Journal of Medicinal Chemistry

Article

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Structure–Activity Relationships in Human Toll-like Receptor 2-Specific Monoacyl Lipopeptides

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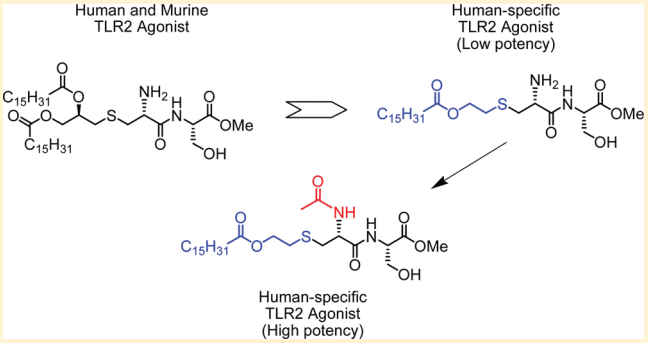
Supporting Information

ABSTRACT: Toll-like receptor 2-agonistic lipopeptides typified by *S*-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-*R*-cysteinyl-*S*-serine (PAM_2CS) compounds are potential vaccine adjuvants. We had previously determined that at least one acyl group of optimal length (C_{16}) and an appropriately orientated ester carbonyl group is essential for TLR2-agonistic activity. We now show that these structurally simpler analogues display agonistic activities with human, but not murine, TLR2. SAR studies on the monoacyl derivatives show that the optimal acyl chain length is C_{16}, and aryl substituents are not tolerated. A variety of alkyl and acyl substituents on the cysteine amine were examined. All *N*-alkyl derivatives were inactive. In contradistinction, short-chain *N*-acyl analogues were found to be highly active, with a clear dependence on the chain length. A cysteine *N*-acetyl analogue was found to be the most potent (EC_{50}: 1 nM), followed by the *N*-butyryl analogue. The *N*-acetyl analogue is human TLR2-specific, with its potency comparable to that of PAM_2CS.



INTRODUCTION

Vaccines are perhaps one of the most successful medical interventions against infectious disease.[1] An important component in the design of effective vaccines is the incorporation of appropriate immune potentiators (also termed adjuvants) along with the antigen; adjuvants initiate early innate immune responses, which lead to the induction of robust and long-lasting adaptive immune responses.[2] More than eight decades have elapsed since the discovery of adjuvanticity of aluminum salts (primarily phosphate and hydroxide) by Glenny and co-workers,[3] and the repertoire of investigational adjuvants has grown to encompass a very wide range of materials;[4] however, aluminum salt-based mineral salts (generically, and incorrectly, termed "alum") have, until the recent introduction of 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL),[5] remained the only adjuvants currently approved by the FDA. Aluminum salts have enjoyed a good safety record but are weak adjuvants for antibody induction, promoting a T_H2-skewed, rather than a T_H1 response.[6,7] Furthermore, not only are aluminum salts ineffective at inducing cytotoxic T lymphocyte (CTL) or mucosal IgA antibody responses but also have an undesirable propensity to induce IgE isotype switching, which has been associated with allergic reactions in some subjects.[6,7]

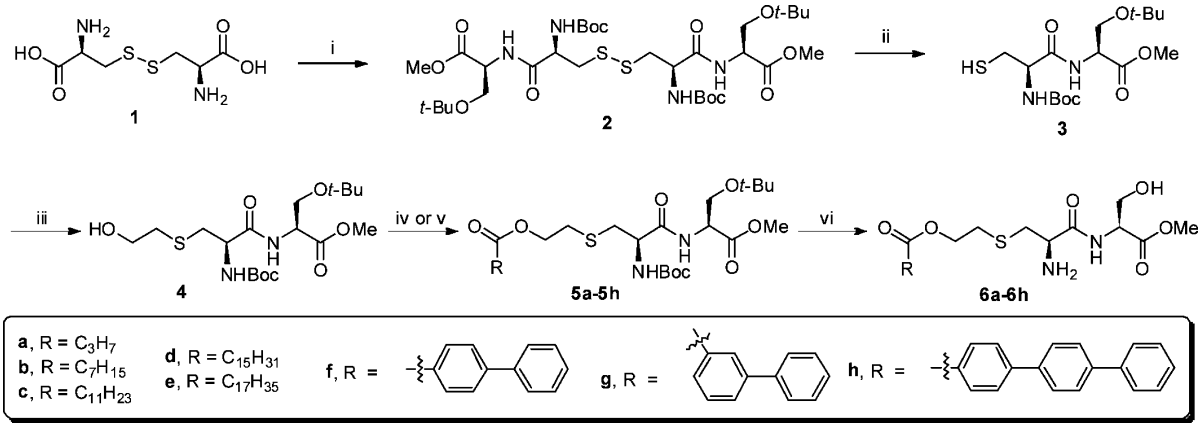
Toll-like receptors (TLRs) are pattern recognition receptors present on diverse cell types. TLRs recognize specific molecular patterns present in molecules that are broadly shared by pathogens but are sufficiently different so as to be distinguishable from host molecules and are collectively referred to as pathogen-associated molecular patterns (PAMPs).[8,9] There are 10 TLRs in the human genome; these are transmembrane proteins with an extracellular domain having leucine-rich repeats (LRR) and a cytosolic domain called the Toll/IL-1 receptor (TIR) domain.[9]

The ligands for these receptors are highly conserved microbial molecules such as lipopolysaccharides (LPS) (recognized by TLR4), lipopeptides (TLR2 in combination with TLR1 or TLR6), flagellin (TLR5), single-stranded RNA (TLR7 and TLR8), double-stranded RNA (TLR3), CpG motif-containing DNA (recognized by TLR9), and profilin present on uropathogenic bacteria (TLR 11).[10,11] TLR1, -2, -4, -5, and -6 respond to extracellular stimuli, while TLR3, -7, -8, and -9 respond to intracytoplasmic PAMPs, being associated with the endolysosomal compartment.[9] The activation of TLRs by their cognate ligands leads to production of inflammatory cytokines, and up-regulation of MHC molecules and costimulatory signals in antigen-presenting cells as well as activating natural killer (NK) cells (innate immune response), in addition to priming and amplifying T- and B-cell effector functions (adaptive immune responses).[12−15] Thus, TLR stimuli serve to link innate and adaptive immunity[13] and can therefore be exploited

Received: January 12, 2012
Published: March 2, 2012

dx.doi.org/10.1021/jm3000533 | *J. Med. Chem.* 2012, 55, 3353−3363

Scheme 1. Syntheses of Monoacyl Lipopeptide 6d and Its Analogues[a]



[a]Reagents and conditions: (i) (a) Boc_2O, Et_3N, H_2O, (b) *H*-Ser(*t*Bu)-OMe·HCl, EDCI, HOBt, Et_3N, DMF; (ii) Bu_3P, CH_2Cl_2; (iii) 2-iodoethanol, Et_3N, DMF. For compounds **5a**–**5g**: (iv) RCOCl, Et_3N, DMAP, CH_2Cl_2. For compounds **5h**: (v) RCOOH, HBTU, Et_3N, DMAP, DMF; (vi) CF_3COOH.

as powerful adjuvants in eliciting both primary and anamnestic immune responses.

We have recently begun to explore in detail structure–activity relationships (SAR) of several immunostimulatory chemotypes.[16−24] Our attention has focused particularly on agonists of TLR2, exemplified by the *S*-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-*R*-cysteinyl-*S*-serine (PAM_2CS) chemotype,[17,23] which distinguishes itself from virtually all other TLR agonists in that although the lipopeptide is devoid of any detectable pro-inflammatory activity in ex vivo human blood models (as defined by the production of detectable levels of TNF-α, IL-1β, IL-6, or IL-8),[25] or of local reactogenicity and pyrogenicity in rabbit models,[18] it is potently adjuvantic in murine models of immunization,[25] suggesting that this chemotype may be a safe and effective adjuvant. Extensive SAR on the PAM_2CS class of compounds[23] led to simplified second-generation monoacyl lipopeptides in which the spacing between the ester-linked acyl group and the thioether was found to play a crucial role in determining activity; homologation of the ethylene-bridged compound (**6d** in Scheme 1) by one methylene unit resulted in complete abrogation of activity.[17]

The structurally simpler, synthetically more accessible, and water-soluble **6d** with its potent TLR2-agonistic properties in both primary screens employing human TLR2, as well as secondary screens in ex vivo human blood models presented an excellent lead. Prior to commencing immunization studies in rodent and nonrodent models, this compound was subjected to further evaluation. We found, entirely to our surprise, that unlike PAM_2CS, **6d** showed exquisite specificity in activating human TLR2 (hTLR2), but not murine TLR2 (mTLR2), suggesting that the binding mode of **6d** to TLR2 may be substantially different from that of PAM_2CS. This conjecture was strengthened by the observation that *N*-acylation of **6d** with a palmitoyl group resulted in complete loss of hTLR2-agonistic activity, which is unexpected given the pronounced activity of the analogous, *N*-palmitoylated PAM_2CS compound (PAM_3CS) and crystallographic evidence for the engagement of the TLR2/TLR1 heterodimer by PAM_3CS.[26,27] These preliminary findings warranted a detailed SAR on the monoacylated lipopeptide chemotype, our goals being not only to identify potentially more potent analogues of **6d** but also to attempt to understand the structural correlates determining human versus murine TLR2 specificity.

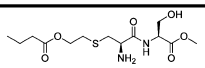
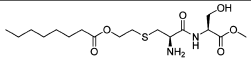
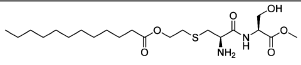
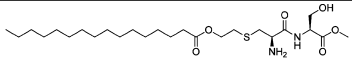
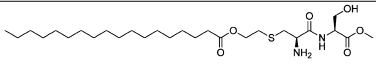
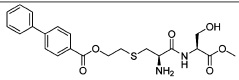
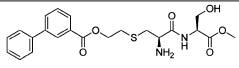
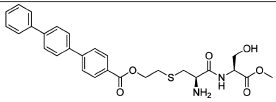
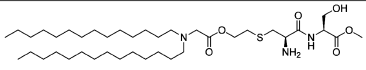
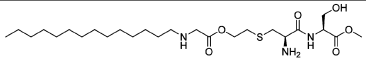
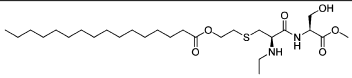
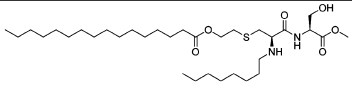
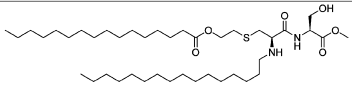
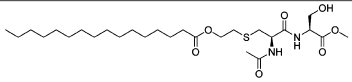
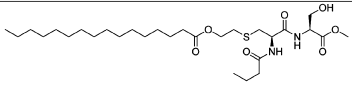
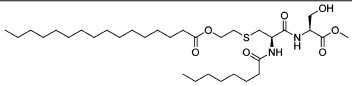
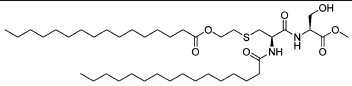
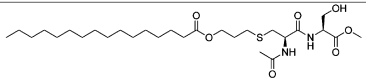
RESULTS AND DISCUSSION

The SAR of the monoacyl derivatives began with a careful exploration of the nature of the ester-linked acyl group on the mercaptoethanol fragment of the lipopeptide; specifically, we wished to determine the optimal chain length corresponding to maximal TLR2-stimulatory activity and whether aryl groups could substitute for the palmitoyl group. The syntheses of these analogues were accomplished (Scheme 1) using the strategy previously described by us;[17] as mentioned earlier, **6d**, with a palmitoyl group (Scheme 1), was the point of departure. Examination of this series of compounds showed a clear-cut and straightforward SAR: **6d** (palmitoyl) and **6e** (stearoyl) were active in engaging hTLR2 (3.63 and 4.79 nM, respectively, Table 1) but entirely inactive in assays using mTLR2 (Figure 1). The shorter-chain analogues **6a**–**6c** were completely inactive (Table 1). Aryl substituents were found to abrogate activity as exemplified by the complete absence of activity in the biphenyl-4-carboxylate (**6f**) and biphenyl-3-carboxylate (**6g**) as well as the *p*-terphenyl-4-carboxylate (**6h**) derived analogues. The latter results were not unexpected given the dimensions of the hydrophobic tunnel in the crystal structure of TLR2;[26,28] however, we were also interested in examining the possibility of these inactive analogues behaving as antagonists of TLR2 because there is only one known report of a low-potency lanthionine-derived antagonist.[29] None of the above-mentioned inactive compounds were antagonistic in homotypic (using PAM_2CS as stimulus) or heterotypic (using lipoteichoic acid[30−32] as stimulus) assays in mTLR2 and hTLR2 assays (data not shown).

Our previous SAR on the lipopeptides demonstrated the absolute requirement of the ester-linked long-chain group and of the importance of the orientation of the carbonyl group of the ester; in these earlier studies, we had found that replacement of the long-chain hydrocarbon functional group with polar polyether or polyamine moieties were not tolerated.[17] We were interested in examining a more conservative modification. We hypothesized that the replacement of the palmitoyl group by a 2-(ditetradecylamino) acetate fragment (**10a**, Scheme 2) may confer to the molecule dual

dx.doi.org/10.1021/jm3000533 | J. Med. Chem. 2012, 55, 3353−3363

Table 1. EC_{50} Values (± standard Error of Means) of Compounds in Human TLR2-Specific Reporter Gene Assay[a]

Structure	Compound Number	TLR2-Agonistic Activity (nM)
	6a	ND
	6b	ND
	6c	ND
	6d	3.63 ± 0.125
	6e	4.79 ± 0.215
	6f	ND
	6g	ND
	6h	ND
	10a	ND
	10b	ND
	11a	ND
	11b	ND
	11c	ND
	11d	1.01 ± 0.051
	11e	1.64 ± 0.712
	11f	3.80 ± 0.156
	11g	ND
	16	5.52 ± 0.195

[a]ND denotes no activity detected at 10 μM.

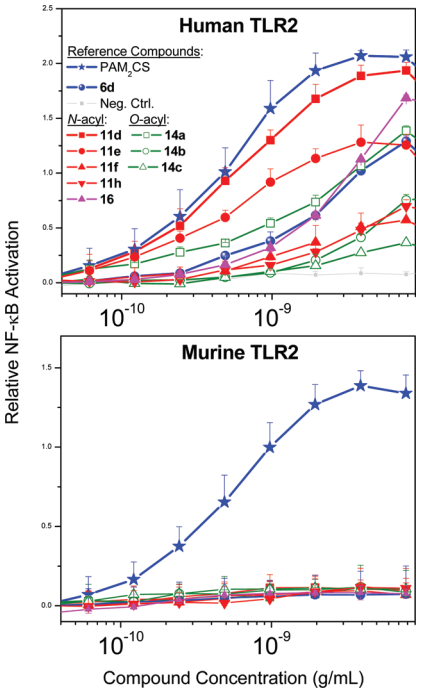


Figure 1. TLR2-specific NF-κB induction by selected analogues in human and murine TLR2 reporter gene assays. Means and standard deviations of quadruplicate samples are shown.

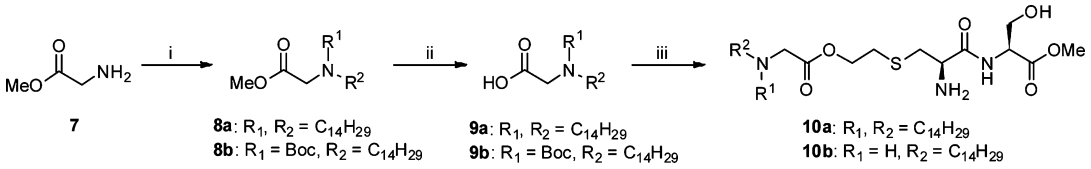
hTLR2/mTLR2 activity by mimicking PAM_2CS. Our assumption was incorrect because both **10a** and its monoalkyl homologue, **10b**, were completely inactive (Table 1).

As mentioned earlier, *N*-palmitoylation of **6d** (**11g**, Scheme 3) resulted in an unanticipated loss of TLR2-agonistic activity (Table 1). We therefore examined in detail a variety of alkyl and acyl substituents on the cysteine amine. The *N*-alkyl derivatives **11a** (ethyl), **11b** (octyl), and **11c** (hexadecyl) analogues (obtained by either reductive amination using appropriate aldehydes for **11a** and **11b** or by direct alkylation in the case of **11c** using hexadecyl bromide) were inactive (Table 1). In contradistinction, the short-chain *N*-acyl analogues were found to be highly active, with a clear dependence on the chain length: the *N*-acetyl analogue **11d** was found to be the most potent (1 nM), followed by the *N*-butyryl analogue **11e** and *N*-octanoyl compound **11f** (3.8 nM). Further homologation (**11g**, palmitoyl) resulted in complete loss of activity. Two points are to be noted; first, the active analogues (**11d**–**11f**) retained specificity toward hTLR2 and displayed no agonistic activity in mTLR2; second, while the EC_{50} of the lead compound (**6d**: 3.6 nM) is, at first glance, comparable to that of the *N*-acetyl derivative (**11d**: 1 nM), the absolute magnitude of TLR2-induced nuclear translocation of NF-κB is far greater for **11d** and approaches that of PAM_2CS (Figure 1), indicating the higher potency of **11d**.

The progressive decrease in activity with increasing *N*-acyl chain length strongly suggested steric issues. We wanted to confirm this hypothesis and also evaluate replacements of the *N*-acetyl group with functionalities differing in electron-withdrawing properties. The trifluoroacetamido derivative **11h** retains weaker activity (EC_{50}: 4.5 nM, but low magnitude of NF-κB activation, Figure 1), while the trichloroacetamido analogue **11i** is inactive; consistent with these results are the observations that the methanesulfonamide (**11j**), trifluoromethanesulfonamide (**11k**), and *p*-toluenesulfonamide (**11l**)

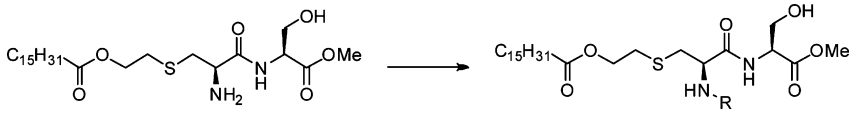
dx.doi.org/10.1021/jm3000533 | J. Med. Chem. 2012, 55, 3353–3363

Scheme 2. Syntheses of Analogues of 6d[a]



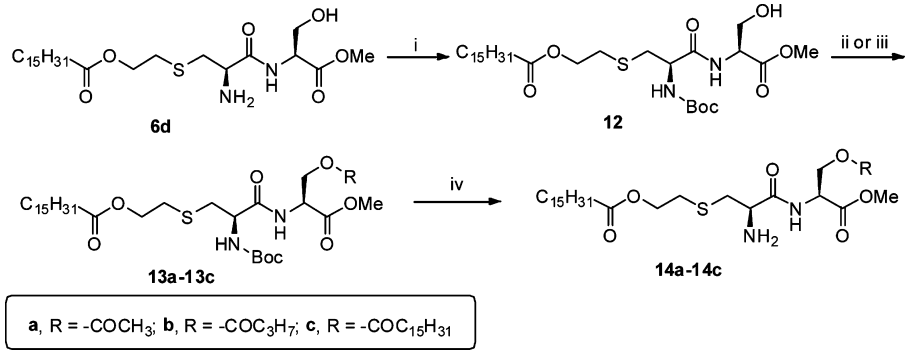
[a]Reagents and conditions: (i) (a) 1-bromotetradecane, Et_3N, DMF, (b) Boc_2O, Et_3N, CH_2Cl_2; (ii) LiOH, H_2O, THF; (iii) (a) **4**, EDCI, DMAP, NMM, CH_2Cl_2, (b) CF_3COOH.

Scheme 3. Syntheses of *N*-Alkyl, *N*-Acyl, and *N*-Sulfonyl Derivatives of 6d



Compounds	R	Reagents and conditions
11a	$-C_2H_5$	CH_3CHO, CH_3COOH, MP-$CNBH_3$, CH_2Cl_2
11b	$-C_8H_{17}$	$C_7H_{15}CHO$, CH_3COOH, MP-$CNBH_3$, CH_2Cl_2
11c	$-C_{16}H_{33}$	$C_{16}H_{33}Br$, Et_3N, CH_2Cl_2
11d	$-COCH_3$	$(CH_3CO)_2O$, Et_3N, CH_2Cl_2.
11e	$-COC_3H_7$	C_3H_7COCl, pyridine
11f	$-COC_7H_{15}$	$C_7H_{15}COCl$, pyridine
11g	$-COC_{15}H_{31}$	$C_{15}H_{31}COCl$, pyridine
11h	$-COCF_3$	$(CF_3CO)_2O$, Et_3N, CH_2Cl_2
11i	$-COCCl_3$	EDCI.HCl, HOBt, CCl_3COOH, CH_2Cl_2
11j	$-SO_2CH_3$	$(CH_3SO_2)_2O$, Et_3N, CH_2Cl_2
11k	$-SO_2CF_3$	$(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2
11l	$-SO_2C_6H_4CH_3$	$CH_3C_6H_4SO_2Cl$, Et_3N, CH_2Cl_2

Scheme 4. Syntheses of *O*-Acyl Derivatives of 6d[a]



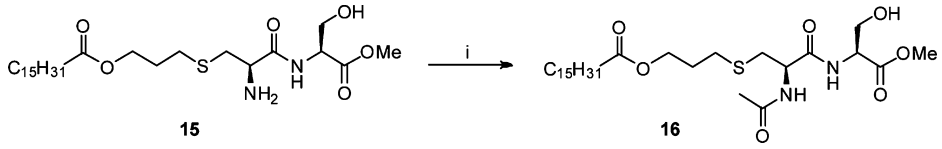
[a]Reagents and conditions: (i) $(Boc)_2O$, Et_3N, CH_2Cl_2. For compound **13a**: (ii) $(CH_3CO)_2O$, pyridine. For compound **13b**–**13c**: (iii) RCl, Et_3N, THF; (iv) CF_3COOH.

derivatives are all inactive. Taken together, these results highlight the simultaneous influence of electronic and steric effects of the substituents on the amine.

Given that the cysteine *N*-acyl substituents showed dramatic differences in activity as described above, it was of interest to explore serine *O*-acyl substituents as well. The syntheses of these analogues (Scheme 4) were straightforward, requiring the protection of the cysteine amine as the *t*-butyl carbamate, followed by *O*-acylation with either anhydride (**14a**) or acyl chlorides (**14b**, **14c**). The activity of the *O*-acetyl analogue **14a** is virtually indistinguishable from that of **6d**; progressive loss in activity was evident with increasing acyl chain lengths (Figure 1 and Table 1). As was observed for all of the compounds

dx.doi.org/10.1021/jm3000533 | J. Med. Chem. 2012, 55, 3353–3363

Scheme 5. Syntheses of *N*-Acetyl Derivative of Homologue of 6d[a]



[a]Reagents and conditions: (i) $(CH_3CO)_2O$, Et_3N, CH_2Cl_2.

described above, the *O*-acyl analogues were also found to be specific for hTLR2.

Given that *N*-acetylation appeared to specifically enhance TLR2-agonistic potency while preserving specificity for human TLR2, it was interesting to examine if this modification on an inactive homologue of **6d** that we had previously identified[17] would also result in augmented agonistic activity. The propylene-bridged 3-((*R*)-2-amino-3-((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-ylamino)-3-oxopropylthio)propyl palmitate compound **15** was *N*-acetylated to furnish **16** as depicted in Scheme 5. The *N*-acetylated derivative was found to be active, albeit much weaker in potency than **11d** (Table 1, Figure 1), clearly emphasizing the role of *N*-acetyl group in determining TLR2-agonistic potency, although the structural basis for this observation remains to be elucidated.

CONCLUSION

Our continuing SAR studies on the TLR2-agonistic lipopeptide chemotype have led to the identification of new analogues possessing strong TLR2-agonistic activity that is exquisitely human TLR2-specific. It is being increasingly appreciated that significant differences between murine and nonrodent species exist not only in receptor specificity to TLR ligands but also in the cellular responses to them. As has been observed with TLR4 ligands such as taxol,[33] lipid IVa, and E5531, a synthetic lipid A analogue,[34] recent evidence suggests that significant interspecies differences exist for TLR2 also, as exemplified by variations in specificities for lipopeptide recognition in chimeric TLR constructs.[35] Furthermore, the coupling of these pattern recognition receptors to downstream adaptor molecules also appear to be distinct as shown by disparities in clinical outcomes in humans with IRAK-4 (interleukin-1 receptor-associated kinase 4) deficiency versus the susceptibility to pathogens in knockout mice.[36] We do not yet understand the structural basis for this specificity, which will have to await detailed crystallographic studies.

As discussed above, TLR2 agonists appear unique among all other TLR agonists in that although the lipopeptides are devoid of any detectable pro-inflammatory activity in ex vivo human blood models,[25] or of local reactogenicity and pyrogenicity in rabbit models,[18] it is potently adjuvantic in murine models of immunization,[25] suggesting that this chemotype may be a safe and effective adjuvant. The human-specific TLR2-agonistic properties of the monoacyl lipopeptides precludes its evaluation in murine models, and it remains to be examined if nonrodent animal models (including nonhuman primates) would be suitable surrogates to evaluate the safety and efficacy of these analogues. These studies are currently underway.

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 nitrogen atmosphere in oven-dried (120 °C) glass apparatus. The solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using RediSep Rf "Gold" high performance silica columns on CombiFlash Rf instrument unless otherwise mentioned, while thin-layer chromatography was carried out on silica gel CCM precoated aluminum sheets. Purity for all final compounds was confirmed to be greater than 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 or H_2O–CH_3CN gradients and an Agilent ESI-TOF mass spectrometer (mass accuracy of 3 ppm) operating in the positive ion (or negative ion, as appropriate) acquisition mode.

General Procedure for the Syntheses of Compounds 5a–5g. *Synthesis of Compound **5a**: 2-(((R)-3-(((S)-3-(tert-butoxy)-1-methoxy-1-oxopropan-2-yl)amino)-2-((tert-butoxycarbonyl)-amino)-3-oxopropyl)thio)ethyl butyrate.* To a solution of compound **4** (100 mg, 0.24 mmol) in anhydrous CH_2Cl_2 (5 mL) were added triethylamine (49 μL, 0.18 mmol) and 4-dimethylaminopyridine (DMAP, 3.0 mg, 0.024 mmol), and the reaction mixture was stirred at room temperature. After 10 min, butyryl chloride (30 μL, 0.28 mmol) was added and the reaction mixture was stirred for further 30 min. The solvent was then removed using vacuum, and the residue was purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain the compound **5a** (99 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, *J* = 7.8 Hz, 1H), 5.44 (s, 1H), 4.65 (dt, *J* = 8.1, 3.0 Hz, 1H), 4.33 (d, *J* = 4.5 Hz, 1H), 4.24 (t, *J* = 6.6 Hz, 2H), 3.82 (dd, *J* = 9.1, 2.9 Hz, 1H), 3.74 (s, 3H), 3.57 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.01 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.92 (dd, *J* = 13.9, 6.7 Hz, 1H), 2.84 (dd, *J* = 12.2, 6.1 Hz, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.65 (m, 2H), 1.45 (s, 9H), 1.13 (s, 9H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.61, 170.59, 170.50, 155.37, 80.40, 77.42, 77.42, 77.16, 76.91, 73.65, 63.15, 61.78, 53.84, 53.32, 52.57, 36.18, 34.98, 31.19, 28.43, 27.43, 18.50, 13.82. MS (ESI) calcd for $C_{22}H_{40}N_2O_8S$, *m*/*z* 492.25; found 515.24 $(M + Na)^+$.

Compounds **5b**, **5c**, and **5e–5g** were synthesized similarly to compound **5a**. Compound **5d** was synthesized as published earlier.[17]

*2-(((R)-3-(((S)-3-(tert-Butoxy)-1-methoxy-1-oxopropan-2-yl)-amino)-2-((tert-butoxycarbonyl) amino)-3-oxopropyl)thio)ethyl octanoate (**5b**).* Yield 119 mg, 92%. ^{1}H NMR (500 MHz, CDCl3) δ 7.13 (d, *J* = 7.9 Hz, 1H), 5.44 (s, 1H), 4.65 (dt, *J* = 8.1, 3.0 Hz, 1H), 4.32 (s, 1H), 4.24 (t, *J* = 6.6 Hz, 2H), 3.82 (dd, *J* = 9.1, 2.9 Hz, 1H), 3.74 (s, 3H), 3.57 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.01 (dd, *J* = 13.9, 5.5 Hz, 1H), 2.92 (dd, *J* = 13.9, 6.8 Hz, 1H), 2.83 (dd, *J* = 12.6, 6.3 Hz, 2H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.61 (dd, *J* = 14.7, 7.4 Hz, 2H), 1.45 (s, 9H), 1.31–1.24 (m, 8H), 1.14 (s, 9H), 0.87 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.05, 170.84, 170.76, 155.61, 80.64, 73.91, 63.39, 62.03, 54.09, 53.57, 52.82, 35.22, 34.57, 32.04, 31.42, 29.48, 29.32, 28.69, 27.68, 25.28, 22.99, 14.47. MS (ESI) calcd for $C_{26}H_{48}N_2O_8S$, *m*/*z* 548.31; found 571.31 $(M + Na)^+$.

*2-(((R)-3-(((S)-3-(tert-Butoxy)-1-methoxy-1-oxopropan-2-yl)-amino)-2-((tert-butoxycarbonyl) amino)-3-oxopropyl)thio)ethyl dodecanoate (**5c**).* Yield 124 mg, 87%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (d, *J* = 7.9 Hz, 1H), 5.44 (s, 1H), 4.65 (dt, *J* = 8.1, 3.0 Hz, 1H), 4.33 (d, *J* = 4.2 Hz, 1H), 4.23 (t, *J* = 6.6 Hz, 2H), 3.82 (dd, *J* = 9.1, 3.0 Hz, 1H), 3.74 (s, 3H), 3.57 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.01 (dd, *J* = 13.9, 5.5 Hz, 1H), 2.92 (dd, *J* = 13.9, 6.8 Hz, 1H), 2.83 (dd, *J* = 12.6, 6.3 Hz, 2H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.60 (dd, *J* = 14.6, 7.3 Hz, 2H), 1.45 (s, 9H), 1.30–1.23 (m, 16H), 1.14 (s, 9H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.05, 170.84, 170.76, 155.61, 80.65, 73.91, 63.39, 62.03, 54.09, 53.57, 52.82, 35.22, 34.58, 32.30,

dx.doi.org/10.1021/jm3000533 | J. Med. Chem. 2012, 55, 3353–3363

31.41, 30.00, 29.86, 29.73, 29.67, 29.54, 28.69, 27.68, 25.28, 23.08, 14.52. MS (ESI) calcd for $C_{30}H_{56}N_2O_8S$, m/z 604.38; found 627.37 $(M + Na)^+$.

*2-(((R)-3-(((S)-3-(tert-Butoxy)-1-methoxy-1-oxopropan-2-yl)amino)-2-((tert-butoxycarbonyl) amino)-3-oxopropyl)thio)ethyl stearate (**5e**).* Yield 142 mg, 87%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, J = 7.9 Hz, 1H), 5.44 (s, 1H), 4.65 (dt, J = 8.1, 3.0 Hz, 1H), 4.33 (d, J = 4.5 Hz, 1H), 4.24 (t, J = 6.6 Hz, 2H), 3.82 (dd, J = 9.1, 2.9 Hz, 1H), 3.74 (s, 3H), 3.57 (dd, J = 9.1, 3.2 Hz, 1H), 3.01 (dd, J = 13.9, 5.5 Hz, 1H), 2.92 (dd, J = 13.9, 6.8 Hz, 1H), 2.83 (dd, J = 12.7, 6.4 Hz, 2H), 2.31 (t, J = 7.6 Hz, 2H), 1.63−1.58 (m, 2H), 1.46 (s, 9H), 1.30−1.22 (m, 28H), 1.14 (s, 9H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.05, 170.84, 170.76, 155.59, 80.65, 73.91, 63.38, 62.03, 54.09, 53.58, 52.82, 35.22, 34.58, 32.32, 31.42, 30.10, 30.06, 30.02, 29.88, 29.76, 29.69, 29.55, 28.69, 27.69, 25.28, 23.09, 14.53. MS (ESI) calcd for $C_{36}H_{68}N_2O_8S$, m/z 688.47; found 711.48 $(M + Na)^+$.

*2-(((R)-3-(((S)-3-(tert-Butoxy)-1-methoxy-1-oxopropan-2-yl)amino)-2-((tert-butoxycarbonyl) amino)-3-oxopropyl)thio)ethyl-[1,1′-biphenyl]-4-carboxylate (**5f**).* Yield 87 mg, 60%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.63 (dd, J = 5.2, 3.3 Hz, 2H), 7.47 (t, J = 7.5 Hz, 2H), 7.40 (dt, J = 9.3, 4.3 Hz, 1H), 7.17 (d, J = 7.9 Hz, 1H), 5.48 (s, 1H), 4.67 (dt, J = 8.1, 3.0 Hz, 1H), 4.52 (t, J = 6.6 Hz, 2H), 4.38 (s, 1H), 3.83 (dd, J = 9.1, 2.9 Hz, 1H), 3.73 (s, 3H), 3.57 (dd, J = 9.1, 3.2 Hz, 1H), 3.09 (dd, J = 13.9, 5.5 Hz, 1H), 3.04−2.94 (m, 3H), 1.46 (s, 9H), 1.13 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.61, 170.52, 166.42, 155.40, 145.94, 140.13, 130.38, 129.08, 128.79, 128.32, 127.43, 127.23, 80.44, 73.67, 63.79, 61.78, 53.88, 53.34, 52.58, 35.05, 31.28, 28.44, 27.43. MS (ESI) calcd for $C_{31}H_{42}N_2O_8S$, m/z 602.27; found 625.27 $(M + Na)^+$.

*2-(((R)-3-(((S)-3-(tert-Butoxy)-1-methoxy-1-oxopropan-2-yl)amino)-2-((tert-butoxycarbonyl) amino)-3-oxopropyl)thio)ethyl-[1,1′-biphenyl]-3-carboxylate (**5g**).* Yield 81 mg, 56%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (t, J = 1.6 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.79 (ddd, J = 7.7, 1.8, 1.2 Hz, 1H), 7.62 (d, J = 7.1 Hz, 2H), 7.52 (d, J = 7.7 Hz, 1H), 7.46 (dd, J = 9.9, 3.5 Hz, 2H), 7.38 (t, J = 7.4 Hz, 1H), 7.17 (d, J = 7.8 Hz, 1H), 5.48 (d, J = 3.4 Hz, 1H), 4.66 (dt, J = 8.1, 3.0 Hz, 1H), 4.52 (t, J = 6.7 Hz, 2H), 4.38 (d, J = 3.6 Hz, 1H), 3.82 (dd, J = 9.1, 2.9 Hz, 1H), 3.72 (s, 3H), 3.56 (dd, J = 9.1, 3.2 Hz, 1H), 3.08 (dd, J = 13.9, 5.4 Hz, 1H), 3.03 − 2.95 (m, 3H), 1.45 (s, 9H), 1.13 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.60, 170.50, 166.48, 155.39, 141.65, 140.21, 131.86, 130.61, 129.04, 128.57, 127.90, 127.32, 80.41, 73.66, 63.83, 61.76, 53.89, 53.34, 52.57, 35.05, 31.23, 28.43, 27.41. MS (ESI) calcd for $C_{31}H_{42}N_2O_8S$, m/z 602.27; found 625.26 $(M + Na)^+$.

General Procedure for Synthesis of Compounds 6a−6g. *Synthesis of Compound **6a**: 2-(((R)-2-Amino-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl butyrate.* Compound **5a** (49 mg, 0.1 mmol) was dissolved in 2 mL of trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the residue, which was further purified using column chromatography to obtain the trifluoroacetate salt of compound **6a** in quantitative yield (49 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 7.7 Hz, 1H), 4.71−4.62 (m, 1H), 4.35 (t, J = 6.4 Hz, 1H), 4.28−4.17 (m, 2H), 3.97−3.90 (m, 1H), 3.87 (dd, J = 11.6, 5.1 Hz, 1H), 3.76 (s, 3H), 3.17 (dd, J = 14.4, 5.4 Hz, 1H), 3.02 (dd, J = 14.4, 7.1 Hz, 1H), 2.81 (t, J = 6.4 Hz, 2H), 2.31 (t, J = 7.4 Hz, 2H), 1.69−1.55 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.40, 170.35, 168.39, 62.71, 61.83, 55.35, 52.91, 52.86, 36.14, 33.16, 30.90, 18.43, 13.71. MS (ESI) calcd for $C_{13}H_{24}N_2O_6S$, m/z 336.14; found 337.15 $(M + H)^+$.

Compounds **6b**, **6c**, and d**6e−6g** were synthesized similarly to compound **6a**. Compound **6d** was synthesized as published earlier.[17]

*2-(((R)-2-Amino-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl octanoate (**6b**).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, J = 7.7 Hz, 1H), 4.71 − 4.64 (m, 1H), 4.35 (t, J = 6.5 Hz, 1H), 4.26−4.18 (m, 2H), 3.96−3.90 (m, 1H), 3.86 (dd, J = 11.7, 5.2 Hz, 1H), 3.75 (s, 3H), 3.48 (s, 1H), 3.16 (dd, J = 14.5, 5.6 Hz, 1H), 3.01 (dd, J = 14.6, 7.2 Hz, 1H), 2.81 (t, J = 6.5 Hz, 2H), 2.31 (t, J = 7.6 Hz, 2H), 1.64−1.55 (m, 2H), 1.35−1.18 (m, 8H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.57, 170.31, 168.25, 62.64, 61.82, 55.38, 52.96, 52.82, 50.99, 34.30, 33.05, 31.78, 30.90, 29.21, 29.04, 24.97, 22.73, 14.19. MS (ESI) calcd for $C_{17}H_{32}N_2O_6S$, m/z 392.20; found 393.21 $(M + H)^+$.

*2-(((R)-2-Amino-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl dodecanoate (**6c**).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, J = 7.3 Hz, 1H), 4.68 (s, 1H), 4.36 (s, 1H), 4.23 (t, J = 6.0 Hz, 2H), 3.93 (d, J = 9.5 Hz, 1H), 3.86 (dd, J = 11.1, 4.7 Hz, 1H), 3.76 (s, 3H), 3.16 (dd, J = 14.3, 5.2 Hz, 1H), 3.02 (dd, J = 14.2, 6.9 Hz, 1H), 2.81 (t, J = 6.4 Hz, 2H), 2.31 (t, J = 7.7 Hz, 2H), 1.59 (dd, J = 14.3, 7.2 Hz, 2H), 1.31−1.22 (m, 16H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.83, 170.54, 168.51, 62.89, 62.07, 55.65, 53.22, 53.07, 34.57, 33.29, 32.31, 31.15, 30.03, 30.02, 29.90, 29.75, 29.69, 29.55, 25.24, 23.08, 14.52. MS (ESI) calcd for $C_{21}H_{40}N_2O_6S$, m/z 448.26; found 449.27 $(M + H)^+$.

*2-(((R)-2-Amino-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl stearate (**6e**).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (d, J = 7.6 Hz, 1H), 4.72−4.64 (m, 1H), 4.35 (t, J = 6.3 Hz, 1H), 4.24−4.16 (m, 2H), 3.94 (d, J = 8.9 Hz, 1H), 3.86 (dd, J = 11.6, 5.1 Hz, 1H), 3.76 (s, 3H), 3.16 (dd, J = 14.4, 5.3 Hz, 1H), 3.03−2.97 (m, 1H), 2.81 (t, J = 6.4 Hz, 2H), 2.31 (t, J = 7.7 Hz, 2H), 1.59 (dd, J = 14.3, 7.1 Hz, 2H), 1.39−1.09 (m, 28H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.43, 170.15, 168.12, 62.49, 61.70, 55.26, 52.83, 52.69, 34.18, 32.94, 31.94, 30.79, 29.73, 29.71, 29.68, 29.54, 29.38, 29.32, 29.18, 24.85, 22.70, 14.13. MS (ESI) calcd for $C_{27}H_{52}N_2O_6S$, m/z 532.35; found 533.37 $(M + H)^+$.

*2-(((R)-2-Amino-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl[1,1′-biphenyl]-4-carboxylate (**6f**).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (d, J = 7.4 Hz, 1H), 8.02 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 7.55−7.52 (m, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.36−7.31 (m, 1H), 4.72−4.67 (m, 1H), 4.50−4.42 (m, 3H), 3.94 (d, J = 9.2 Hz, 1H), 3.87 (dd, J = 11.4, 4.7 Hz, 1H), 3.65 (s, 3H), 3.25 (dd, J = 14.3, 5.1 Hz, 1H), 3.10 (dd, J = 14.3, 6.9 Hz, 1H), 2.95 (t, J = 6.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.59, 168.60, 167.20, 146.33, 140.15, 130.62, 129.31, 128.63, 128.60, 127.62, 127.45, 63.57, 62.12, 55.67, 53.18, 51.27, 33.42, 31.25. MS (ESI) calcd for $C_{22}H_{26}N_2O_6S$, m/z 446.15; found 447.16 $(M + H)^+$.

*2-(((R)-2-Amino-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl [1,1′-biphenyl]-3-carboxylate (**6g**).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, J = 7.5 Hz, 1H), 8.21 (s, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.56 (d, J = 7.2 Hz, 2H), 7.42 (dt, J = 13.5, 7.8 Hz, 3H), 7.33 (t, J = 7.4 Hz, 1H), 4.71−4.64 (m, 1H), 4.48−4.42 (m, 3H), 3.92 (d, J = 9.2 Hz, 1H), 3.85 (dd, J = 11.5, 4.9 Hz, 1H), 3.63 (s, 3H), 3.48 (s, 2H), 3.23 (dd, J = 14.4, 5.3 Hz, 1H), 3.08 (dd, J = 14.3, 6.9 Hz, 1H), 2.93 (t, J = 6.5 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.55, 168.54, 167.25, 141.87, 140.27, 132.28, 130.50, 129.33, 129.28, 128.84, 128.71, 128.18, 127.50, 63.56, 62.10, 55.67, 53.16, 51.27, 33.38, 31.21. MS (ESI) calcd for $C_{22}H_{26}N_2O_6S$, m/z 446.15; found 447.16 $(M + H)^+$.

*Synthesis of Compound **6h**: 2-(((R)-2-Amino-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl[1,1′-biphenyl]-3-carboxylate.* To a solution of *p*-terphenyl-4-carboxylic acid (33 mg, 0.12 mmol) and compound **4** (100 mg, 0.24 mmol) in anhydrous dimethylformamide (DMF) were added triethylamine (33 μL, 0.24 mmol), DMAP (2.9 mg, 0.024 mmol), and *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium hexafluorophosphate (HBTU, 55 mg, 0.144 mmol), and the reaction mixture was stirred at room temperature for 14 h, followed by removal of the solvent under reduced pressure. The crude was then dissolved in ethyl acetate and washed with water. The organic fraction was dried over anhydrous sodium sulfate, filtered, concentrated, and purified using column chromatography (5% $MeOH/CH_2Cl_2$) to obtain the compound **5h**. The product obtained was dissolved in 2 mL of trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the residue, which was further purified using column chromatography to obtain the trifluoroacetate salt of compound **6h** (37 mg, 59%). ^{1}H NMR (500 MHz, MeOD) δ 8.14 (d, J = 8.5 Hz, 2H), 7.83 (d, J = 8.5 Hz, 2H), 7.81−7.74 (m, 2H), 7.68 (dd, J = 8.3, 1.1 Hz, 1H), 7.46 (t, J = 7.7 Hz,

1H), 7.37 (t, J = 7.4 Hz, 1H), 4.62 (t, J = 5.0 Hz 1H), 4.59−4.53 (m, 1H), 4.17 (dd, J = 8.8, 4.7 Hz, 1H), 3.97 (dd, J = 11.2, 4.8 Hz, 1H), 3.85 (dd, J = 11.2, 3.8 Hz, 1H), 3.74 (s, 3H), 3.35 (d, J = 4.6 Hz, 1H), 3.07 (td, J = 6.5, 2.5 Hz, 2H), 3.02 (dd, J = 14.7, 8.8 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 171.67, 169.09, 167.76, 146.80, 142.49, 141.63, 139.84, 131.30, 129.98, 129.89, 128.69, 128.66, 128.61, 128.01, 127.92, 64.82, 62.57, 56.41, 53.59, 53.00, 34.29, 31.68. MS (ESI) calcd for $C_{28}H_{30}N_2O_6S$, m/z 522.18; found 523.19 (M + H)$^+$.

*Syntheses of Methyl 2-(Ditetradecylamino)acetate (**8a**) and Methyl 2-((tert-Butoxycarbonyl)(tetradecyl)amino)acetate (**8b**).* To a solution of glycine methyl ester 7 (1.0 g, 8 mmol) in DMF (5 mL), triethylamine (4.5 mL, 32 mmol) was added, followed by 1-bromotetradecane (3.6 mL, 12 mmol), and the reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated, water (50 mL) was added, and the product was extracted in CH_2Cl_2 (100 mL). The organic layer was dried over sodium sulfate and evaporated to afford a mixture of methyl 2-(tetradecylamino)acetate and methyl 2-(ditetradecylamino)acetate. Di-*tert*-butyl dicarbonate (1.0 g) was added to the solution of this crude mixture in anhydrous CH_2Cl_2 (10 mL) followed by triethylamine (0.7 mL, 5.25 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed, and the product was column purified to furnish compound **8a** (330 mg, 9%) and compound **8b** (1.01 g, 33%). Spectroscopic evidence for two conformers for **8b** was observed in both ^{1}H and ^{13}C NMR.

8a: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.69 (s, 3H), 3.32 (s, 2H), 2.53 (dd, J = 8.6, 6.7 Hz, 4H), 1.46−1.39 (m, 4H), 1.35−1.15 (m, 44H), 0.88 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.39, 55.26, 54.71, 51.54, 32.08, 29.85, 29.84, 29.83, 29.81, 29.79, 29.73, 29.52, 27.56, 27.53, 22.85, 14.28. MS (ESI) calcd for $C_{31}H_{63}NO_2$, m/z 481.49; found 482.67 (M + H)$^+$.

8b: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.95 (s, 1H), 3.86 (s, 1H), 3.73 (d, J = 2.6 Hz, 3H), 3.25 (dt, J = 19.4, 7.5 Hz, 2H), 1.42−1.50 (m, 11H), 1.34−1.19 (m, 22H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.91, 170.81, 156.02, 155.27, 80.16, 80.13, 52.11, 52.05, 49.27, 48.60, 48.49, 48.43, 32.06, 29.83, 29.81, 29.79, 29.75, 29.54, 29.50, 29.45, 28.50, 28.44, 28.39, 28.27, 26.93, 26.86, 22.83, 14.27. MS (ESI) calcd for $C_{22}H_{43}NO_4$, m/z 385.32; found 386.33 (M + H)$^+$.

*Synthesis of 2-(Ditetradecylamino)acetic Acid (**9a**).* Compound **8a** (275 mg, 0.57 mmol) was dissolved in tetrahydrofuran (THF, 10 mL) and LiOH (68 mg, 2.85 mmol) in 2 mL of H_2O was added, and the reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated, water was added, the pH of the aqueous layer was rendered acidic by the addition of 1N HCl, and the product was extracted in CH_2Cl_2. The organic layer was dried over anhydrous sodium sulfate, evaporated, and column purified to afford compound **9a** as white solid (250 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.29 (s, 2H), 3.13 (s, 1H), 2.72−2.46 (m, 4H), 1.47 (m, 4H), 1.19 (m, 44H), 0.81 (t, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.66, 58.26, 54.51, 31.94, 29.72, 29.71, 29.68, 29.64, 29.63, 29.51, 29.39, 27.43, 25.00, 22.70, 14.09. MS (ESI) calcd for $C_{30}H_{61}NO_2$, m/z 467.47; found 468.49 (M + H)$^+$.

Compound **9b** was synthesized similarly as compound **9a**.

***9b**: 2-((tert-Butoxycarbonyl)(tetradecyl)amino)acetic Acid.* Spectroscopic evidence for two conformers was observed in both ^{1}H and ^{13}C NMR. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.88 (d, J = 9.7 Hz, 2H), 3.30−3.18 (m, 2H), 1.52−1.38 (m, 11H), 1.32−1.20 (m, 22H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.85, 175.28, 156.82, 155.32, 80.83, 80.31, 49.70, 49.16, 48.82, 48.29, 32.07, 29.84, 29.82, 29.81, 29.76, 29.68, 29.64, 29.57, 29.51, 29.46, 28.50, 28.39, 28.35, 28.25, 26.95, 26.84, 22.84, 14.28. MS (ESI) calcd for $C_{21}H_{41}NO_4$, m/z 371.30; found 394.30 (M + Na)$^+$.

*Synthesis of (S)-Methyl 2-((R)-2-Amino-3-((2-(2-(ditetradecylamino)acetoxy)ethyl) thio)propan-amido)-3-hydroxypropanoate (**10a**).* To the solution of compound **4** (80 mg, 0.189 mmol) and **9a** (177 mg, 0.379 mmol) in dry CH_2Cl_2 (5 mL), *N*-methylmorpholine (41 μL, 0.379 mmol) and DMAP (9.0 mg, 0.076 mmol) were added, and the reaction mixture was stirred in an ice bath. After 20 min, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI·HCl, 58 mg, 0.379 mmol) was added, and the reaction mixture was stirred at room temperature for 18 h. Then 50 mL of CH_2Cl_2 was added to the reaction mixture and the organic layer was washed with water, brine, dried over anhydrous sodium sulfate, and evaporated. The crude product was further column purified to furnish the ester intermediate *N*-Boc, *O*-^{t}Bu protected **10a**. The product obtained was dissolved in 4 mL of trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the residue which was further purified using column chromatography to obtain the trifluoroacetate salt of compound **10a** in quantitative yield. ^{1}H NMR (500 MHz, MeOD) δ 4.60 (t, J = 4.1 Hz, 1H), 4.48 (dtd, J = 17.9, 11.7, 6.2 Hz, 2H), 4.19 (s, 2H), 4.15 (dd, J = 8.4, 4.8 Hz, 1H), 3.97 (dd, J = 11.3, 4.6 Hz, 1H), 3.84 (dd, J = 11.3, 3.7 Hz, 1H), 3.76 (s, 3H), 3.28 (dd, J = 14.8, 4.9 Hz, 1H), 3.20 (dd, J = 9.6, 7.1 Hz, 4H), 3.03−2.87 (m, 3H), 1.72 (s, 4H), 1.45−1.25 (m, 44H), 0.90 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 171.87, 169.24, 167.79, 66.48, 62.57, 56.35, 55.98, 54.39, 53.31, 53.01, 34.00, 33.09, 30.81, 30.79, 30.78, 30.76, 30.64, 30.51, 30.49, 30.19, 27.56, 25.03, 23.75, 14.45. MS (ESI) calcd for $C_{39}H_{77}N_3O_6S$, m/z 715.55; found 716.59 (M + H)$^+$.

Compound **10b** was synthesized similarly to compound **10a**.

***10b**: (S)-Methyl 2-((R)-2-Amino-3-((2-(2-(tetradecylamino)-acetoxy)ethyl)thio)propanamido)-3-hydroxypropanoate.* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 4.63 (dt, J = 7.6, 3.8 Hz, 1H), 4.36 − 4.24 (m, 2H), 3.95 (ddd, J = 17.8, 11.2, 3.8 Hz, 2H), 3.80 (s, 3H), 3.67 − 3.58 (m, 1H), 3.44 (s, 2H), 3.05 (dd, J = 13.6, 4.3 Hz, 1H), 2.95 (dd, J = 13.6, 6.9 Hz, 1H), 2.81 (t, J = 6.6 Hz, 2H), 2.63−2.55 (m, 2H), 1.72 (bs, 4H), 1.49 (m, 2H), 1.29−1.23 (m, 22H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.78, 172.27, 170.90, 63.76, 63.14, 54.87, 54.12, 52.87, 50.86, 49.74, 37.99, 32.07, 31.00, 29.96, 29.84, 29.83, 29.82, 29.80, 29.77, 29.74, 29.66, 29.51, 27.35, 22.84, 14.28. MS (ESI) calcd for $C_{25}H_{49}N_3O_6S$, m/z 519.33; found 520.35 (M + H)$^+$.

*Synthesis of Compound **11a**: 2-(((R)-2-(Ethylamino)-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)-ethyl palmitate.* To a solution of compound **6d** (50 mg, 0.081 mmol) in anhydrous CH_2Cl_2 were added acetaldehyde (3.5 mg, 0.081 mmol), 4 drops of acetic acid, and macroporous polystyrene-bound cyanoborohydride (73 mg, 0.162 mmol). The reaction mixture was stirred for 2 h and then filtered to remove the solid resin. The filtrate was evaporated under vacuum to obtain the residue, which was purified using column chromatography (4% MeOH/CH_2Cl_2), yielding compound **11a** (8 mg, 20%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, J = 7.7 Hz, 1H), 4.64 (dt, J = 7.7, 3.8 Hz, 1H), 4.22 (t, J = 6.7 Hz, 2H), 4.01−3.90 (m, 2H), 3.80 (s, 3H), 3.28 (dd, J = 8.2, 3.9 Hz, 1H), 3.10 (dd, J = 13.5, 3.9 Hz, 1H), 2.84−2.71 (m, 3H), 2.68 (qd, J = 7.1, 2.9 Hz, 2H), 2.32 (t, J = 7.6 Hz, 2H), 2.08 (s, 2H), 1.66−1.54 (m, 2H), 1.35−1.21 (m, 24H), 1.15 (t, J = 7.1 Hz, 3H), 0.87 (d, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.93, 173.55, 170.77, 63.50, 63.05, 61.55, 54.72, 52.88, 43.30, 35.83, 34.32, 32.07, 30.93, 29.84, 29.83, 29.80, 29.76, 29.62, 29.51, 29.42, 29.28, 25.04, 22.84, 15.50, 14.28. MS (ESI) calcd for $C_{27}H_{52}N_2O_6S$, m/z 532.35; found 533.37 (M + H)$^+$.

Compound **11b** was synthesized similarly as compound **11a**.

***11b**: 2-(((R)-3-(((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)-amino)-2-(octylamino)-3-oxopropyl) thio)ethyl Palmitate.* Yield 10 mg, 20%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 7.7 Hz, 1H), 4.63 (dt, J = 7.7, 3.8 Hz, 1H), 4.22 (t, J = 6.7 Hz, 2H), 4.03−3.91 (m, 2H), 3.79 (s, 3H), 3.30 (dd, J = 7.8, 3.5 Hz, 1H), 3.10 (dd, J = 13.5, 3.9 Hz, 1H), 2.86−2.71 (m, 3H), 2.63 (td, J = 7.2, 2.2 Hz, 2H), 2.32 (t, J = 7.6 Hz, 2H), 2.05 (s, 2H), 1.67−1.55 (m, 2H), 1.51 (dd, J = 14.2, 7.1 Hz, 2H), 1.37−1.19 (m, 34H), 0.92−0.82 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.94, 173.41, 170.67, 63.47, 63.05, 61.57, 54.78, 52.85, 48.95, 35.71, 34.32, 32.07, 31.98, 30.94, 30.19, 29.84, 29.81, 29.77, 29.62, 29.51, 29.43, 29.41, 29.29, 27.32, 25.04, 22.84, 22.81, 14.28, 14.25. MS (ESI) calcd for $C_{33}H_{64}N_2O_6S$, m/z 616.45; found 617.46 (M + H)$^+$.

*Synthesis of Compound **11c**: 2-(((R)-2-(Hexadecylamino)-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)-ethyl Palmitate.* To a solution of compound **6d** (100 mg, 0.162 mmol) in anhydrous DMF, triethylamine (56 μL, 0.405 mmol) was

dx.doi.org/10.1021/jm3000533 | J. Med. Chem. 2012, 55, 3353−3363

added, followed by 1-bromohexadecane (123 μL, 0.405 mmol). The reaction mixture was stirred at room temperature for 14 h. DMF was evaporated at 50 °C, and the crude product obtained was purified using column chromatography to give compound **11c** (18 mg, 15%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 7.7 Hz, 1H), 4.63 (dt, *J* = 7.6, 3.7 Hz, 1H), 4.22 (t, *J* = 6.7 Hz, 2H), 4.00−3.90 (m, 2H), 3.80 (s, 3H), 3.26 (dd, *J* = 8.1, 3.7 Hz, 1H), 3.09 (dd, *J* = 13.5, 3.7 Hz, 1H), 2.83−2.72 (m, 3H), 2.61 (t, *J* = 7.0 Hz, 2H), 2.32 (t, *J* = 7.6 Hz, 2H), 1.88 (bs, 2H), 1.65−1.54 (m, 2H), 1.54−1.45 (m, 2H), 1.25 (s, 50H), 0.88 (t, *J* = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.90, 173.60, 170.69, 63.51, 63.06, 61.66, 54.76, 52.85, 49.01, 35.82, 34.32, 32.07, 30.92, 30.30, 29.86, 29.85, 29.81, 29.78, 29.70, 29.63, 29.51, 29.43, 29.29, 27.36, 25.04, 22.84, 14.28. MS (ESI) calcd for $C_{41}H_{80}N_2O_6S$, *m*/*z* 728.57; found 729.58 $(M + H)^+$.

Synthesis of Compound ***11d****: 2-(((R)-2-Acetamido-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)-ethyl Palmitate.* To a solution of compound **6d** (50 mg, 0.081 mmol) in anhydrous CH_2Cl_2 were added triethylamine (17 μL, 0.121 mmol) and acetic anhydride (8 μL, 0.081 mmol). The reaction mixture was stirred for 2 h. The solvent was removed under vacuum to obtain the residue, which was purified using column chromatography (6% MeOH/CH_2Cl_2), furnishing compound **11d** (35 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.7 Hz, 1H), 6.63 (d, *J* = 7.1 Hz, 1H), 4.67−4.57 (m, 2H), 4.37−4.17 (m, 2H), 4.01−3.90 (m, 2H), 3.79 (s, 3H), 3.26 (t, *J* = 6.3 Hz, 1H), 2.96 (dd, *J* = 6.4, 3.6 Hz, 2H), 2.83 (t, *J* = 6.6 Hz, 2H), 2.32 (t, *J* = 7.6 Hz, 2H), 2.05 (s, 3H), 1.60 (dd, *J* = 14.4, 7.2 Hz, 2H), 1.25 (s, 24H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.32, 170.95, 170.58, 170.55, 62.88, 62.76, 55.18, 52.93, 52.89, 34.56, 34.39, 32.06, 31.30, 29.84, 29.80, 29.76, 29.62, 29.50, 29.42, 29.29, 25.02, 23.23, 22.84, 14.27. MS (ESI) calcd for $C_{27}H_{50}N_2O_7S$, *m*/*z* 546.33; found 547.35 $(M + H)^+$.

Compounds **11h**, **11j**, and **11k** were synthesized similarly as compound **11d**.

11h*: 2-(((R)-3-(((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)-amino)-3-oxo-2-(2,2,2-trifluoro-acetamido)propyl)thio)ethyl Palmitate.* Yield 10 mg, 21%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.0 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 4.72 − 4.63 (m, 2H), 4.40−4.30 (m, 1H), 4.27 (dd, *J* = 12.0, 5.7 Hz, 1H), 4.00 (ddd, *J* = 54.9, 11.5, 3.3 Hz, 2H), 3.80 (s, 3H), 2.99 (t, *J* = 6.2 Hz, 2H), 2.92−2.80 (m, 2H), 2.77 (s, 1H), 2.38−2.26 (m, 2H), 1.66−1.55 (m, 2H), 1.38−1.18 (m, 24H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.69, 170.38, 168.95, 157.74, 157.44, 157.13, 156.83, 119.13, 116.85, 114.56, 112.28, 62.81, 62.75, 55.14, 53.06, 52.77, 34.51, 34.41, 32.06, 31.38, 29.84, 29.83, 29.80, 29.76, 29.61, 29.50, 29.40, 29.27, 24.98, 22.83, 14.27. MS (ESI) calcd for $C_{27}H_{47}F_3N_2O_7S$, *m*/*z* 600.31; found 601.31 $(M + H)^+$ and 618.34 $(M + NH_4^+)$.

11j*: 2-(((R)-3-(((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)-amino)-2-(methylsulfonamido)-3-oxo-propyl)thio)ethyl Palmitate.* Yield 7 mg, 15%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.6 Hz, 1H), 5.60 (d, *J* = 7.9 Hz, 1H), 4.71−4.60 (m, 1H), 4.24 (dt, *J* = 14.1, 6.4 Hz, 3H), 4.10−3.92 (m, 2H), 3.80 (s, 3H), 3.09 (s, 3H), 3.05 (t, *J* = 5.7 Hz, 2H), 2.82 (t, *J* = 6.6 Hz, 2H), 2.71 (s, 1H), 2.33 (t, *J* = 7.6 Hz, 2H), 1.60 (q, *J* = 7.3 Hz, 3H), 1.28−1.25 (m, 23H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.49, 170.46, 170.05, 62.83, 62.78, 56.26, 55.17, 53.06, 41.84, 35.62, 34.40, 32.07, 31.27, 29.84, 29.81, 29.77, 29.63, 29.51, 29.41, 29.29, 25.02, 22.84, 14.27. MS (ESI) calcd for $C_{26}H_{50}N_2O_8S_2$, *m*/*z* 582.30; found 583.31 $(M + H)^+$.

11k*: 2-(((R)-3-(((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)-amino)-3-oxo-2-(trifluoromethyl-sulfonamido)propyl)thio)ethyl Palmitate.* Yield 39 mg, 78%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.9 Hz, 1H), 4.75−4.64 (m, 1H), 4.36 (t, *J* = 6.4 Hz, 1H), 4.28−4.20 (m, 2H), 4.05 (dd, *J* = 11.5, 3.5 Hz, 1H), 3.92 (dd, *J* = 11.5, 3.3 Hz, 1H), 3.79 (s. 3H), 3.07−2.94 (m, 2H), 2.86−2.72 (m, 2H), 2.33 (t, *J* = 7.6 Hz, 2H), 1.59 (dd, *J* = 14.5, 7.2 Hz, 2H), 1.33−1.18 (m, 26H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.71, 170.56, 169.23, 123.42, 120.87, 118.32, 115.77, 62.79, 62.75, 57.35, 54.99, 53.13, 35.73, 34.37, 32.06, 31.29, 29.83, 29.79, 29.76, 29.61, 29.50, 29.40, 29.26, 24.97, 22.83, 14.26. MS (ESI) calcd for $C_{26}H_{47}F_3N_2O_8S_2$, *m*/*z* 636.27; found 637.28 $(M + H)^+$ and 654.31 $(M + NH_4^+)$.

Synthesis of Compound ***11e****: 2-(((R)-2-Butyramido-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)-ethyl Palmitate.* To a solution of compound **6d** (50 mg, 0.081 mmol) in pyridine (1 mL), butyryl chloride (10.2 μL, 0.097 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. The volatiles were removed by evaporation, and the crude product obtained was purified using column chromatography (10% MeOH/CH_2Cl_2) to obtain compound **11e** as a white solid (35 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* = 7.7 Hz, 1H), 6.59 (d, *J* = 7.1 Hz, 1H), 4.63 (dt, *J* = 8.8, 5.0 Hz, 2H), 4.35−4.19 (m, 2H), 3.96 (dt, *J* = 11.5, 8.0 Hz, 2H), 3.78 (s, 3H), 3.02−2.90 (m, 2H), 2.84 (t, *J* = 6.6 Hz, 2H), 2.32 (t, *J* = 7.6 Hz, 2H), 2.27−2.19 (m, 2H), 1.73−1.55 (m, 5H), 1.35−1.19 (m, 24H), 0.95 (t, *J* = 7.4 Hz, 3H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.31, 173.88, 170.62, 170.57, 62.93, 62.77, 55.19, 52.91, 52.71, 38.45, 34.54, 34.39, 32.06, 31.31, 29.83, 29.80, 29.76, 29.62, 29.50, 29.42, 29.29, 25.02, 22.83, 19.13, 14.27, 13.84. MS (ESI) calcd for $C_{29}H_{54}N_2O_7S$, *m*/*z* 574.37; found 575.38 $(M + H)^+$.

Compounds **11f** and **11g** were synthesized similarly as compound **11e**.

11f*: 2-(((R)-3-(((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)-amino)-2-octanamido-3-oxopropyl) thio)ethyl Palmitate.* Yield 32 mg, 62%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.6 Hz, 1H), 6.55 (d, *J* = 7.0 Hz, 1H), 4.66−4.55 (m, 2H), 4.35−4.17 (m, 2H), 4.03−3.89 (m, 2H), 3.79 (s, 3H), 3.19 (s, 1H), 2.96 (qd, *J* = 13.9, 6.4 Hz, 2H), 2.84 (t, *J* = 6.6 Hz, 2H), 2.32 (t, *J* = 7.6 Hz, 2H), 2.26−2.22 (m, 2H), 1.77 (s, 1H), 1.61 (dd, *J* = 14.5, 7.5 Hz, 4H), 1.34−1.19 (m, 31H), 0.90−0.83 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.33, 174.01, 170.60, 170.56, 62.93, 62.79, 55.20, 52.92, 52.73, 36.62, 34.54, 34.40, 32.07, 31.80, 31.34, 29.84, 29.80, 29.77, 29.63, 29.51, 29.43, 29.34, 29.30, 29.12, 25.68, 25.03, 22.84, 22.75, 14.27, 14.21. MS (ESI) calcd for $C_{33}H_{62}N_2O_7S$, *m*/*z* 630.43; found 631.44 $(M + H)^+$.

11g*: 2-(((R)-3-(((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)-amino)-3-oxo-2-palmitamidopropyl) thio)ethyl Palmitate.* Yield 41 mg, 68%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (d, *J* = 7.6 Hz, 1H), 6.55 (d, *J* = 7.0 Hz, 1H), 4.61 (ddd, *J* = 19.9, 10.3, 5.0 Hz, 2H), 4.33 (dt, *J* = 11.4, 6.7 Hz, 1H), 4.24 (dt, *J* = 11.4, 6.4 Hz, 1H), 3.97 (qd, *J* = 11.6, 3.5 Hz, 2H), 3.79 (s, 3H), 2.95 (ddd, *J* = 30.5, 14.0, 6.5 Hz, 2H), 2.85 (t, *J* = 6.6 Hz, 2H), 2.34−2.30 (m, 2H), 2.27−2.21 (m, 2H), 1.62 (dd, *J* = 14.6, 7.3 Hz, 4H), 1.29−1.23 (m, 49H), 0.87 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.38, 174.01, 170.63, 170.53, 62.94, 62.80, 55.21, 52.94, 52.76, 36.63, 34.48, 34.41, 32.07, 31.36, 29.85, 29.81, 29.79, 29.78, 29.76, 29.65, 29.64, 29.62, 29.52, 29.49, 29.43, 29.41, 29.30, 25.69, 25.03, 22.84, 14.28. MS (ESI) calcd for $C_{41}H_{78}N_2O_7S$, *m*/*z* 742.55; found 743.56 $(M + H)^+$.

Synthesis of Compound ***11i****: 2-(((R)-3-(((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxo-2-(2,2,2-trichloroacetamido)propyl)thio)ethyl Palmitate.* To a solution of compound **6d** (40 mg, 0.079 mmol) in anhydrous CH_2Cl_2 were added triethylamine (11 μL, 0.079 mmol), trichloroacetic acid (13 mg, 0.079 mmol), EDCI·HCl (19 mg, 0.095 mmol), and a catalytic amount of 1-hydroxybenzotriazole (HOBt). The reaction mixture was stirred for 2 h, and the solvent was then removed under vacuum. The residue was purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **11i** (6 mg, 12%). ^{1}H NMR (500 MHz, DMSO) δ 8.61 (d, *J* = 7.6 Hz, 1H), 5.16 (t, *J* = 5.4 Hz, 1H), 4.57 (dd, *J* = 10.4, 4.0 Hz, 1H), 4.38 (dt, *J* = 7.7, 4.7 Hz, 1H), 4.14 (tt, *J* = 12.9, 5.5 Hz, 2H), 3.75 (dt, *J* = 10.7, 4.0 Hz, 1H), 3.63 (s, 3H), 3.03 (dd, *J* = 13.9, 4.0 Hz, 1H), 2.94 (dd, *J* = 13.9, 10.5 Hz, 1H), 2.81 (qd, *J* = 13.9, 7.2 Hz, 2H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.54−1.47 (m, 2H), 1.23 (s, 24H), 0.85 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.80, 170.72, 169.26, 161.53, 92.55, 63.00, 61.09, 54.79, 54.46, 51.98, 33.42, 33.20, 31.32, 29.81, 29.06, 29.03, 29.00, 28.90, 28.73, 28.71, 28.45, 24.45, 22.13, 13.99. MS (ESI) calculated for $C_{27}H_{47}Cl_3N_2O_7S$, *m*/*z* 648.22; found 649.22 $(M + H)^+$ and 666.25 $(M + NH_4^+)$.

Synthesis of Compound ***11l****: 2-(((R)-3-(((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-2-(4-methylphenylsulfonamido)-3-oxopropyl)thio)ethyl Palmitate.* To a solution of compound **6d** (40 mg, 0.079 mmol) in anhydrous CH_2Cl_2 were added triethylamine (11 μL, 0.079 mmol) and 4-methylbenzene-1-sulfonyl chloride (15 mg, 0.079 mmol). The reaction mixture was stirred for 2 h, and the solvent

dx.doi.org/10.1021/jm3000533 | J. Med. Chem. 2012, 55, 3353−3363

was then removed under vacuum. The residue was purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **11l** (25 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 7.3 Hz, 1H), 7.33 (d, J = 8.0 Hz, 2H), 5.82 (d, J = 7.6 Hz, 1H), 4.53 (dt, J = 7.2, 3.6 Hz, 1H), 4.13 (td, J = 6.5, 1.6 Hz, 2H), 3.99−3.90 (m, 2H), 3.90−3.81 (m, 1H), 3.78 (s, 3H), 3.02 (dd, J = 14.2, 5.5 Hz, 1H), 2.73−2.56 (m, 4H), 2.43 (s, 3H), 2.31 (t, J = 7.6 Hz, 2H), 1.65−1.54 (m, 2H), 1.27 (d, J = 16.0 Hz, 24H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.23, 170.23, 169.91, 144.47, 136.20, 130.06, 127.60, 62.77, 62.73, 55.66, 55.32, 53.01, 34.79, 34.33, 32.07, 31.08, 29.84, 29.80, 29.77, 29.63, 29.51, 29.42, 29.29, 25.00, 22.84, 21.74, 14.28. MS (ESI) calcd for $C_{32}H_{54}N_2O_8S_2$, m/z 658.33; found 659.34 (M + H)$^+$ and 676.37 (M + NH_4^+).

*Synthesis of Compound **12**: 2-(((2R)-2-((tert-Butoxycarbonyl)-amino)-3-((3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl Palmitate.* To a solution of compound **6d** (180 mg, 0.29 mmol) in anhydrous CH_2Cl_2 were added triethylamine (67 μL, 0.49 mmol) and di-*tert*-butyl dicarbonate (107 mg, 0.49 mmol). The reaction mixture was stirred for 2 h, and the solvent was then removed under vacuum. The residue was purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain the compound **12** (160 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (s, 1H), 5.47 (s, 1H), 4.66 (dt, J = 7.0, 3.3 Hz, 1H), 4.28 (ddd, J = 10.3, 8.8, 4.4 Hz, 3H), 3.99 (d, J = 3.1 Hz, 2H), 3.82 (s, 3H), 3.02 (ddd, J = 32.3, 13.9, 6.1 Hz, 2H), 2.84 (t, J = 6.6 Hz, 3H), 2.34 (t, J = 7.6 Hz, 2H), 1.62 (dd, J = 14.3, 7.0 Hz, 2H), 1.48 (s, 9H), 1.27 (s, 24H), 0.90 (t, J = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.19, 170.83, 170.57, 155.69, 80.92, 63.09, 62.88, 55.21, 54.17, 52.96, 34.56, 34.36, 32.07, 31.31, 29.84, 29.80, 29.76, 29.62, 29.50, 29.41, 29.28, 28.40, 25.01, 22.84, 14.27. MS (ESI) calcd for $C_{30}H_{56}N_2O_8S$, m/z 604.38; found 605.38 (M + H)$^+$.

*Synthesis of Compounds **14a**: 2-(((R)-3-(((S)-3-Acetoxy-1-methoxy-1-oxopropan-2-yl)amino)-2-amino-3-oxopropyl)thio)ethyl Palmitate.* To a solution of compound **12** (40 mg, 0.079 mmol) in pyridine (1 mL) was added acetic anhydride (15 μL, 0.158 mmol). The reaction mixture was stirred at room temperature for 1 h, and the solvent was removed to obtain the crude *N*-Boc intermediate **13a**. The *N*-Boc group was then removed by stirring compound **13a** in 1 mL of trifluoroacetic acid for 15 min, followed by removal of solvent by purging nitrogen. The residue was dried under vacuum to obtain compound **14a** in quantitative yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, J = 7.7 Hz, 1H), 6.55 (s, 2H), 4.80 (dd, J = 7.5, 3.8 Hz, 1H), 4.41 (ddd, J = 27.2, 11.6, 3.9 Hz, 2H), 4.35−4.16 (m, 3H), 3.77 (s, 3H), 3.11 (ddd, J = 43.7, 14.6, 6.5 Hz, 2H), 2.82 (t, J = 6.6 Hz, 2H), 2.31 (t, J = 7.6 Hz, 2H), 2.03 (s, 3H), 1.66−1.53 (m, 2H), 1.35−1.09 (m, 24H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.39, 171.23, 169.23, 63.19, 62.55, 53.13, 52.48, 52.39, 34.31, 33.04, 32.07, 30.89, 29.85, 29.83, 29.81, 29.79, 29.65, 29.51, 29.45, 29.31, 24.99, 22.84, 20.66, 14.27. MS (ESI) calcd for $C_{27}H_{50}N_2O_7S$, m/z 546.33; found 547.34 (M + H)$^+$.

*Synthesis of Compound **14b**: 2-(((R)-2-Amino-3-(((S)-3-(butyryloxy)-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)ethyl Palmitate.* To a solution of compound **12** (50 mg, 0.083 mmol) in anhydrous THF were added triethylamine (36 μL, 0.25 mmol) and butyryl chloride (25 μL, 0.25 mmol). The reaction mixture was stirred for 2 h, and the solvent was then removed under vacuum. The residue was purified using column chromatography (15% ethylacetate/hexanes) to obtain the *N*-Boc protected intermediate **13b** (25 mg, 45%). The *N*-Boc group was then removed by stirring compound **13b** in 1 mL of trifluoroacetic acid for 15 min, followed by removal of the acid by purging nitrogen. The residue was thoroughly dried under vacuum to obtain compound **14b** in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 7.6 Hz, 1H), 6.17 (s, 4H), 4.79 (dt, J = 7.6, 3.7 Hz, 1H), 4.49 (dd, J = 11.6, 4.1 Hz, 1H), 4.39−4.30 (m, 2H), 4.31−4.16 (m, 2H), 3.77 (s, 3H), 3.18 (dd, J = 14.7, 6.2 Hz, 1H), 3.05 (dd, J = 14.7, 7.1 Hz, 1H), 2.82 (t, J = 6.5 Hz, 2H), 2.29 (dt, J = 19.0, 7.5 Hz, 4H), 1.60 (dd, J = 14.7, 7.4 Hz, 4H), 1.25 (s, 24H), 0.89 (dt, J = 14.0, 7.2 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.61, 173.77, 169.15, 167.87, 62.94, 62.39, 53.16, 52.64, 52.47, 35.77, 34.31, 32.80, 32.07, 30.71, 29.85, 29.82, 29.81, 29.78, 29.64, 29.51, 29.42, 29.29, 24.97, 22.84, 18.31, 14.27, 13.62. MS (ESI) calcd for $C_{29}H_{54}N_2O_7S$, m/z 574.37; found 575.37 (M + H)$^+$.

Compound **14c** was synthesized similarly as compound **14b.**

***14c**: 2-(((R)-2-Amino-3-(((S)-1-methoxy-1-oxo-3-(palmitoyloxy)-propan-2-yl)amino)-3-oxopropyl) thio)ethyl Palmitate.* Yield 44 mg, 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 7.6 Hz, 1H), 6.80 (s, 4H), 4.82−4.73 (m, 1H), 4.49 (dd, J = 11.6, 4.1 Hz, 1H), 4.42−4.17 (m, 4H), 3.77 (s, 3H), 3.21 (dd, J = 14.7, 5.8 Hz, 1H), 3.02 (dd, J = 14.7, 7.7 Hz, 1H), 2.81 (t, J = 6.5 Hz, 2H), 2.30 (dt, J = 19.4, 7.6 Hz, 4H), 1.68−1.50 (m, 4H), 1.25 (s, 46H), 0.88 (t, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.85, 174.03, 169.05, 167.77, 62.88, 62.31, 53.20, 52.74, 52.48, 34.34, 33.91, 32.78, 32.08, 30.63, 29.86, 29.83, 29.82, 29.79, 29.65, 29.52, 29.42, 29.29, 29.21, 24.96, 24.85, 22.84, 14.27. MS (ESI) calcd for $C_{41}H_{78}N_2O_7S$, m/z 742.55; found 743.56 (M + H)$^+$.

*Synthesis of Compound **16**: 3-(((R)-2-Acetamido-3-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)thio)-propyl Palmitate.* To a solution of compound **15**[17] (20 mg, 0.032 mmol) in anhydrous CH_2Cl_2 were added triethylamine (5 μL, 0.038 mmol) and acetic anhydride (3 μL, 0.032 mmol). The reaction mixture was stirred for 2 h. The solvent was removed under vacuum to obtain the residue, which was purified using column chromatography (6% MeOH/CH_2Cl_2), furnishing compound **16** (10 mg, 59%). ^{1}H NMR (500 MHz, DMSO) δ 8.41 (d, J = 7.6 Hz, 1H), 8.12 (d, J = 8.5 Hz, 1H), 5.07 (t, J = 5.6 Hz, 1H), 4.54 (td, J = 8.9, 4.9 Hz, 1H), 4.33 (dt, J = 7.7, 4.9 Hz, 1H), 4.10−4.03 (m, 2H), 3.70 (dt, J = 11.1, 5.6 Hz, 1H), 3.66−3.58 (m, 4H), 2.82 (dd, J = 13.7, 4.9 Hz, 1H), 2.57 (ddd, J = 9.2, 5.3, 3.0 Hz, 3H), 2.28 (td, J = 7.4, 2.8 Hz, 2H), 1.85 (s, 3H), 1.84−1.76 (m, 2H), 1.57−1.43 (m, 2H), 1.23 (s, 24H), 0.85 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.96, 170.78, 170.75, 169.25, 62.41, 61.10, 54.75, 51.96, 51.88, 33.66, 33.44, 31.31, 29.06, 29.05, 29.02, 28.98, 28.89, 28.72, 28.69, 28.46, 28.15, 27.69, 24.45, 22.50, 22.12, 13.98. MS (ESI) calcd for $C_{28}H_{52}N_2O_7S$, m/z 560.35; found 561.35 (M + H)$^+$.

TLR2-Specific NF-κB Induction. The induction of NF-κB in a TLR2-specific reporter gene assay was quantified using HEK-Blue cells as previously described by us.[23] HEK293 cells stably transfected with either human TLR2 or murine TLR2 and alkaline phosphatase (sAP) were obtained 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 promoters is inducible by TLR2 agonists, and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK-Blue cells were incubated at a density of ~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 then stimulated with serially diluted aliquots of compounds for 12 h. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in the HEK-detection medium as supplied by the vendor) at 620 nm. For antagonism assays, HEK-Blue cells were incubated at a density of ~10^5 cells/mL in a volume of 80 μL/well and stimulated with either PAM_2CS (1 μg/mL) or lipoteichoic acid (1 μg/mL) in the presence of graded concentrations of the test compounds as described for TLR7 previously.[19,37]

■ ASSOCIATED CONTENT

Supporting Information

Characterization of intermediates and final compounds (^{1}H, ^{13}C, mass spectra). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by NIH/NIAID contract HHSN272200900033C.

■ ABBREVIATIONS USED

DMF, dimethylformamide; DMAP, 4-dimethylaminopyridine; EC_{50}, half-maximal effective concentration; EDCI, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; ESI-TOF, electrospray ionization-time-of-flight; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium hexafluorophosphate; HEK, human embryonic kidney; HOBt, 1-hydroxybenzotriazole; IRAK-4, interleukin-1 receptor-associated kinase 4; MPL, monophosphoryl lipid A; MHC, major histocompatibility complex; NF-κB, nuclear factor-κB; PAM, palmitoyl; sAP, secreted alkaline phosphatase; SAR, structure−activity relationship; T_H1, helper T lymphocyte, type 1; T_H2, helper T lymphocyte, type 2; THF, tetrahydrofuran; TLR, Toll-like receptor

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