4.34 (dd, J = 3.5, 11.9, 1H), 4.15 (dd, J = 6.0, 11.9, 1H), 3.30 (d, J = 6.1, 2H), 2.68 (dd, J = 6.5, 11.5, 4H), 2.38−2.24 (m, 4H), 1.62−1.54 (m, 4H), 1.42 (s, 9H), 1.25 (d, J = 10.6, 48H), 0.86 (t, J = 6.9, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.42, 173.16, 155.77, 79.50, 70.30, 63.56, 39.59, 34.31, 34.12, 43.14, 31.94, 29.72, 29.69, 29.66, 29.61, 29.52, 29.46, 29.38, 29.31, 29.26, 29.15, 29.13, 29.09, 28.39, 24.91, 24.74, 22.71, 14.15. MS (ESI) calculated for $C_{42}H_{81}NO_6S$, m/z 727.58, found 728.59 $(M + H)^+$ and 750.57 $(M + Na)^+$.

5b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (m, 1H), 4.88 (s, 1H), 4.34 (dd, J = 3.5, 11.9, 1H), 4.15 (dd, J = 6.0, 11.9, 1H), 3.30 (d, J = 6.1, 2H), 2.68 (dd, J = 6.4, 11.5, 4H), 2.34−2.25 (m, 4H), 1.64−1.56 (m, 4H), 1.42 (s, 9H), 1.23 (s, 48H), 0.86 (t, J = 6.8, 6H). MS (ESI) calculated for $C_{42}H_{81}NO_6S$, m/z 727.58, found 728.59 $(M + H)^+$ and 750.57 $(M + Na)^+$.

General Procedure for *N*-Boc Deprotection. Syntheses of Compounds 6a ((*R*)-3-(2-Aminoethylthio)propane-1,2-diyl Dipalmitate) and 6b ((*S*)-3-(2-Aminoethylthio)propane-1,2-diyl Dipalmitate). To **5a** and **5b** was added excess dry trifluoroacetic acid (TFA), and the mixture was stirred at room temperature for 30 min. TFA was removed by purging nitrogen and the residue was thoroughly washed with diethyl ether to obtain the title compounds as flaky white solids (98−100%).

6a: ^{1}H NMR (400 MHz, DMSO) δ 7.83 (s, 1H), 5.18−5.06 (m, 1H), 4.30 (dd, J = 2.7, 11.9, 1H), 4.10 (dd, J = 7.2, 12.0, 1H), 2.99 (t, J = 7.2, 2H), 2.90−2.65 (m, 4H), 2.32−2.14 (m, 4H), 1.58−1.42 (m, 4H), 1.23 (s, 48H), 0.84 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.47, 172.29, 69.64, 63.47, 33.62, 33.53, 33.37, 31.29, 30.87, 29.06, 29.03, 29.01, 28.93, 28.75, 28.71, 28.50, 28.42, 28.38, 24.47, 24.38, 22.08, 13.91. MS (ESI) calculated for $C_{37}H_{73}NO_4S$, m/z 627.53, found 628.51 $(M + H)^+$.

6b: ^{1}H NMR (400 MHz, DMSO) δ 7.80 (s, 1H), 5.16−5.05 (m, 1H), 4.30 (dd, J = 2.8, 11.9, 1H), 4.10 (dd, J = 7.1, 12.0,

1H), 2.99 (t, J = 7.1, 2H), 2.87−2.65 (m, 4H), 2.33−2.20 (m, 4H), 1.50 (d, J = 6.2, 4H), 1.23 (s, 48H), 0.84 (t, J = 6.8, 6H). MS (ESI) calculated for $C_{37}H_{73}NO_4S$, m/z 627.53, found 628.51 $(M + H)^+$.

Syntheses of Compounds 7a ((6*R*,13*R*)-6-(Hydroxymethyl)-2,2-dimethyl-4,7-dioxo-3-oxa-11-thia-5,8-diazatetradecane-13,14-diyl Dipalmitate) and 7b ((6*S*,13*R*)-6-(Hydroxymethyl)-2,2-dimethyl-4,7-dioxo-3-oxa-11-thia-5,8-diazatetradecane-13,14-diyl Dipalmitate). The terminal L-serine of the dipeptide unit was appended to **6a** and **6b** by conventional solution phase coupling procedures. To **6a** and **6b** (200 mg, 0.27 mmol) in anhydrous DCM (8 mL) at 0 °C were added Boc-L-Ser-OH (Bachem Americas, Inc., Torrance, CA, 83 mg, 0.40 mmol), (3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI; 103 mg, 0.54 mmol), *N*,*N*-diisopropylethylamine (EDIPA; 53.4 μL, 0.32 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred at 0 °C for 30 min, then brought to room temperature, and stirred for an additional 8 h. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 3:2) to afford the title compounds **7a** or **7b** as white solids (75−80%).

7a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (s, 1H), 5.54 (s, 1H), 5.11 (dt, J = 5.0, 9.8, 1H), 4.36 (dd, J = 3.3, 11.9, 1H), 4.16−4.04 (m, 3H), 3.64 (s, 1H), 3.56−3.37 (m, 2H), 2.90 (s, 1H), 2.77−2.61 (m, 4H), 2.29 (td, J = 4.5, 7.5, 4H), 1.58 (s, 4H), 1.44 (s, 9H), 1.23 (s, 48H), 0.86 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.30, 172.13, 170.33, 154.96, 79.32, 69.11, 62.38, 61.73, 53.57, 37.04, 33.11, 32.90, 31.01, 30.72, 28.50, 28.46, 28.44, 28.29, 28.16, 28.09, 27.92, 27.10, 23.68, 21.49, 12.92. MS (ESI) calculated for $C_{45}H_{86}N_2O_8S$, m/z 814.61, found 837.56 $(M + Na)^+$.

7b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 5.53 (s, 1H), 5.12 (qd, J = 3.4, 6.5, 1H), 4.35 (dd, J = 3.4, 11.9, 1H), 4.16−4.03 (m, 3H), 3.64 (s, 1H), 3.57−3.34 (m, 2H), 2.91 (s, 1H), 2.78−2.61 (m, 4H), 2.29 (td, J = 4.1, 7.6, 4H), 1.60 (s, 4H), 1.44 (s, 9H), 1.23 (s, 48H), 0.86 (t, J = 6.8, 6H). MS (ESI) calculated for $C_{45}H_{86}N_2O_8S$, m/z 814.61, found 837.56 $(M + Na)^+$.

Syntheses of Compounds 8a ((*R*)-3-(2-((*R*)-2-Amino-3-hydroxypropanamido)ethylthio)propane-1,2-diyl Dipalmitate) and 8b ((*S*)-3-(2-((*R*)-2-Amino-3-hydroxypropanamido)ethylthio)propane-1,2-diyl Dipalmitate). **7a** and **7b** were deprotected with TFA as described earlier in the general procedure for *N*-Boc deprotection (see syntheses of **6a**, **6b**). The title compounds were obtained as flaky white solids (99−100%).

8a: ^{1}H NMR (400 MHz, DMSO) δ 8.51 (t, J = 5.7, 1H), 8.08 (s, 1H), 5.47 (s, 1H), 5.08 (dd, J = 4.5, 11.4, 1H), 4.31 (dd, J = 2.7, 11.9, 1H), 4.10 (dd, J = 7.1, 12.0, 1H), 3.69 (dd, J = 9.1, 33.4, 3H), 3.28 (dd, J = 6.5, 13.3, 2H), 2.74 (ddd, J = 6.6, 14.1, 21.4, 2H), 2.62 (t, J = 6.9, 2H), 2.32−2.19 (m, 4H), 1.56−1.45 (m, 4H), 1.23 (s, 48H), 0.85 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.50, 172.27, 166.69, 69.88, 63.45, 60.27, 54.35, 38.52, 33.55, 33.37, 31.28, 31.04, 30.80, 29.05, 29.02, 29.00, 28.92, 28.91, 28.74, 28.71, 28.70, 28.41, 28.36, 24.47, 24.39, 22.08, 13.92. MS (ESI) calculated for $C_{40}H_{78}N_2O_6S$, m/z 714.56, found 715.54 $(M + H)^+$.

8b: ^{1}H NMR (400 MHz, DMSO) δ 8.51 (t, J = 5.4, 1H), 8.08 (s, 1H), 5.48 (s, 1H), 5.09 (d, J = 5.8, 1H), 4.30 (dd, J = 2.6, 11.9, 1H), 4.10 (dd, J = 7.1, 11.9, 1H), 3.85−3.57 (m, 3H), 3.32−3.26 (m, 2H), 2.80 (dd, J = 5.8, 14.1, 1H), 2.65 (ddd, J = 7.0, 13.9, 20.3, 3H), 2.31−2.22 (m, 4H), 1.50 (d, J = 6.8, 4H), 1.23 (s, 48H), 0.84 (t, J = 6.7, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.85, 172.63, 167.10, 70.25, 63.84, 60.65, 54.71, 38.92, 33.92, 33.75, 31.66, 31.42, 31.17, 29.44, 29.41, 29.39, 29.30, 29.13, 29.10, 29.09, 28.80, 28.75, 24.80, 22.46, 14.18. MS (ESI) calculated for $C_{40}H_{78}N_2O_6S$, m/z 714.56, found 715.54 $(M + H)^+$.

Synthesis of Compound 9 ((*R*)-4-(Iodomethyl)-2,2-dimethyl-1,3-dioxolane). To a solution of (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (compound **1a,** 3.0 g, 22.70 mmol) in toluene (50 mL) was added triphenylphosphine (PPh_3; 37.15 g, 27.38 mmol), imidazole (4.64 g, 68.10 mmol), and iodine (7.0 g, 29.51 mmol).[64] The reaction mixture was stirred at 90 °C for 2 h. After removal of toluene under reduced pressure, the residue was dissolved in DCM, washed with saturated $Na_2S_2O_3$ (to quench the unreacted iodine), brine, and dried over Na_2SO_4. The solvent was then removed under vacuum and the residue was purified by flash column chromatography (hexane/EtOAc = 49:1) to afford the literature compound **9**[65] as a colorless liquid (5.44 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30−4.21 (m, 1H), 4.12 (dd, J = 6.1, 8.6, 1H), 3.76 (dd, J = 5.4, 8.6, 1H), 3.23 (dd, J = 4.6, 9.8, 1H), 3.12 (dd, J = 8.5, 9.8, 1H), 1.43 (s, 1H), 1.32 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 109.47, 74.63, 68.57, 26.11, 24.57, 5.67.

Syntheses of Compounds 10a ((*R*)-Methyl 2-(*tert*-Butoxycarbonylamino)-3-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methylthio)propanoate) and 10b ((*S*)-Methyl 2-(*tert*-Butoxycarbonylamino)-3-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methylthio)propanoate). *N*-Boc protected L- and D-cysteine methyl esters were first obtained by treating H-L- and D-Cys-OMe·HCl (Sigma-Aldrich) with di-*tert*-butyl dicarbonate (Boc_2O, 2.0 equiv) in DCM, followed by slow addition of triethylamine (TEA, 1.0 equiv) at 0 °C. DCM was removed under vacuum after the reaction was stirred at 0 °C for 2 h, and the residue was then purified by flash column chromatography with 10% EtOAc/hexane). S-Alkylation of Boc-L-Cys-OMe and Boc-D-Cys-OMe with **9** was then carried out. Compound **10** was synthesized as follows. To a solution of **9** (900 mg, 3.72 mmol) in anhydrous DMF (8 mL) was added TEA (1.55 mL, 11.15 mmol), followed by Boc-L- or D-Cys-OMe (673 mg, 2.86 mmol). The reaction solution was stirred at 85 °C for 4 h. After removal of DMF under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 9:1) to afford **10a** or **10b**, respectively, as viscous oils (60−63%).

10a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.39 (d, J = 7.1, 1H), 4.52 (d, J = 7.3, 1H), 4.21 (p, J = 6.2, 1H), 4.06 (dd, J = 6.1, 8.3, 1H), 3.74 (s, 3H), 3.66 (dd, J = 6.4, 8.3, 1H), 3.02 (d, J = 3.2, 2H), 2.73 (dd, J = 6.0, 13.4, 1H), 2.62 (dd, J = 6.5, 13.4, 1H), 1.43 (s, 9H), 1.40 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.49, 155.23, 109.73, 80.18, 75.64, 68.68, 53.45, 52.58, 38.78, 35.22, 28.31, 26.84, 25.55. MS (ESI) calculated for $C_{15}H_{27}NO_6S$, m/z 349.16, found 372.10 $(M + Na)^+$.

10b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 (d, J = 7.0, 1H), 4.52 (s, 1H), 4.26−4.18 (m, 1H), 4.06 (dd, J = 6.1, 8.3, 1H), 3.76 (d, J = 11.0, 3H), 3.66 (dd, J = 6.5, 8.3, 1H), 3.02 (ddd, J = 5.2, 13.8, 19.4, 2H), 2.75 (dd, J = 5.9, 13.5, 1H), 2.63 (dd, J = 6.3, 13.5, 1H), 1.43 (s, 9H), 1.41 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.49, 155.23, 109.73, 80.18, 75.64, 68.68, 53.45, 52.58, 35.78, 35.22, 28.31, 26.84, 25.55. MS (ESI) calculated for $C_{15}H_{27}NO_6S$, m/z 349.16, found 372.10 $(M + Na)^+$.

General Procedure for Acetonide Deprotection and Palmitoylation of the 1,2-Isopropylideneglycerol Unit. Synthesis of Compound 11 ((*R*)-3-((*R*)-2-Amino-3-methoxy-3-oxopropylthio)propane-1,2-diyl Dipalmitate). To **10a** (200 mg, 0.57 mmol) was added 15 mL of 70% acetic acid ($AcOH/H_2O$ = 7:3), and the mixture was stirred at room temperature for 24 h. After complete removal of AcOH and water under reduced pressure, O-palmitoylation of the diol was carried out by sequential addition of pyridine (278 μL, 3.44 mmol), a catalytic amount of DMAP, followed by palmitoyl chloride (709 μL, 2.29 mmol) to the diol dissolved in anhydrous DCM (15 mL), and the mixture was precooled to 0 °C. After being stirred at 0 °C for 30 min, the reaction solution was brought to room temperature and stirred for 8 h. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 19:1) to afford the *N*-Boc-protected intermediate as a colorless oil. Finally, *N*-Boc deprotection was carried out as described earlier (see syntheses of **6a**, **6b**) to obtain the title compound **11** as a white glassy solid (435 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 2H), 5.13 (s, 1H), 4.41−4.06 (m, 3H), 3.81 (s, 3H), 3.20 (d, J = 41.1, 2H), 2.74 (s, 2H), 2.29 (dd, J = 7.9, 15.5, 4H), 1.58 (d, J = 4.7, 4H), 1.23 (s, 48H), 0.86

(t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.66, 173.63, 168.19, 69.95, 63.47, 53.73, 52.50, 34.24, 34.02, 32.91, 32.46, 31.94, 29.72, 29.70, 29.68, 29.52, 29.38, 29.30, 29.13, 24.85, 22.70, 14.13. MS (ESI) calculated for $C_{39}H_{75}NO_6S$, m/z 685.53, found 686.59 $(M + H)^+$.

General Procedure for Deesterification of 10a and 10b and Subsequent Coupling of Serine. Syntheses of Compounds 12a−d. **10a** and **10b** were deesterified with aqueous LiOH (165 mg, 6.87 mmol dissolved in 6 mL of water/15 mL of THF) at room temperature for 10 h. Then 1 M HCl solution was added (to quench unreacted LiOH and to convert the lithium salt to the free-acid form) until a pH of 4 was reached. After removal of THF under vacuum, the residue was extracted thrice with DCM. The combined DCM layers were washed with brine and dried over Na_2SO_4. After removal of DCM under vacuum, H-L-Ser(^{t}Bu)-O^{t}Bu·HCl or H-L-Ser(^{t}Bu)-OMe·HCl or H-D-Ser-(^{t}Bu)-OMe·HCl (all from Bachem Americas, Inc., Torrance, CA, 437 mg, 2.06 mmol) was coupled to the deesterified intermediates as described earlier for the syntheses of **7a** and **7b**. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 4:1) to afford the corresponding compounds **12a−d** as viscous oils (32−38%).

12a ((*S*)-*tert*-Butyl 3-*tert*-butoxy-2-((*R*)-2-(*tert*-butoxycarbonylamino)-3-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methylthio)propanamido)propanoate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 5.48 (s, 1H), 4.70−4.47 (m, 2H), 4.38−4.22 (m, 2H), 4.07 (dd, J = 6.1, 8.2, 1H), 3.79−3.71 (m, 1H), 3.67 (dd, J = 6.6, 8.2, 1H), 3.59−3.45 (m, 1H), 3.10 (d, J = 6.5, 1H), 2.96 (ddd, J = 6.1, 13.9, 43.9, 1H), 2.74 (ddd, J = 6.1, 13.5, 44.1, 1H), 1.43 (s, 18H), 1.33 (s, 3H), 1.22 (s, 3H), 1.11 (d, J = 4.0, 9H). MS (ESI) calculated for $C_{25}H_{46}N_2O_8S$, m/z 534.30, found 557.29 $(M + Na)^+$.

12b ((*S*)-Methyl 3-*tert*-butoxy-2-((*R*)-2-(*tert*-butoxycarbonylamino)-3-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methylthio)propanamido)propanoate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 8.3, 1H), 5.48 (s, 1H), 4.71−4.59 (m, 1H), 4.41−4.22 (m, 2H), 4.15−4.02 (m, 1H), 3.80 (dd, J = 2.9, 9.1, 1H), 3.72 (s, 3H), 3.68 (dd, J = 6.5, 8.3, 1H), 3.55 (dd, J = 3.2, 9.1, 1H), 3.06 (ddd, J = 5.9, 12.2, 19.8, 1H), 2.98−2.87 (m, 1H), 2.85−2.76 (m, 1H), 2.71 (dt, J = 6.8, 13.5, 1H), 1.44 (s, 9H), 1.42 (s, 3H), 1.34 (s, 3H), 1.12 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.47, 170.41, 155.28, 109.70, 75.70, 74.81, 73.52, 68.73, 61.69, 54.10, 53.12, 52.43, 35.61, 35.22, 28.31, 27.30, 26.87, 25.56. MS (ESI) calculated for $C_{22}H_{40}N_2O_8S$, m/z 492.25, found 515.18 $(M + Na)^+$.

12c ((*R*)-Methyl 3-*tert*-butoxy-2-((*R*)-2-(*tert*-butoxycarbonylamino)-3-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methylthio)propanamido)propanoate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (s, 1H), 5.55 (s, 1H), 4.71 (d, J = 8.2, 1H), 4.39 (s, 1H), 4.35−4.25 (m, 1H), 4.16−4.08 (m, 1H), 3.85 (d, J = 9.0, 1H), 3.76 (s, 3H), 3.70 (t, J = 7.3, 1H), 3.57 (d, J = 9.0, 1H), 3.13 (d, J = 15.0, 1H), 2.93 (dd, J = 6.1, 13.9, 1H), 2.86−2.71 (m, 2H), 1.48 (s, 9H), 1.45 (s, 3H), 1.38 (s, 3H), 1.15 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.60, 170.34, 155.39, 109.70, 75.52, 74.79, 73.50, 68.68, 61.75, 53.99, 52.86, 52.41, 35.51, 30.95, 28.31, 27.30, 26.88, 25.52. MS (ESI) calculated for $C_{22}H_{40}N_2O_8S$ m/z 492.25, found 515.18 $(M + Na)^+$.

12d ((*S*)-Methyl 3-*tert*-butoxy-2-((*S*)-2-(*tert*-butoxycarbonylamino)-3-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methylthio)propanamido)propanoate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 8.3, 1H), 5.48 (s, 1H), 4.71−4.59 (m, 1H), 4.41−4.22 (m, 2H), 4.15−4.02 (m, 1H), 3.80 (dd, J = 2.9, 9.1, 1H), 3.72 (s, 3H), 3.68 (dd, J = 6.5, 8.3, 1H), 3.55 (dd, J = 3.2, 9.1, 1H), 3.06 (ddd, J = 5.9, 12.2, 19.8, 1H), 2.98−2.87 (m, 1H), 2.85−2.76 (m, 1H), 2.71 (dt, J = 6.8, 13.5, 1H), 1.44 (s, 9H), 1.42 (s, 3H), 1.34 (s, 3H), 1.12 (s, 9H). MS (ESI) calculated for $C_{22}H_{40}N_2O_8S$, m/z 492.25, found 515.18 $(M + Na)^+$.

Syntheses of Compounds 13a−d. Acetonide deprotection followed by O-palmitoylation of **12a−d** were performed as per the general procedure outlined earlier (synthesis of compound **11**) to afford the corresponding compounds **13a−d** in 90−95% yields.

13a ((*R*)-3-((*R*)-2-(*tert*-Butoxycarbonylamino)-3-((*S*)-1,3-di-*tert*-butoxy-1-oxopropan-2-ylamino)-3-oxopropylthio)propane-1,2-diyl dipalmitate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (d, J = 7.9, 1H), 5.37 (s, 1H), 5.19−5.09 (m, 1H), 4.51 (dt, J = 2.9, 8.0, 1H), 4.31 (dd, J = 3.5, 11.9, 2H), 4.14 (dd, J = 5.9, 11.9, 1H), 3.76 (dd, J = 2.9, 8.8, 1H), 3.51 (dd, J = 3.0, 8.8, 1H), 2.93 (d, J = 6.2, 2H), 2.79 (d, J = 6.1, 2H), 2.30 (ddd, J = 5.8, 10.8, 12.0, 4H), 1.59 (dd, J = 6.6, 12.8, 5H), 1.43 (d, J = 2.6, 17H), 1.23 (s, 48H), 1.12 (d, J = 2.6, 9H), 0.86 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.40, 173.13, 170.13, 168.92, 81.97, 73.17, 70.32, 63.52, 61.97, 53.51, 35.61, 34.31, 34.11, 33.71, 33.13, 31.94, 29.72, 29.68, 29.66, 29.53, 29.38, 29.33, 29.32, 29.16, 29.15, 28.30, 28.02, 27.34, 24.90, 24.74, 22.71, 14.14. MS (ESI) calculated for $C_{54}H_{102}N_2O_{10}S$, m/z 970.73, found 993.82 $(M + Na)^+$.

13b ((*R*)-3-((*R*)-3-((*S*)-3-*tert*-Butoxy-1-methoxy-1-oxopropan-2-ylamino)-2-(*tert*-butoxycarbonylamino)-3-oxopropylthio)propane-1,2-diyl dipalmitate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, J = 8.1, 1H), 5.17 (s, 1H), 4.69−4.58 (m, 1H), 4.31 (dd, J = 3.5, 11.9, 2H), 4.15 (dd, J = 5.9, 11.9, 1H), 3.80 (dd, J = 3.0, 9.1, 1H), 3.72 (s, 3H), 3.55 (dd, J = 3.2, 9.1, 1H), 2.94 (d, J = 6.1, 2H), 2.79 (d, J = 5.9, 2H), 2.29 (td, J = 4.9, 7.5, 4H), 1.59 (s, 4H), 1.43 (s, 9H), 1.23 (s, 48H), 1.12 (s, 9H), 0.86 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.37, 173.14, 170.44, 170.30, 155.22, 73.51, 70.29, 63.49, 61.67, 53.14, 52.41, 35.52, 34.30, 34.09, 33.20, 31.93, 29.71, 29.51, 29.37, 29.32, 29.31, 29.15, 29.14, 28.29, 27.29, 24.90, 24.88, 22.70, 14.13. MS (ESI) calculated for $C_{51}H_{96}N_2O_{10}S$, m/z 928.68, found 951.57 $(M + Na)^+$.

13c ((*R*)-3-((*R*)-3-((*R*)-3-*tert*-Butoxy-1-methoxy-1-oxopropan-2-ylamino)-2-(*tert*-butoxycarbonylamino)-3-oxopropylthio)propane-1,2-diyl dipalmitate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15−7.06 (m, 1H), 5.19−5.11 (m, 1H), 4.65 (d, J = 8.4, 1H), 4.31 (dd, J = 3.6, 11.9, 2H), 4.14 (dd, J = 5.9, 11.9, 1H), 3.81 (dd, J = 2.9, 9.0, 1H), 3.72 (s, 3H), 3.53 (dd, J = 3.3, 9.0, 1H), 2.94 (dd, J = 14.0, 20.2, 2H), 2.78 (s, 2H), 2.29 (dd, J = 7.5, 13.4, 4H), 1.59 (d, J = 6.7, 4H), 1.44 (s, 9H), 1.23 (s, 47H), 1.12 (d, J = 1.9, 9H), 0.86 (t, J = 6.9, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.76, 172.63, 171.22, 170.29, 73.10, 70.42, 63.59, 55.56, 52.96, 34.36, 34.05, 31.93, 30.96, 29.73, 29.68, 29.61, 29.55, 29.38, 29.32, 29.15, 19.12, 24.84, 22.70, 14.13. MS (ESI) calculated for $C_{51}H_{96}N_2O_{10}S$, m/z 928.68, found 951.57 $(M + Na)^+$.

13d ((*R*)-3-((*S*)-3-((*S*)-3-*tert*-Butoxy-1-methoxy-1-oxopropan-2-ylamino)-2-(*tert*-butoxycarbonylamino)-3-oxopropylthio)propane-1,2-diyl dipalmitate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, J = 8.1, 1H), 5.17 (s, 1H), 4.69−4.58 (m, 1H), 4.31 (dd, J = 3.5, 11.9, 2H), 4.15 (dd, J = 5.9, 11.9, 1H), 3.80 (dd, J = 3.0, 9.1, 1H), 3.72 (s, 3H), 3.55 (dd, J = 3.2, 9.1, 1H), 2.94 (d, J = 6.1, 2H), 2.79 (d, J = 5.9, 2H), 2.29 (td, J = 4.9, 7.5, 4H), 1.59 (s, 4H), 1.43 (s, 9H), 1.23 (s, 48H), 1.12 (s, 9H), 0.86 (t, J = 6.8, 6H). MS (ESI) calculated for $C_{51}H_{96}N_2O_{10}S$, m/z 928.68, found 951.57 $(M + Na)^+$.

General Procedure for One-Step Deprotection of *N*-Boc and *O*-^{t}Bu. Syntheses of Compounds 14a−d. **13a−d** were deprotected with dry TFA as described earlier (general procedure for *N*-Boc deprotection) which allowed for the simultaneous deprotection of the *N*-Boc and *O*-^{t}Bu groups. Consequently, **14a** was obtained as the -Ser-OH (free acid), while **14b−d** were the -Ser-OMe esters. All title compounds (**14a−d**) were obtained as white glassy solids in near-quantitative yields.

14a ((*S*)-2-((*R*)-2-Amino-3-((*R*)-2,3-bis(palmitoyloxy)propylthio)propanamido)-3-hydroxypropanoic acid): ^{1}H NMR (400 MHz, DMSO) δ 9.10 (d, J = 7.4, 1H), 8.82 (d, J = 7.9, 1H), 8.23 (s, 1H), 5.16 (d, J = 7.5, 1H), 4.81−4.58 (m, 1H), 4.42−4.23 (m, 2H), 4.18−4.07 (m, 1H), 3.79 (dd, J = 4.8, 11.0, 1H), 3.70−3.58 (m, 1H), 3.07 (dt, J = 6.7, 13.0, 1H), 2.94−2.65 (m, 3H), 2.31−2.13 (m, 4H), 1.55−1.44 (m, 4H), 1.22 (s, 48H), 0.84 (t, J = 6.7, 6H). ^{13}C NMR (126 MHz, DMSO) δ 179.71, 177.74, 177.51, 176.39, 74.88, 68.75, 66.29, 60.04, 56.60, 38.87, 38.78, 38.61, 38.23, 36.53, 34.30, 34.25, 34.20, 34.17, 34.13, 34.03, 33.98, 33.95, 33.76, 33.68, 33.63, 29.71, 29.62, 27.32, 19.16. MS (ESI) calculated for $C_{41}H_{78}N_2O_8S$, m/z 758.55, found 759.66 $(M + H)^+$.

14b ((*R*)-3-((*R*)-2-Amino-3-((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-ylamino)-3-oxopropylthio)propane-1,2-diyl dipalmitate): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 5.28 (s, 1H), 5.20−5.07 (m, 1H), 4.79−4.60 (m, 2H), 4.47−4.26 (m, 2H), 4.14−4.04 (m, 1H), 3.99−3.90 (m, 1H), 3.74 (s, 3H), 3.24−2.97 (m, 2H), 2.75 (s, 2H), 2.36−2.23 (m, 4H), 1.57 (s, 4H), 1.23 (s, 48H), 0.86 (t, J = 7.0, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.39, 173.97, 169.90, 70.27, 70.08, 63.65, 63.52, 61.90, 52.92, 34.32, 34.05, 31.93, 29.73, 29.68, 29.55, 59.53, 29.38, 29.0, 29.14, 29.11, 24.89, 24.86, 24.82, 22.70, 14.12. MS (ESI) calculated for $C_{42}H_{80}N_2O_8S$, m/z 772.56, found 773.53 $(M + H)^+$.

14c ((*R*)-3-((*R*)-2-Amino-3-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-ylamino)-3-oxopropylthio)propane-1,2-diyl dipalmitate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86−8.37 (m, 1H), 5.40−5.22 (m, 1H), 5.21−5.01 (m, 1H), 4.72−4.42 (m, 2H), 4.42−4.16 (m, 2H), 4.08 (s, 1H), 4.01−3.88 (m, 1H), 3.73 (s, 3H), 3.39−2.98 (m, 2H), 2.73 (s, 2H), 2.29 (s, 4H), 1.56 (s, 4H), 1.23 (s, 48H), 0.85 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.22, 173.92, 70.02, 69.83, 63.66, 63.60, 53.02, 34.30, 34.05, 31.94, 29.73, 29.69, 29.56, 29.55, 29.39, 29.32, 29.16, 29.13, 24.88, 24.83, 22.70, 14.13. MS (ESI) calculated for $C_{42}H_{80}N_2O_8S$, m/z 772.56, found 773.53 $(M + H)^+$.

14d ((*R*)-3-((*S*)-2-Amino-3-((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-ylamino)-3-oxopropylthio)propane-1,2-diyl dipalmitate): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.87 (s, 1H), 5.32 (s, 1H), 5.13 (s, 1H), 4.46 (d, J = 80.5, 3H), 4.13 (s, 1H), 3.99 (s, 2H), 3.78 (s, 3H), 3.23 (s, 2H), 2.78 (s, 2H), 2.33 (s, 4H), 1.61 (s, 4H), 1.61 (s, 3H), 1.27 (s, 48H), 0.89 (t, J = 6.7, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.49, 173.76, 70.42, 63.59, 55.56, 53.70, 52.96, 48.56, 34.36, 34.05, 31.93, 29.73, 29.68, 29.55, 29.38, 29.32, 29.15, 29.12, 24.84, 22.70, 14.13. MS (ESI) calculated for $C_{42}H_{80}N_2O_8S$, m/z 772.56, found 773.53 $(M + H)^+$.

Synthesis of Compound 15 (Ethyl 3-(((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy)-2-hydroxypropanoate). To a solution of ethyl 2,3-epoxypropanoate (Sigma-Aldrich, Inc., 375 mg, 2.91 mmol) in anhydrous DCM (10 mL) was added boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$; 83 mg, 0.58 mmol), followed by (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (**1a**, 1.20 g, 8.72 mmol).[66] The reaction mixture was stirred at room temperature for 5 h. After removal of solvent under reduced pressure, a mixture of ethyl 3-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-hydroxypropanoate (compound **15**) and (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (unreacted starting material, **1a**) were obtained. The R_f values of both the required product and the starting material were identical, which presented difficulty in chromatographic isolation. The mixture was therefore subjected to selective protection of the primary hydroxyl group of the acetonide-protected glycerol **1a** with TBDMSCl (*tert*-butyldimethylsilyl chloride), which allowed the facile isolation of compound **15** by flash column chromatography using 20% EtOAc/hexane (433 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34−4.22 (m, 4H), 4.05 (dd, J = 6.4, 8.3, 1H), 3.88−3.80 (m, 2H), 3.75 (ddd, J = 6.3, 7.5, 8.2, 1H), 3.66−3.52 (m, 2H), 3.15 (dd, J = 6.6, 15.6, 1H), 1.44−1.27 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.49, 171.44, 108.42, 73.59, 73.55, 72.11, 72.04, 71.56, 69.95, 69.85, 65.57, 65.54, 60.85, 28.68, 25.68, 25.67, 24.37, 24.33, 13.17. MS (ESI) calculated for $C_{11}H_{10}O_6$, m/z 248.13, found 271.12 $(M + Na^+)$ and 519.245 $(M + M + Na)^+$.

Synthesis of Compound 16 (Ethyl 3-(((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy)-2-tosyloxy)propanoate). O-Tosylation was performed by sequentially adding TEA (590 μL, 4.23 mmol) and *p*-TsCl (806 mg, 4.23 mmol) to a solution of **15** (350 mg, 1.41 mmol) in anhydrous DCM (10 mL) cooled to 0 °C. The reaction mixture was brought to room temperature and stirred for 8 h. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 9:1) to afford the title compound **16** (443 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.4, 2H), 7.31 (d, J = 8.0, 2H), 5.02−4.96 (m, 1H), 4.18−4.06 (m, 3H), 3.96−3.77 (m, 3H), 3.62 (ddd, J = 3.4, 6.3, 8.4, 1H), 3.54−3.38 (m, 2H), 2.42 (s, 3H), 1.35 (s, 3H), 1.31 (s, 3H), 1.19 (t, J = 7.1, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.34, 143.94, 132.12, 132.09, 128.55, 126.92, 108.23, 108.20, 75.73, 73.22, 71.20, 69.59, 65.31, 60.90, 25.48, 24.18, 20.49, 12.77. MS (ESI) calculated for $C_{18}H_{26}O_8$, m/z 402.13, found 425.13 $(M + Na)^+$.

Synthesis of Compound 17 (Ethyl 2-Azido-3-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)propanoate). To a solution of compound **16** (380 mg, 0.94 mmol) in anhydrous DMF (10 mL) was added sodium azide (184 mg, 2.83 mmol). The reaction mixture was stirred at room temperature for 12 h. After removal of DMF under vacuum, the reaction residue was dissolved in DCM, washed with water, brine, and dried over Na_2SO_4. After removal of solvent under reduced pressure, the residue was purified by flash column chromatography (hexane/EtOAc = 9:1) to afford the title compound **17** (245 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29−4.18 (m, 3H), 3.74 (ddd, J = 6.2, 8.4, 11.2, 1H), 3.61−3.48 (m, 2H), 1.39 (d, J = 0.9, 3H), 1.32 (d, J = 5.0, 3H), 1.29 (t, J = 7.1, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.54, 107.70, 107.67, 72.67, 70.55, 69.89, 64.82, 60.53, 59.86, 24.94, 23.58, 12.37. MS (ESI) calculated for $C_{11}H_{19}N_3O_5$, m/z 273.13, found 296.14 $(M + Na)^+$.

Synthesis of Compound 18 (Ethyl 2-(*tert*-Butoxycarbonylamino)-3-(((*S*)2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)propanoate). Reduction of the Azide to the Amine under Staudinger Conditions.[67] To **17** (230 mg, 0.84 mmol) dissolved in THF (8 mL) and H_2O (30.3 mg, 1.68 mmol) was added PPh_3 (265 mg, 1.01 mmol). The reaction mixture was refluxed for 6 h. After removal of solvent, the reaction residue was dissolved in anhydrous DCM (5 mL) and di-*tert*-butyl dicarbonate (Boc_2O; 551 mg, 2.52 mmol). TEA (351 μL, 2.52 mmol) was added, and the mixture was stirred at room temperature for 8 h. The solvent was removed under vacuum and the residue was purified by flash column chromatography (hexane/EtOAc = 17:3) to afford the title compound **18** (272 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 (t, J = 8.2, 1H), 4.42−4.32 (m, 1H), 4.26−4.13 (m, 3H), 3.99 (dd, J = 6.4, 8.3, 1H), 3.94−3.86 (m, 1H), 3.75−3.63 (m, 2H), 3.55−3.40 (m, 2H), 1.43 (s, 9H), 1.38 (d, J = 1.9, 3H), 1.33 (d, J = 0.6, 3H), 1.25 (t, J = 7.1, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.21, 153.15, 107.09, 77.59, 70.07, 69.60, 69.36, 64.18, 59.17, 51.70, 25.95, 24.32, 23.02, 11.81. MS (ESI) calculated for $C_{16}H_{29}NO_7$, m/z 347.19, found 370.28 $(M + Na)^+$.

Syntheses of Compounds 19a ((*S*)-Methyl 3-*tert*-Butoxy-2-(2-(*tert*-butoxycarbonylamino)-3-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)propanamido)propanoate) and 19b ((*R*)-Methyl 3-*tert*-Butoxy-2-(2-(*tert*-butoxycarbonylamino)-3-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)propanamido)propanoate). Deesterification of the ethyl ester of **18** and subsequent coupling to H-L- and -D-Ser(^{t}Bu)-OMe·HCl were performed as described for the syntheses of compounds **12a**−**d** to yield **19a** and **19b** (70−75%).

19a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 1H), 5.48 (s, 1H), 4.71−4.62 (m, 1H), 4.25 (dt, J = 5.9, 11.7, 2H), 4.06−3.98 (m, 1H), 3.89 (ddd, J = 2.4, 4.0, 9.5, 1H), 3.82−3.68 (m, 5H), 3.66−3.50 (m, 4H), 1.44 (s, 9H), 1.40 (d, J = 2.4, 3H), 1.33 (d, J = 2.2, 3H), 1.15−1.08 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.88, 168.87, 168.81, 108.18, 73.14, 62.15, 71.24, 71.00, 69.90, 65.30, 65.06, 60.56, 51.78, 51.03, 28.38, 26.98, 25.97, 25.43, 24.10. MS (ESI) calculated for $C_{22}H_{40}N_2O_9$, m/z 476.27, found 499.19 $(M + Na)^+$.

19b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 1H), 5.49 (s, 1H), 4.66 (ddd, J = 3.2, 7.2, 11.3, 1H), 4.25 (ddd, J = 5.7, 11.6, 17.0, 2H), 4.06−3.98 (m, 1H), 3.89 (ddd, J = 2.4, 4.0, 9.5, 1H), 3.84−3.76 (m, 1H), 3.75−3.67 (m, 4H), 3.64−3.50 (m, 4H), 1.44 (s, 9H), 1.42−1.38 (m, 3H), 1.36−1.31 (m, 3H), 1.13−1.08 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.90, 167.83, 107.19, 72.16, 71.16, 70.27, 70.03, 68.94, 64.32, 64.09, 59.58, 50.80, 50.05, 27.40, 26.00, 24.99, 24.46, 23.12. MS (ESI) calculated for $C_{22}H_{40}N_2O_9$, m/z 476.27, found 499.19 $(M + Na)^+$.

Syntheses of Compounds 20a ((*S*)-3-(3-((*S*)-3-*tert*-Butoxy-1-methoxy-1-oxopropan-2-ylamino)-2-(*tert*-butoxycarbonylamino)-3-oxopropoxy)propane-1,2-diyl Dipalmitate) and 20b ((*S*)-3-(3-((*R*)-3-*tert*-Butoxy-1-methoxy-1-oxopropan-2-ylamino)-2-(*tert*-butoxycarbonylamino)-3-oxopropoxy)propane-1,2-diyl Dipalmitate). Acetonide deprotection and *O*-palmitoylation were performed as described earlier (see syntheses of **13a**−**d**). **20a** and **20b** were obtained in 80−83% yield.

20a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (t, J = 8.3, 1H), 5.40 (s, 1H), 5.16 (dd, J = 3.3, 5.5, 1H), 4.66 (tt, J = 3.0, 8.8, 1H), 4.38−4.22 (m, 2H), 4.18−4.06 (m, 1H), 3.96−3.76 (m, 2H), 3.71 (d, J = 1.5, 3H), 3.66−3.49 (m, 4H), 2.28 (dd, J = 7.7, 15.5, 4H), 1.58 (d, J = 5.7, 4H), 1.44 (s, 9H), 1.23 (s, 48H), 1.13−1.08 (m, 9H), 0.85 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.18, 171.88, 169.40, 168.79, 154.25, 79.11, 72.29, 69.96, 69.69, 68.70, 68.63, 68.58, 68.55, 68.48, 68.44, 68.41, 61.19, 60.94, 51.90, 51.66, 51.17, 33.06, 32.91, 30.74, 28.52, 28.48, 28.32, 28.18, 18.12, 27.94, 27.11, 26.09, 23.70, 21.51, 12.94. MS (ESI) calculated for $C_{51}H_{96}N_2O_{11}$, m/z 912.70, found 935.54 $(M + Na)^+$.

20b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (t, J = 9.0, 1H), 5.40 (s, 1H), 5.23−5.11 (m, 1H), 4.66 (tt, J = 3.0, 8.6, 1H), 4.30 (ddd, J = 4.3, 10.2, 15.3, 2H), 4.11 (ddt, J = 5.0, 6.0, 10.3, 1H), 3.91−3.76 (m, 2H), 3.71 (d, J = 1.3, 3H), 3.68−3.49 (m, 4H), 2.28 (dd, J = 7.8, 15.5, 4H), 1.58 (dd, J = 6.9, 12.8, 4H), 1.44 (s, 9H), 1.23 (s, 48H), 1.14−1.07 (m, 9H), 0.85 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.84, 171.56, 168.96, 168.46, 153.92, 78.72, 71.96, 69.62, 69.40, 68.37, 68.30, 68.25, 68.22, 68.15, 68.08, 60.86, 60.31, 51.57, 51.32, 50.84, 32.74, 32.58, 30.41, 28.19, 28.15, 27.99, 27.85, 27.79, 27.62, 26.78, 25.72, 23.38, 21.18, 12.61. MS (ESI) calculated for $C_{51}H_{96}N_2O_{11}$, m/z 912.70, found 935.54 $(M + Na)^+$.

Syntheses of Compound 21a ((*S*)-3-(2-Amino-3-((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-ylamino)-3-oxopropoxy)propane-1,2-diyl dipalmitate, Trifluoroacetate) and 21b ((*S*)-3-(2-Amino-3-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-ylamino)-3-oxopropoxy)propane-1,2-diyl dipalmitate, Trifluoroacetate). One-step deprotection of the *N*-Boc and *O*-^{t}Bu groups utilizing TFA was carried out as described earlier (syntheses of **14a**−**d**). Title compounds were obtained as white glassy solid **21a** or **21b** (99−100%).

21a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.03 (d, J = 28.1, 1H), 5.28 (s, 1H), 5.15 (s, 1H), 4.71 (dd, J = 69.3, 103.2, 2H), 4.17 (ddd, J = 7.3, 17.4, 18.6, 2H), 4.06−3.81 (m, 3H), 3.76 (d, J = 14.3, 3H), 3.58 (dd, J = 10.2, 15.7, 2H), 2.28 (dd, J = 7.3, 11.9, 4H), 1.56 (s, 4H), 1.23 (s, 48H), 0.86 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.76, 172.47, 172.41, 172.34, 168.73, 68.50, 68.31, 68.22, 67.59, 61.22, 61.10, 60.49, 54.08, 53.97, 51.92, 51.63, 51.47. 32.90, 32.78, 30.63, 28.42, 28.37, 28.24, 28.08, 28.02, 27.85, 27.80, 23.55, 21.40, 12.82. MS (ESI) calculated for $C_{42}H_{80}N_2O_9$, m/z 756.59, found 757.45 $(M + H)^+$.

21b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.04 (s, 1H), 5.28 (s, 1H), 5.15 (s, 1H), 4.58 (d, J = 51.7, 2H), 4.18 (d, J = 42.7, 2H), 3.90 (s, 3H), 3.76 (d, J = 14.5, 3H), 3.59 (s, 2H), 2.28 (dd, J = 7.4, 11.7, 4H), 1.56 (s, 4H), 1.23 (s, 48H), 0.86 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.11, 171.82, 171.76, 171.69, 168.06, 67.84, 67.64, 67.55, 66.89, 60.55, 60.41, 59.84, 53.42, 53.32, 51.27, 50.98, 50.82, 32.24, 32.14, 29.97, 27.76, 27.72, 27.58, 27.42, 27.35, 27.19, 27.14, 22.89, 20.74, 12.16. MS (ESI) calculated for $C_{42}H_{80}N_2O_9$, m/z 756.59, found 757.45 $(M + H)^+$.

Synthesis of Compound 22 ((*S*)-4-(Benzyloxymethyl)-2,2-dimethyl-1,3-dioxolane). O-Benzylation of **1a** was performed by first adding sodium hydride (NaH; 60% in mineral oil, 363 mg) to a solution of (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (**1a**, 200 mg, 1.51 mmol) in anhydrous DMF (5 mL) on ice. After the mixture was stirred for 10 min, benzyl bromide (BnBr; 360 μL, 3.03 mmol) was added slowly to the reaction mixture. The reaction was brought to room temperature after 30 min and stirred for an additional 8 h. NaH was quenched by slowly adding methanol to the reaction vessel at 0 °C. After removal of solvent, the residue was dissolved in DCM and washed with water (1×) and the resulting water layer was extracted with DCM (3×). The combined DCM layers were washed with brine and dried over Na_2SO_4. After removal of solvent, the residue was purified by flash column chromatography (hexane/EtOAc = 19:1) to yield the title compound **22** (330 mg, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.30 (m, 4H), 7.29−7.24 (m, 1H), 4.62−4.51 (m, 2H), 4.33−4.25 (m, 1H), 4.04 (dd, J = 6.4, 8.3, 1H), 3.73 (dd, J = 6.3, 8.3, 1H), 3.54 (dd, J = 5.7, 9.8, 1H), 3.46 (dd, J = 5.5, 9.8, 1H), 1.40 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.87, 127.39, 126.73, 126.71, 108.39, 73.68, 72.47, 70.00, 65.81, 25.74, 24.35. MS (ESI) calculated for $C_{13}H_{18}O_3$, m/z 222.13, found 245.13 $(M + Na)^+$.

Synthesis of Compound 23 ((*R*)-((2,3-Bis(hexadecyloxy)propoxy)methyl)benzene). Deprotection of the acetonide protecting group of **22** was achieved by stirring the compound (180 mg, 0.81 mmol) in 70% acetic acid ($AcOH/H_2O$ = 7:3) as described for the syntheses of **4a** and **4b**. After complete removal of acetic acid and water under reduced pressure, the residue was dissolved in DMF (5 mL) and cooled to 0 °C. The resulting diol was O-alkylated with 1-iodohexadecane (1.14 g, 3.24 mmol) in the presence of NaH (60% in mineral oil, 770 mg) in anhydrous DMF (8 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C, and the temperature was then raised to room temperature for 8 h. The excess NaH was quenched by slowly adding methanol to the reaction at 0 °C. After removal of solvent, the residue was dissolved in DCM and washed with water (1×). And the resulting water layer was extracted with DCM (3×). The combined DCM layers were washed with brine and dried over Na_2SO_4. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 49:1) to afford a white solid (470 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J = 4.3, 4H), 7.28−7.24 (m, 1H), 4.54 (s, 2H), 3.62−3.44 (m, 7H), 3.41 (t, J = 6.7, 2H), 1.60−1.47 (m, 4H), 1.26 (d, 52H), 0.86 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.42, 128.31, 127.58, 77.88, 73.34, 71.66, 70.79, 70.62, 70.24, 31.93, 30.09, 29.72, 29.66, 29.52, 29.38, 26.10, 22.70, 14.14. MS (ESI) calculated for $C_{42}H_{78}O_3$, m/z 630.60, found 653.61 $(M + Na)^+$.

Synthesis of Compound 24 ((*R*)-2,3-Bis(hexadecyloxy)propyl 4-Methylbenzenesulfonate). O-Debenzylation followed by O-tosylation of **23** en route to the iodo intermediate **25** was obtained as follows: Hydrogenolysis of **23** (500 mg, 0.79 mmol) dissolved in a mixture of 20 mL MeOH/DCM (1:1) was carried out using $Pd(OH)_2/C$ (500 mg) and H_2 at 55 psi in a Parr apparatus for 8 h. The catalyst was removed by filtration over Celite, and the solvent was removed to afford the O-debenzylated intermediate as a white solid (425 mg, ∼100%). After drying completely, the solid was dissolved in anhydrous CH_3CN (15 mL), followed by addition of pyridine (320 μL, 3.96 mmol) and a catalytic amount of DMAP. Then *p*-TsCl (755 mg, 3.96 mmol) was added and the reaction mixture was heated at 70 °C for 12 h. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 17:1) to afford the title compound **24** (527 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.2, 2H), 7.31 (d, J = 8.5, 2H), 4.13 (dd, J = 4.1, 10.3, 1H), 4.00 (dd, J = 5.8, 10.3, 1H), 3.62−3.54 (m, 1H), 3.47−3.41 (m, 2H), 3.38 (t, 2H), 3.33 (t, J = 6.7, 2H), 2.42 (s, 3H), 1.45 (s, 4H), 1.23 (s, 52H), 0.86 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.66, 131.93, 128.75, 126.98, 75.16, 70.75, 69.80, 68.64, 68.30, 30.91, 28.85, 28.69, 28.65, 28.63, 28.60, 28.50, 28.46, 28.44, 28.35, 25.01, 24.94, 21.68, 20.62, 13.11. MS (ESI) calculated for $C_{42}H_{78}O_5S$, m/z 694.56, found 717.52 $(M + Na)^+$.

Synthesis of Compound 25 ((*R*)-1-(1-(Hexadecyloxy)-3-iodopropan-2-yloxy)hexadecane). The *O*-tosyl derivative **24** was converted to the iodo derivative **25**. Iodine (310 mg, 12.23 mmol) and potassium iodide (KI; 2.03 g, 12.23 mmol) were added to a

solution of **25** (850 mg, 1.22 mmol) in anhydrous DMF (15 mL), and the mixture was stirred at 80 °C for 24 h. After removal of DMF under vacuum, the residue was diluted with DCM, washed with water (1×), brine (1×), and dried over Na_2SO_4. The solvent was removed and the residue was purified by flash column chromatography (hexane/EtOAc = 49:1) to yield the title compound **25** (700 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.51 (m, 3H), 3.45−3.39 (m, 3H), 3.37−3.24 (m, 3H), 1.60−1.50 (m, 4H), 1.23 (s, 52H), 0.86 (t, J = 6.9, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 76.40, 70.86, 70.73, 69.25, 30.91, 28.91, 28.69, 28.66, 28.65, 28.61, 28.45, 28.43, 28.35, 25.09, 25.07, 21.68, 13.11, 6.39. MS (ESI) calculated for $C_{35}H_{71}IO_2$, m/z 650.45, found 673.42 $(M + Na)^+$.

Synthesis of Compound 26 ((6*R*,10*R*)-Methyl 10-(Hexadecyloxy)-2,2-dimethyl-4-oxo-3,12-dioxa-8-thia-5-azaoctacosane-6-carboxylate). S-Alkylation of Boc-L-Cys-OMe with **25** was carried out as follows: Boc-L-Cys-OMe (752 mg, 3.20 mmol) and TEA (445 μL, 3.20 mmol) were added to a solution of **25** (260 mg, 0.40 mmol) in anhydrous DMF (8 mL). The reaction was stirred at 85 °C for 2 h. After removal of DMF under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 19:1) to afford the title compound **26** (291 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.53 (d, J = 8.0, 1H), 4.51 (s, 1H), 3.73 (s, 3H), 3.48 (m, 5H), 3.40 (m, 6.7, 2H), 3.07−2.93 (m, 2H), 2.75 (dd, J = 4.9, 13.7, 1H), 2.63 (dd, J = 6.0, 13.7, 1H), 1.57−1.49 (m, 4H), 1.42 (s, 9H), 1.31−1.20 (m, 52H), 0.85 (t, J = 7.0, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.63, 154.23, 28.97, 77.55, 70.68, 70.28, 69.55, 52.47, 51.47, 34.44, 33.51, 30.90, 28.97, 28.69, 28.64, 28.35, 27.28, 25.08, 25.05, 21.68, 13.13. MS (ESI) calculated for $C_{44}H_{87}O_6S$, m/z 757.63, found 780.61 $(M + Na)^+$.

Synthesis of Compound 27 ((5*S*,8*R*,12*R*)-Methyl 8-(*tert*-Butoxycarbonylamino)-12-(hexadecyloxy)-2,2-dimethyl-7-oxo-3,14-dioxa-10-thia-6-azatriacontane-5-carboxylate). Compound **26** was obtained as a viscous oil (228 mg, 64%) via procedures similar to those described for the syntheses of compounds **12a**−**d**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.60 (s, 1H), 4.64 (d, J = 8.1, 1H), 4.30 (s, 1H), 3.79 (dd, J = 3.0, 9.1, 1H), 3.71 (s, 3H), 3.60−3.47 (m, 7H), 3.45−3.35 (m, 2H), 2.95 (dd, J = 5.8, 13.9, 1H), 2.87 (dd, J = 6.9, 13.8, 1H), 2.78 (s, 2H), 1.58−1.49 (m, 4H), 1.43 (s, 6H), 1.30−1.20 (m, 52H), 1.11 (s, 8H), 0.85 (t, J = 7.0, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.58. 169.46, 154.24, 77.23, 72.40, 70.68, 70.27, 69.54, 60.72, 52.13, 51.33, 34.57 33.34, 30.90, 28.95, 28.69, 28.64, 28.62, 28.50, 28.35, 27.28, 26.26, 25.08, 25.03, 21.68, 13.13. MS (ESI) calculated for $C_{51}H_{100}N_2O_8S$, m/z 900.72, found 923.69 $(M + Na)^+$.

Synthesis of Compound 28 ((*S*)-Methyl 2-((*R*)-2-Amino-3-((*R*)-2,3-bis(hexadecyloxy)propylthio)propanamido)-3-hydroxypropanoate, Trifluoroacetate). *N*-Boc deprotection with neat TFA was performed as described for the synthesis of **6a** and **6b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 4.64 (s, 1H), 4.32 (s, 1H), 4.00−3.82 (m, 1H), 3.74 (s, 3H), 3.61−3.48 (m, 3H), 3.47−3.37 (m, 3H), 3.18−3.07 (m, 1H), 3.01−2.89 (m, 1H), 2.86−2.66 (m, 1H), 1.59−1.47 (m, 4H), 1.23 (s, 48H), 0.86 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.05, 77.85, 71.80, 71.12, 70.91, 61.75, 55.32, 53.06, 52.74, 34.98, 34.32, 31.93, 29.73, 29.68, 29.55, 29.51, 29.42, 29.38, 26.06, 25.87, 22.69, 14.12. MS (ESI) calculated for $C_{42}H_{84}N_2O_6S$, m/z 744.61, found 645.61 $(M + H)^+$.

Synthesis of Compound 30 ((*R*)-Methyl 3-Hydroxy-2-palmitamidopropanoate). N-Palmitoylation of **29** was carried out as follows: H-D-Ser-OMe·HCl (**29**) was N-acylated by first generating the free base by the addition of 10 mL of saturated aqueous $NaHCO_3$ solution to a solution of **29** (200 mg, 1.04 mmol, Bachem) in EtOAc (10 mL) and then adding palmitoyl chloride (377 μL, 1.24 mmol) dropwise to the reaction. The reaction mixture, after being stirred for 1 h, was extracted with EtOAc (3×). The combined EtOAc layers were washed with brine and dried over Na_2SO_4. After removal of solvent under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 7:3) to afford the title compound as a white solid (286 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.61 (s, 1H), 4.65−4.58 (m, 1H), 3.92 (dd, J = 3.6, 11.1, 1H), 3.83 (d, J = 11.1, 1H), 3.73 (s, 3H), 2.25−2.18 (m, 3H), 1.64−1.54 (m, 2H), 1.27−1.18 (m, 24H), 0.83 (t, J = 6.9, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ173.98, 171.65, 63.23, 54.59, 52.68, 36.48, 31.82, 29.70, 29.67, 29.66, 29.64, 29.52, 29.37, 29.36, 29.26, 25.59, 22.69, 14.12. MS (ESI) calculated for $C_{20}H_{39}NO_4$, m/z 357.29, found 358.30 $(M + H)^+$ and 380.28 $(M + Na)^+$.

Synthesis of Compound 31 ((*R*)-Methyl 2,2-Dimethyl-3-palmitoyloxazolidine-4-carboxylate). Compound **30** was acetonide protected with 2,2-dimethyoxypropane (2,2-DMP; 24 mL, 195.8 mmol) and pyridinium *p*-toluenesulfonate (PPTS; 281 mg, 1.12 mmol) by adding the above reagents to compound **30** (2.0 g, 5.59 mmol) in toluene (25 mL), and the mixture was refluxed at 90 °C for 22 h. After the mixture was concentrated under reduced pressure, the residue was purified by flash column chromatography (hexane/EtOAc = 9:1) to afford the title compound as a white solid (2.0 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.42 (dd, J = 1.3, 6.3, 1H), 4.20 (dd, J = 1.4, 9.3, 1H), 4.14 (dd, J = 6.3, 9.3, 1H), 3.78 (s, 3H), 2.18−2.03 (m, 2H), 1.67 (s, 2H), 1.63−1.57 (m, 2H), 1.54 (s, 3H), 1.28−1.20 (m, 24H), 0.85 (t, J = 7.0, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.15, 170.17, 96.62, 66.99, 59.49, 52.89, 35.64, 31.93, 29.70, 29.66, 29.64, 29.56, 29.48, 29.37, 29.25, 25.15, 24.57, 23.57, 22.70, 14.13. MS (ESI) calculated for $C_{23}H_{43}NO_4$, m/z 397.59, found 398.33 $(M + H)^+$ and 420.31 $(M + Na)^+$.

Synthesis of Compound 32 ((*S*)-1-(4-(Hydroxymethyl)-2,2-dimethyloxazolidin-3-yl)hexadecan-1-one). The ester functional group of **31** was reduced to the primary alcohol by first diluting lithium borohydride ($LiBH_4$; 2.0 M in THF, 1.89 mL, 3.77 mmol) in 5 mL of THF (5 mL) cooled to 0 °C for 30 min. Comound **31** (500 mg, 1.26 mmol), dissolved in THF (5 mL), was then added dropwise, after which the mixture was stirred at 0 °C for 3 h and then maintained at room temperature for an additional 6 h. After the unreacted $LiBH_4$ was quenched with water, the reaction mixture was extracted with EtOAc (3×) and the combined EtOAc layers were washed with brine and dried over Na_2SO_4. The solvent was removed under vacuum and the resulting residue was purified by flash column chromatography (hexane/EtOAc = 4:1) to yield a white solid (489 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.40 (s, 1H), 4.04 (d, J = 9.0, 1H), 4.01−3.88 (m, 2H), 3.64 (d, J = 10.5, 2H), 2.39−2.26 (m, 2H), 1.62 (s, 2H), 1.59 (d, J = 17.8, 4H), 1.52 (s, 2H), 1.25 (d, J = 22.7, 24H), 0.86 (t, J = 6.9, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.41, 95.27, 65.43, 62.93, 58.55, 35.49, 31.94, 29.71, 29.68, 29.65, 29.54, 29.38, 26.83, 25.20, 22.93, 22.71, 14.15. MS (ESI) calculated for $C_{23}H_{43}NO_4$, m/z 369.58, found 370.33 $(M + H)^+$, 392.31 $(M + Na)^+$.

Synthesis of Compound 33 ((*R*)-1-(4-(Iodomethyl)-2,2-dimethyloxazolidin-3-yl)hexadecan-1-one). Compound **33** (151 mg, 97%) was obtained following the procedure described for the synthesis of **9**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17−4.10 (m, 2H), 4.03−3.96 (m, 1H), 3.26 (dd, J = 9.9, 11.4, 1H), 3.16 (dt, J = 2.5, 9.9, 1H), 2.34−2.16 (m, 2H), 1.67−1.62 (m, 3H), 1.53−1.46 (m, 3H), 1.39−1.16 (m, 26H), 0.86 (t, J = 6.9, 3H). MS (ESI) calculated for $C_{22}H_{42}INO_2$ m/z 479.23, found 502.21 $(M + Na)^+$.

General Procedure for S-Alkylation. Synthesis of Compound 34 (Methyl 2-(*tert*-Butoxycarbonylamino)-3-(((*R*)-2,2-dimethyl-3-palmitoyloxazolidin-4-yl)methylthio)propanoate). To a solution of **33** (300 mg, 0.63 mmol) in anhydrous DMF (8 mL) was added TEA (872 μL, 6.26 mmol), followed by Boc-L-Cys-OMe (1.03 g, 4.38 mmol). The reaction solution was stirred at 85 °C for 2 h. After removal of DMF under vacuum, the residue was purified by flash column chromatography (hexane/EtOAc = 9:1) to afford **34** as a viscous oil (356 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32−5.24 (m, 1H), 4.61−4.48 (m, 1H), 4.03 (d, J = 9.2, 1H), 3.95−3.89 (m, 1H), 3.87−3.81 (m, 1H), 3.76 (s, 3H), 3.07−2.92 (m, 2H), 2.79−2.63 (m, 2H), 2.36−2.17 (m, 2H),

1.70−1.58 (m, 6H), 1.43 (s, 9H), 1.36−1.17 (m, 27H), 0.86 (t, J = 6.9, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.10, 169.73, 155.02, 95.54, 80.37, 66.36, 57.42, 53.39, 52.79, 35.86, 35.49, 35.46, 31.92, 29.70, 29.69, 29.68, 29.66, 29.59, 29.37, 28.27, 26.96, 25.08, 22.90, 22.70, 14.15. MS (ESI) calculated for $C_{31}H_{158}N_2O_6S$, m/z 586.40, found 609.39 $(M + Na)^+$.

Synthesis of Compound 35 ((*S*)-Methyl 3-*tert*-Butoxy-2-((*R*)-2-(*tert*-butoxycarbonylamino)-3-(((*R*)-2,2-dimethyl-3-palmitoyloxazolidin-4-yl)methylthio)propanamido)propanoate). Compound **34** (400 mg, 0.68 mmol) in 15 mL of THF was deesterified using procedures as described for the syntheses of **12a**−**d** with the exception that barium hydroxide octahydrate (645 mg, 2.04 mmol, in 6 mL H_2O at 60 °C, 1 h), and not LiOH, was used; we elected to use the much milder $Ba(OH)_2$ conditions in view of potential lability of the amide bond in **34**. Subsequent coupling to H-L-Ser(^{t}Bu)-OMe·HCl was carried out as described earlier for **12a**−**d**. Compound **35** was obtained as a viscous oil (274 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (d, J = 8.2, 1H), 5.40 (s, 1H), 4.63 (d, J = 8.2, 1H), 4.35−4.24 (m, 1H), 4.11− 4.02 (m, 1H), 3.96−3,87 (m, 2H), 3.81 (dd, J = 2.8, 9.1, 1H), 3.74−3.69 (m, 3H), 3.54 (dd, J = 3.2, 9.1, 1H), 3.05−2.94 (m, 1H), 2.93−2.79 (m, 2H), 2.77−2.67 (m, 1H), 2.38−2.23 (m, 2H), 1.61 (s, 5H), 1.53−1.48 (m, 3H), 1.31−1.20 (m, 24H), 1.12 (s, 9H), 0.85 (t, J = 6.8, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.80, 170.63, 170.51, 170.02, 95.57, 80.38, 73.69, 73.58, 66.43, 63.58, 61.68, 61.61, 57.45, 53.15, 52.50, 52.48, 51.49, 36.71, 35.40, 35.13, 34.94, 32.92, 31.93, 30.95, 29.69, 29.66, 29.65, 29.54, 29.52, 29.38, 29.37, 29.31, 28.32, 28.28, 27.30, 27.28, 26.96, 25.71, 25.16, 22.96, 22.70. MS (ESI) calculated for $C_{38}H_{71}N_3O_8S$, m/z 729.50, found 752.48 $(M + Na)^+$.

Synthesis of Compound 36 ((5*S*,8*R*,12*R*)-Methyl 8-(*tert*-Butoxycarbonylamino)-2,2-dimethyl-7,14-dioxo-12-(palmitoyloxymethyl)-3-oxa-10-thia-6,13-diazanonacosane-5-carboxylate). The general procedure of acetonide deprotection and subsequent O-palmitoylation described earlier for the synthesis of **11** was utilized. The reaction residue was purified by flash column chromatography (hexane/EtOAc = 3:1) to afford the intermediate as a colorless oil (203 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.21 (d, J = 7.9, 1H), 5.56 (d, J = 6.7, 1H), 4.68−4.58 (m, 1H), 4.43−4.31 (m, 2H), 4.21 (dd, J = 5.0, 11.3, 1H), 4.08 (dd, J = 4.9, 11.3, 1H), 3.81 (dd, J = 3.0, 9.1, 1H), 3.72 (s, 3H), 3.55 (dd, J = 3.3, 9.1, 1H), 3.01 (dd, J = 5.5, 13.9, 1H), 2.91−2.79 (m, 2H), 2.75−2.64 (m, J = 5.4, 14.0, 1H), 2.35−2.26 (m, 2H), 2.23−2.10 (m, 2H), 1.63−1.55 (br s, 4H), 1.43 (s, 6H), 1.30−1.19 (m, 52H), 1.14−1.10 (m, 8H), 0.85 (t, J = 7.0, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.90, 173.29, 170.59, 170.47, 155.40, 80.21, 73.57, 64.46, 61.65, 53.72, 53.14, 52.47, 48.83, 36.70, 34.12, 31.93, 29.71, 29.67, 29.55, 29.50, 29.46, 29.42, 29.37, 29.30, 29.27, 29.18, 28.31, 27.27, 25.65, 24.88, 22.70, 14.15. MS (ESI) calculated for $C_{51}H_{97}N_3O_9S$, m/z 927.69, found 950.68 $(M + Na)^+$.

Synthesis of Compound 37 ((*R*)-3-((*R*)-2-Amino-3-((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-ylamino)-3-oxopropylthio)-2-palmitamidopropyl Palmitate, Trifluoroacetate). The previously described *N*-Boc/*O*-^{t}Bu deprotection procedure (see syntheses of **14a**−**d**) was used. The title compound was obtained as a flaky yellow solid (99%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (s, 1H), 6.57 (s, 1H), 4.73−4.59 (m, 1H), 4.53−4.34 (m, 1H), 4.34−4.23 (m, 1H), 4.23−4.15 (m, 1H), 4.15−4.06 (br s, 1H), 4.03−3.84 (m, 2H), 3.79−3.67 (m, 3H), 3.21−2.93 (m, 2H), 2.90−2.63 (m, 2H), 2.37−2.10 (m, 4H), 1.64−1.49 (m, 4H), 1.32−1.16 (m, 48H), 0.85 (t, J = 6.9, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.32, 170.36, 170.21, 64.40, 53.13, 52.76, 52.64, 50.29, 49.20, 36.61, 34.02, 31.94, 29.75, 29.73, 29.72, 29.70, 29.68, 29.60, 29.55, 29.38, 29.34, 29.28, 29.23, 29.20, 29.12, 25.70, 24.85, 24.78, 22.70, 14.13. MS (ESI) calculated for $C_{42}H_{81}N_3O_7S$, m/z 771.58, found 772.59 $(M + H)^+$ and 794.57 $(M + Na)^+$.

Synthesis of Compound 38 ((*R*)-Methyl 2,2,11,11-Tetramethyl-4,9-dioxo-3,10-dioxa-5,8-diazadodecane-6-carboxylate). D-2,3-Diaminopropionic acid monohydrochloride (Sigma-Aldrich, 500 mg, 3.56 mmol) was *N*-Boc protected by Boc_2O (2.33 g, 10.67 mmol) in the presence of TEA (1.49 mL, 10.67 mmol) in DCM (10 mL). After the mixture was stirred at room temperature for 2 h, the solvent was removed under vacuum and the residue was purified by silica flash chromatography (DCM/MeOH = 17:3). The free carboxyl group of the intermediate was converted to the methyl ester by dissolving it in anhydrous DCM (10 mL), followed by sequential addition of EDCI (1.11 g, 5.81 mmol), 1-hydroxybenzotriazole hydrate (HOBt; 785 mg, 5.81 mmol), TEA (810 μL, 5.81 mmol), anhydrous MeOH (353 μL, 8.71 mmol), and a catalytic amount of DMAP. After the mixture was stirred at room temperature for 10 h, solvent was removed from the reaction under vacuum and the reaction residue was dissolved in DCM, washed with water (1×), brine (1×), and dried over Na_2SO_4. The residue was purified by silica flash chromatography (hexane/EtOAc = 22: 3) to afford the title compound **38** (702 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (s, 1H), 4.82 (s, 1H), 4.32 (s, 1H), 3.73 (s, 3H), 3.56−3.40 (m, 2H), 1.44−1.39 (m, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.30, 156.13, 155.41, 80.14, 79.85, 54.17, 52.65, 42.40, 30.95, 28.29, 28.28. MS (ESI) calculated for $C_{14}H_{26}N_2O_6$, m/z 318.18, found 341.17 $(M + Na)^+$ and 659.35 $(M + M + Na)^+$.

Synthesis of Compound 39 ((*R*)-*tert*-Butyl 3-Hydroxypropane-1,2-diyldicarbamate). Sodium borohydride ($NaBH_4$; 285 mg, 7.54 mmol) was added to a solution of **39** (600 mg, 2.20 mmol) for reducing the ester to the corresponding primary alcohol. We used $NaBH_4$ as an alternative to $LiBH_4$ described in the synthesis of **32** with significantly better yields. The reaction mixture was refluxed at 75 °C, and then anhydrous methanol (3 mL) was added dropwise over a period of 1 h. After refluxing for an additional 3 h, the mixture was acidified to pH 2.0 with 1 M HCl and THF was removed under vacuum. The residue was extracted with DCM (3×), and the combined DCM layers were washed with brine and dried over Na_2SO_4. The residue was purified by silica flash chromatography (hexane/EtOAc = 3:1) to yield **39** as a colorless oil (525 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (s, 1H), 4.97 (s, 1H), 3.76−3.45 (m, 4H), 3.41−3.16 (m, 2H), 1.58−1.36 (m, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.75, 155.74, 80.41, 79.62, 61.56, 52.27, 40.12, 30.95, 28.36, 28.29. MS (ESI) calculated for $C_{13}H_{16}N_2O_5$, m/z 290.18, found 313.18 $(M + Na)^+$ and 603.37 $(M + M + Na)^+$.

Synthesis of Compound 40 ((*R*)-*tert*-Butyl 3-Iodopropane-1,2-diyldicarbamate). A procedure essentially identical to that described for the synthesis of **9** was followed; however, the reaction was carried out at 0 °C and the mixture was then warmed to room temperature instead of at 90 °C in an effort to minimize side reactions and improve yield. No significant improvements in yield, unfortunately, were obtained. Compound **40** was obtained as a white solid (489 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (s, 1H), 4.83 (s, 1H), 3.62 (s, 1H), 3.44−3.21 (m, 4H), 1.46 (s, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.76, 154.44, 79.00, 50.54, 43.16 29.92, 27.31, 27.19, 7.69. MS (ESI) calculated for $C_{13}H_{25}N_2O_4$, m/z 400.09, found 423.09 $(M + Na)^+$ and 823.18 $(M + M + Na)^+$.

Synthesis of Compound 41 ((6*R*,10*R*)-Methyl 10-(*tert*-Butoxycarbonylamino)-1,1,1-trichloro-15,15-dimethyl-4,13-dioxo-3,14-dioxa-8-thia-5,12-diazahexadecane-6-carboxylate). Orthogonal protection of the amines of the 2,3-diaminopropionic acid fragment and of the amine of the cysteine unit was necessary. We elected to utilize Troc-L-Cys-OMe, which was obtained from L-cystine dimethyl ester (Sigma-Aldrich) as follows. 2,2,2-Trichloroethyl chloroformate (805 μL, 5.86 mmol) was slowly added to a stirring solution of L-cystine dimethyl ester dihydrochloride (500 mg, 1.47 mmol) in anhydrous DCM (10 mL) and pyridine (10 mL) cooled to 0 °C. The mixture was brought to room temperature and stirred for 8 h. After removal of solvent under vacuum, the residue was purified by flash chromatography (hexane/EtOAc = 4:1) to afford *N*-Troc cystine dimethyl ester as a white solid. The disulfide bond of the *N*-Troc-protected cystine ester was reduced by dissolving the solid in THF (10 mL) and adding tributylphosphine (Bu_3P; 542 μL, 2.20

mmol) and H_2O (132 μL, 7.33 mmol). The mixture was stirred at room temperature for 2 h. After removal of solvent, the residue was purified by flash column chromatography (hexane/EtOAc = 9:1) to afford the title compound as a oil (410 mg, 90%). The characterization data for Troc-L-Cys-OMe (Scheme 6, step d) is provided in the Supporting Information. S-Alkylation of Troc-L-Cys-OMe with **40** was performed as described earlier for **26**. Compound **41** was obtained as a mixture with the oxidized *N*-Troc cystine dimethyl ester as ascertained by LC−MS and ^{1}H NMR. The R_f values of both components were virtually identical, rendering the isolation of **41** very difficult by flash chromatography. Reverse-phase HPLC employing several solvent combinations also did not yield good separations. We reasoned, however, that *N*-Troc cystine dimethyl ester would be inert to both *N*-Boc deprotection and N-acylation conditions that were to follow (see synthesis of **42** below). We therefore proceeded without further purification.

Synthesis of Compound 42 ((6*R*,10*R*)-Methyl 1,1,1-Trichloro-4,13-dioxo-10-palmitamido-3-oxa-8-thia-5,12-diazaoctacosane-6-carboxylate). A crude sample of **41** (200 mg, 0.34 mmol) was dissolved in excess dry TFA and stirred at room temperature for 30 min to effect *N*-Boc deprotection on the 2,3-diaminopropionic acid fragment. Excess TFA was removed by purging nitrogen. The resulting diamino intermediate was coupled with palmitic acid (264 mg, 1.03 mmol) using EDCI (197 mg, 1.03 mmol), HOBt (139 mg, 1.03 mmol), TEA (287 mL, 2.06 mmol), and a catalytic amount of DMAP in anhydrous DMF (8 mL). The mixture was stirred at 60 °C for 10 h. After DMF was removed under reduced pressure, the residue was dissolved with DCM, washed with water (1×), brine (1×), and dried over Na_2SO_4. The residue was purified by silica flash chromatography (hexane/EtOAc = 39:11) to afford the title compound **42** (185 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.90 (d, J = 6.5, 1H), 6.24−6.14 (m, 1H), 4.82−4.73 (m, 1H), 4.73−4.63 (m, 1H), 4.62−4.54 (m, 1H), 3.98 (br s, 1H), 3.76 (d, J = 4.3, 3H), 3.53−3.43 (m, 1H), 3.41−3.30 (m, 1H), 3.12−2.97 (m, 2H), 2.92−2.78 (m, 2H), 2.57−2.43 (m, 1H), 2.23−2.09 (m, 4H), 1.63−1.50 (m, 4H), 1.22 (s, 48H), 0.85 (t, J = 6.9, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.07, 171.90, 168.27, 151.86, 92.87 72.24, 52.01, 50.44, 48.60, 34.42, 34.16, 32.08, 31.71, 29.48, 27.27, 27.24, 27.22, 27.10, 27.07, 26.92, 26.85, 23.17, 20.25, 11.69. MS (ESI) calculated for $C_{42}H_{78}Cl_3N_3O_6S$, m/z 857.47, found 880.45, 881.45, 882.45 (M + Na^+ with expected chlorine isotopic mass-spectral envelope).

Synthesis of Compound 43 ((6*R*,10*R*)-Methyl 2,2-Dimethyl-4,13-dioxo-10-palmitamido-3-oxa-8-thia-5,12-diazaoctacosane-6-carboxylate). The base-labile *N*-Troc protecting group on the cysteine unit had to be converted to the *N*-Boc derivative in order to carry out the subsequent base-catalyzed deesterfication step (see synthesis of **44** below). Accordingly, **42** (350 mg, 0.41 mmol) was dissolved in THF (3 mL), and zinc dust (266 mg, 4.07 mmol), AcOH (15 mL), and H_2O (1 mL) were added to the reaction. After the mixture was stirred at room temperature for 1 h, zinc was filtered on Celite and the filtrate was concentrated under reduced pressure. Reprotection with Boc_2O was achieved by dissolving the completely dried intermediate in DCM, adding Boc_2O (444 mg, 2.04 mmol) in the presence of TEA (284 μL, 2.04 mmol), and stirring the reaction mixture at room temperature for 1 h. After the solvent was removed, the residue was purified by silica flash chromatography (hexane/EtOAc = 7:3) to yield the desired product **43** as a white solid (217 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (s, 1H), 5.43 (s, 1H), 4.55 (s, 1H), 4.03 (s, 1H), 3.82−3.77 (m, 3H), 3.53 (s, 1H), 3.42 (s, 1H), 3.11−2.86 (m, 3H), 2.30−2.13 (m, 4H), 1.69−1.56 (m, 4H), 1.54−1.44 (m,7H), 1.27 (s, 48H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.14, 141.89, 169.32, 169.22, 78.11, 72.52, 51.39, 50.52, 48.69, 48.31, 40.02, 39.64, 34.60, 34.46, 33.77, 33.19, 32.16, 29.76, 27.55, 27.52, 27.50, 27.38, 27.36, 27.21, 27.17, 27.14, 27.12, 26.13, 23.57, 23.50, 23.48, 23.37, 20.53. MS (ESI) calculated for $C_{44}H_{85}N_3O_6S$, m/z 783.62, found 806.61 (M + $Na)^+$.

Synthesis of Compound 44 ((5*S*,8*R*,12*R*)-Methyl 8-(*tert*-Butoxycarbonylamino)-2,2-dimethyl-7,15-dioxo-12-palmitamido-3-oxa-10-thia-6,14-diazatriacontane-5-carboxylate). Deesterification followed by serine coupling was carried out as described for the synthesis of **35** (75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 6.52 (s, 1H), 5.52 (s, 1H), 4.68−4.61 (m, 1H), 3.83−3.77 (m, 1H), 3.74−3.69 (m, 3H), 3.59−3.51 (m, 1H), 3.50−3.32 (m, 2H), 2.98−2.83 (m, 2H), 2.23−2.09 (m, 4H), 1.65−1.51 (m, 4H), 1.46−1.40 (m, 8H), 1.22 (s, 48H), 1.14−1.09 (m, 9H), 0.85 (t, J = 6.8, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.15, 173.17, 169.65, 169.56, 154.54, 79.12, 72.61, 60.69, 52.18, 51.40, 50.55, 41.27, 35.75, 35.61, 30.91, 29.92, 28.69, 28.66, 28.65, 28.54, 28.52, 28.39, 28.38, 28.35, 28.32, 28.30, 27.32, 27.29, 26.26, 24.76, 24.67, 21.67, 13.11. MS (ESI) calculated for $C_{51}H_{98}N_4O_8S$, m/z 926.71, found 949.70 (M + $Na)^+$.

Synthesis of Compound 45 ((*S*)-Methyl 2-((*R*)-2-Amino-3-((*R*)-2,3-dipalmitamidopropylthio)-propanamido)-3-hydroxypropanoate, Trifluoroacetate). Compound **45** was deprotected with TFA as described earlier. The title compounds were obtained as a flaky yellow solid (99%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21−7.11 (m, 1H), 7.20−7.11 (m, 1H), 4.70−4.54 (m, 1H), 4.51−4.37 (m, 1H), 3.90 (s, 2H), 3.71 (s, 3H), 3.49−3.35 (m, 1H), 3.33−3.22 (m, 1H), 3.21−3.01 (m, 1H), 2.80−2.53 (m, 1H), 2.15 (s, 3H), 1.53 (s, 3H), 1.25 (d, J = 21.5, 49H), 0.85 (t, J = 6.9, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 132.65, 131.11, 129.02, 68.37, 52.91, 38.92, 36.93, 36.56, 36.55, 32.15, 30.56, 30.37, 29.97, 29.88, 29.83, 29.76, 29.68, 29.60, 29.56, 29.54, 29.51, 29.47, 29.46, 29.13, 25.98, 25.86, 25.77, 23.94, 23.20, 22.92, 14.41, 14.34, 14.28, 11.17. MS (ESI) calculated for $C_{42}H_{82}N_4O_6S$, m/z 770.60, found 771.60 (M + $H)^+$.

NF-κB Induction. The induction of NF-κB was quantified using HEK-Blue-2 cells as previously described by us.[42] HEK-Blue-2 cells (HEK293 cells stably transfected with TLR2, MD2, CD14, and sAP) were from InvivoGen (San Diego, CA) and were maintained in HEK-Blue Selection medium containing zeocin and normocin. Stable expression of secreted alkaline phosphatase (sAP) under control of NF-κB/AP-1 promoters is inducible by the TLR2 agonists, and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK-Blue-2 cells were incubated at a density of $\sim 10^5$ cells/mL in a volume of 80 μL/well, in 384-well, flat-bottomed, cell culture-treated microtiter plates until confluency was achieved and subsequently graded concentrations of stimuli. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in HEK-detection medium as supplied by the vendor) at 620 nm.

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Supporting Information Available: ^{1}H, ^{13}C, and mass spectral data of all intermediates and final target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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