Journal of Medicinal Chemistry

ARTICLE

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Structure−Activity Relationships in Nucleotide Oligomerization Domain 1 (Nod1) Agonistic γ-Glutamyldiaminopimelic Acid Derivatives

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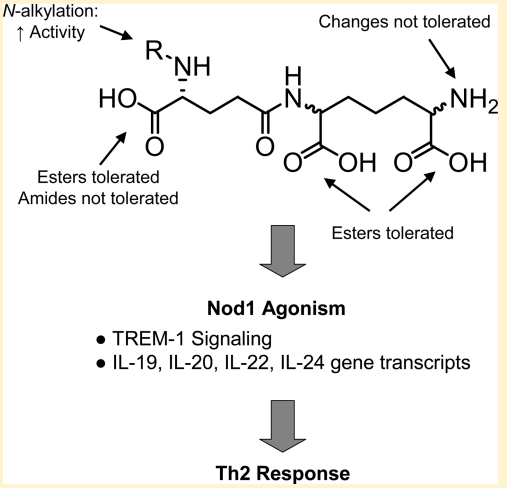
Supporting Information

ABSTRACT: *N*-Acyl-γ-glutamyldiaminopimelic acid is a prototype ligand for Nod1. We report a detailed SAR of C_{12}-γ-D-Glu-DAP. Analogues with glutaric or γ-aminobutyric acid replacing the glutamic acid show greatly attenuated Nod1-agonistic activity. Substitution of the *meso*-diaminopimelic (DAP) acid component with monoaminopimelic acid, L- or D-lysine, or cadaverine also results in reduced activity. The free amine on DAP is crucial. However, the *N*-acyl group on the D-glutamyl residue can be substituted with *N*-alkyl groups with full preservation of activity. The free carboxylates on the DAP and Glu components can also be esterified, resulting in more lipophilic but active analogues. Transcriptomal profiling showed a dominant up-regulation of IL-19, IL-20, IL-22, and IL-24, which may explain the pronounced Th2-polarizing activity of these compounds and also implicate cell signaling mediated by TREM-1. These results may explain the hitherto unknown mechanism of synergy between Nod1 and TLR agonists and are likely to be useful in designing vaccine adjuvants.



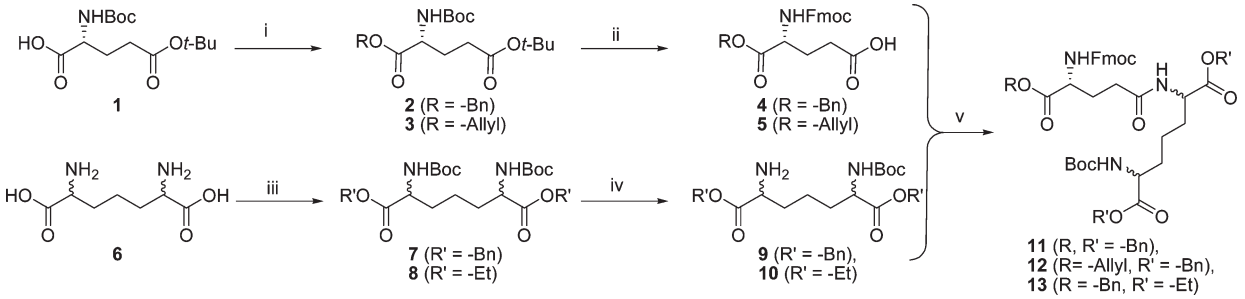
INTRODUCTION

Cellular pattern recognition receptors (PRRs) are ubiquitous innate immune sensors that recognize specific molecular patterns present in molecules that are broadly shared by pathogens but are structurally distinct from host molecules.[1−4] PRRs include not only membrane-bound Toll-like receptors (TLRs), members of which are expressed on the cell surface and the endocytoplasmic reticulum, but also cytosolic receptors encompassing the Nod and RIG-I families of proteins.[4,5] Nod1 and Nod2 are prototype members of the nucleotide oligomerization domain (NOD) and ligand-recognizing leucine-rich repeat (NLR)-containing proteins that serve to signal the presence of intracytoplasmic peptidoglycan fragments by sensing diaminopimelic acid peptides and muramyl dipeptides, respectively.[6−9] Our interest in understanding the structural determinants of the biological properties of ligands of PRRs stems from the potential value in harnessing innate immune stimulatory properties in marshalling and specifically directing subsequent adaptive immune responses with desired immunophenotypes and profiles.[10,11] We had previously reported SAR studies on TLR2 and TLR7,[12,13] and while studies on several other TLR ligands continue, our attention is focused also on the intracytoplasmic NOD family of receptors, especially Nod1. Whereas the muramyldipeptide chemotype has been extensively studied since the early 1970s[14−19] (preceding by several decades the discovery of Nod2 as its primary receptor[9,20]), structure−activity relationships and signaling pathways involving Nod1-agonistic γ-glutamyl-*meso*-diaminopimelic acid peptides[21,22] have remained sparse. Investigators at the Fujisawa Pharmaceutical Company reported in the early 1980s that D-lactoyl-L-alanyl-γ-D-glutamyl-(L)-*meso*-diaminopimelyl-(L)-glycine and heptanoyl-γ-D-glutamyl-(L)-*meso*-diaminopimelyl-D-alanine enhanced host defense in murine models of fatal *E. coli* sepsis.[23−27] Subsequent to the discovery that the γ-glutamyl-*meso*-diaminopimelic acid (iE-DAP) fragment is the crucial pharmacophore mediating recognition of peptidoglycan fragments by Nod1,[21,22] synthetic, lipophilic, *N*-acylglutamyl derivatives have been shown to be potent Nod1 agonists.[28,29] *N*-Dodecanoyl-γ-D-glutamyldiaminopimelic acid (C_{12}-iE-DAP) is now available commercially as a reference Nod1 agonist. Also noteworthy is work by Boons and colleagues who have synthesized and evaluated DAP-containing muramyl peptides.[30,31]

We were keen to explore the structural space around the iE-DAP scaffold for the reason that Nod1 agonists are associated

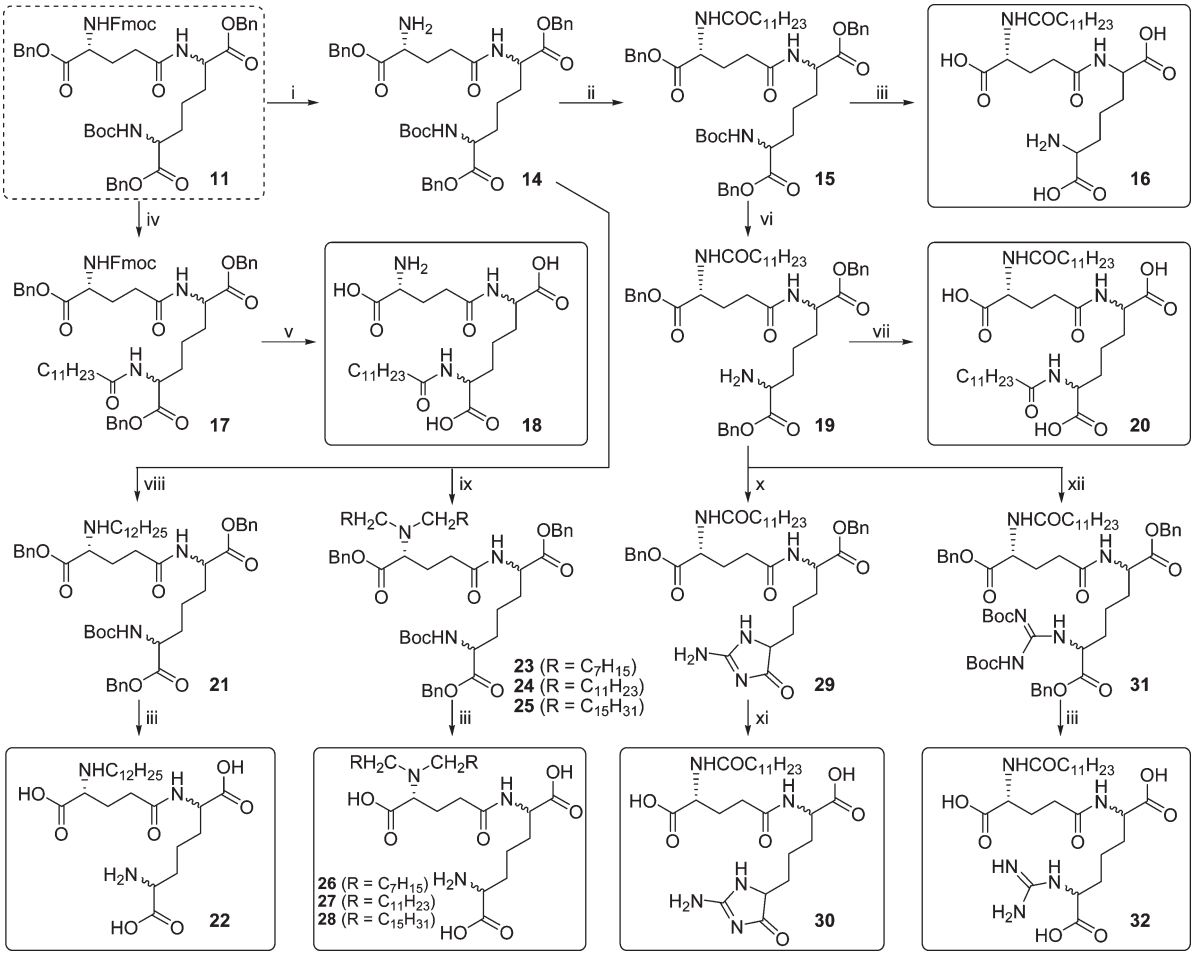
Received: November 30, 2010
Published: February 07, 2011

Scheme 1[a]



[a] Reagents: (i) ROH, HBTU, TEA, DMAP, DMF; (ii) (a) CF_3COOH, (b) FmocCl, Na_2CO_3, dioxane, H_2O; (iii) (a) $(Boc)_2O$, TEA, H_2O, (b) R′OH, HBTU, DMAP, TEA, DMF; (iv) (a) HCl−dioxane, (b) $(Boc)_2O$ (1 equiv), TEA, MeOH; (v) PS-carbodiimide, PS-DMAP, CH_2Cl_2.

Scheme 2[a]

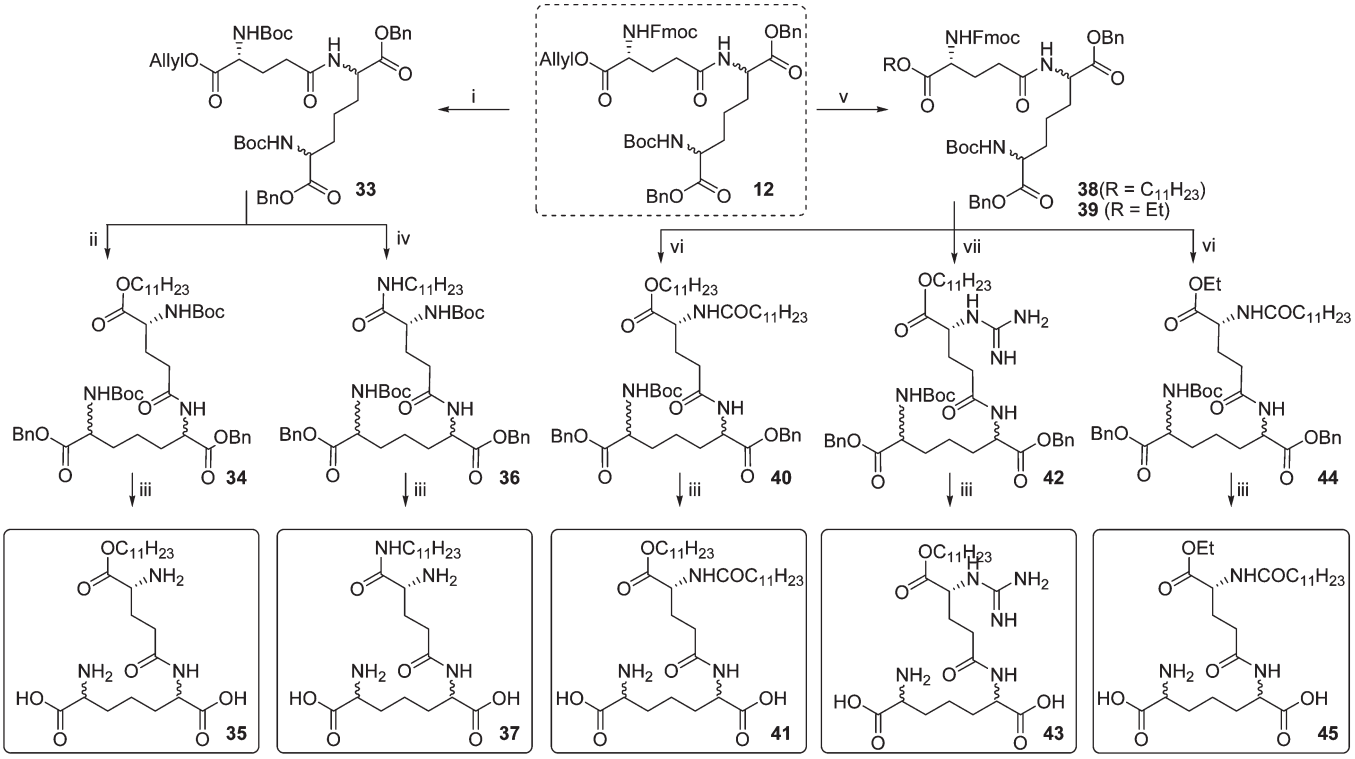


[a] Reagents: (i) 30% piperidine, CH_2Cl_2; (ii) $C_{11}H_{23}COCl$, pyridine, DMAP; (iii) (a) H_2, $Pd(OH)_2/C$, MeOH, 60 psi, (b) CF_3COOH; (iv) (a) CF_3COOH, (b) $C_{11}H_{23}COCl$, TEA, CH_2Cl_2; (v) H_2, $Pd(OH)_2/C$, AcOH, MeOH, 60 psi; (vi) CF_3COOH; (vii) (a) $C_{11}H_{23}COCl$, pyridine, CH_2Cl_2, (b) H_2, $Pd(OH)_2/C$, MeOH, 60 psi; (viii) $C_{11}H_{23}CHO$ (1 equiv), MP-$CNBH_3$, CH_2Cl_2, MeOH, AcOH; (ix) RCHO (excess), MP-$CNBH_3$, CH_2Cl_2, MeOH, AcOH; (x) 1*H*-pyrazole-1-carboxamidine·HCl, pyridine, microwave irradiation, 60 °C; (xi) H_2, $Pd(OH)_2/C$, MeOH, 60 psi; (xii) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine·HCl, pyridine, THF, 50 °C.

with the mobilization of adaptive immune responses with a dominant Th2 bias, which is undesirable when a strong CD8+ cytotoxic T lymphocytic response is required.[32] We hypothesized that covalent coupling to iE-DAP of the TLR7/8-agonistic imidazoquinolines[12,33] that we have found to be extraordinarily Th1-polarizing[10,11] could lead to vaccine adjuvants with balanced

dx.doi.org/10.1021/jm101535e |*J. Med. Chem.* 2011, 54, 1490–1510

Scheme 3[a]



[a] Reagents: (i) (a) 30% piperidine, CH_2Cl_2, (b) $(Boc)_2O$, TEA, MeOH; (ii) (a) chlorotris(triphenylphosphine)rhodium(I), EtOH, H_2O, reflux, (b) $C_{11}H_{23}OH$, HBTU, DMAP, TEA, DMF; (iii) (a) H_2, $Pd(OH)_2/C$, MeOH, 60 psi, (b) CF_3COOH; (iv) (a) chlorotris(triphenylphosphine)rhodium(I), EtOH, H_2O, reflux, (b) $C_{11}H_{23}NH_2$, HBTU, TEA, DMAP, CH_2Cl_2; (v) (a) chlorotris(triphenylphosphine)rhodium(I), EtOH, H_2O, reflux, (b) ROH, HBTU, DMAP, DMF; (vi) (a) 30% piperidine, CH_2Cl_2, (b) $C_{11}H_{23}COCl$, pyridine, DMAP; (vii) (a) 30% piperidine, CH_2Cl_2, (b) 1*H*-pyrazole-1- carboxamidine·HCl, pyridine, microwave irradiation, 60 °C.

Th1/Th2 immunostimulatory phenotypes. In order to accomplish this goal, it was necessary to first determine optimal positions on the iE-DAP backbone that would permit the generation of dual-active "hybrid" molecules, combining the TLR7-agonistic imidazoquinoline and Nod1-active iE-DAP chemotypes in a single covalently coupled construct. We report in this paper an SAR study of Nod1-agonistic iE-DAP derivatives. Secondary screens including transcriptomal profiling in ex vivo models using whole human blood have revealed a possible basis for the pronounced Th2 bias of this chemotype.

RESULTS AND DISCUSSION

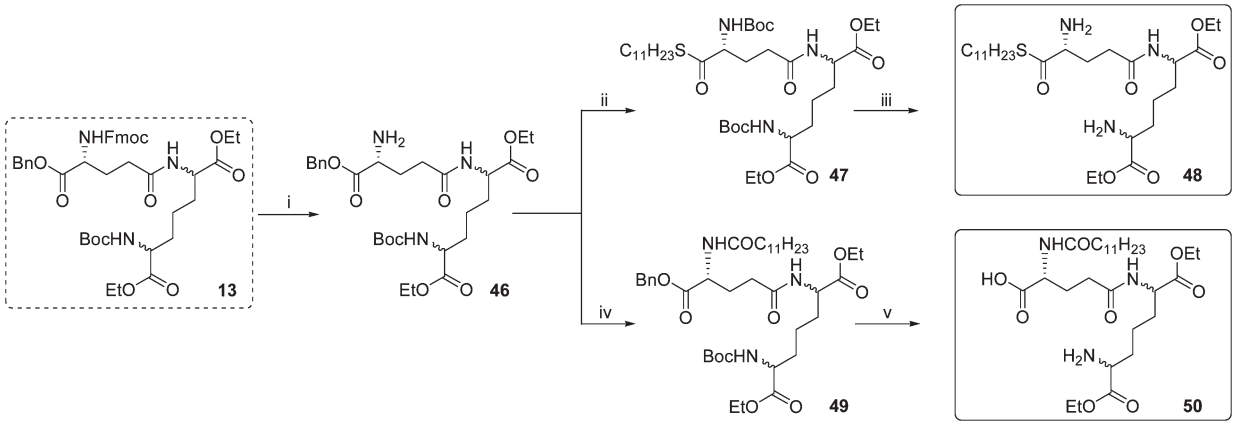
The construction of orthogonally protected γ-D-glutamyldiaminopimelic acid synthons (Scheme 1) allowed convenient access to a number of analogues wherein the carboxyl and amino substituents on both amino acids could be varied (Schemes 2−4). For the sake of consistency, we designate the α-amino group on the D-Glu as *N*, and the γ-amino group on the diaminopimelic acid (DAP) residue as *N*′. We used the commercially available diaminopimelic acid (**6**, mixture of four isomers), since derivatives of all four isomers have been shown to be active.[28,29] Esters of DAP proved to be surprisingly difficult to obtain by a number of conventional methods, necessitating sequential *N*-Boc protection of the amines, esterification (as benzyl or ethyl) of the carboxylic acids, deprotection of the *N*-Boc groups, followed by mono-*N*-Boc protection using stoichiometric equivalents of Boc_2O. *N*-Fmoc-D-Glu-α-benzyl/allyl esters (**4** or **5**, respectively) were coupled to the mono-*N*-Boc-diaminopimelic acid diesters (**9** or **10**) using standard protocols to afford the orthogonally protected synthons (**11**−**13**, Scheme 1). *N*-Fmoc (D-Glu) deprotection followed by acylation with lauroyl chloride and subsequent deprotection of the *N*-Boc and *O*-Bn groups yielded C_{12}-iE-DAP (**16**, Scheme 2), the activity of which was identical to that of the commercially available reference compound with EC_{50} of ∼30 pM (Figure 1A).

The *N*′-acyl (DAP) compound (**18**) and the *N*,*N*′-diacyl (D-Glu, and DAP, respectively) analogue (**20**), as well as *N*-alkyl (**22**) and *N*,*N*-dialkyl analogues (**26**−**28**) were also synthesized from synthon **11** (Scheme 2). Our initial attempts at converting the *N*′ amine to a guanidine group resulted in an undesired cyclization−elimination reaction to form the aminoimidazolone **30**; this was obviated by the use of *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine as the guanidination reagent, affording the desired product **32** (Scheme 2).

Next, analogues were synthesized from **12** with the acyl group on the α-amine of D-Glu transposed to the α-carboxylic acid group as the undecanoyl ester (**35**), the 1-aminoundecane-derived amide (**37**), or the *N*-acyl long-chain ester **41** (Scheme 3). The *N*-guanidino ester analogue **43** and the α-D-Glu ethyl ester derivative of C_{12}-iE-DAP (**45**) were also obtained from **12** (Scheme 3). The α-D-Glu thioester DAP ethyl ester

dx.doi.org/10.1021/jm101535e |*J. Med. Chem.* 2011, 54, 1490–1510

Scheme 4[a]



[a] Reagents: (i) 30% piperidine, CH_2Cl_2; (ii) (a) $(Boc)_2O$, TEA, MeOH, (b) H_2, $Pd(OH)_2/C$, MeOH, 60 psi, (c) $C_{11}H_{23}SH$, HBTU, DMAP, TEA, DMF; (iii) CF_3COOH; (iv) $C_{11}H_{23}COCl$, pyridine; (v) (a) H_2, $Pd(OH)_2/C$, MeOH, 60 psi, (b) CF_3COOH.

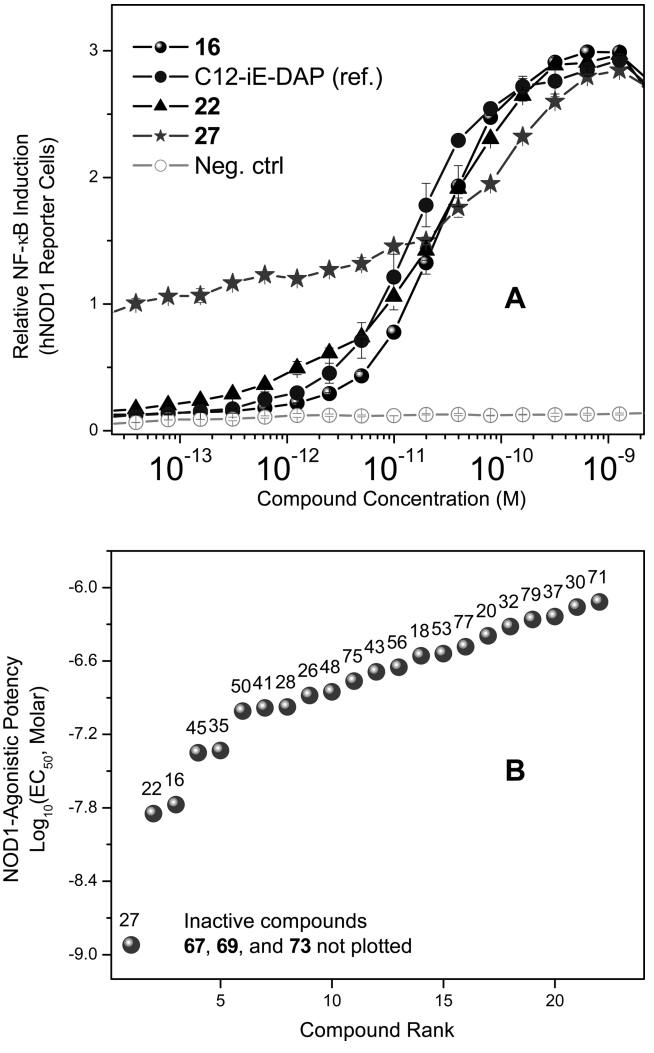


Figure 1. (A) NF-κB induction activity in stable transfectants of HEK-293 cells expressing human Nod1. (B) Rank-order plot of potencies (EC_{50} values) of iE-DAP analogues.

analogue (**48**) and DAP ethyl ester derivative of C_{12}-iE-DAP (**50**) were synthesized from synthon **13** (Scheme 4).

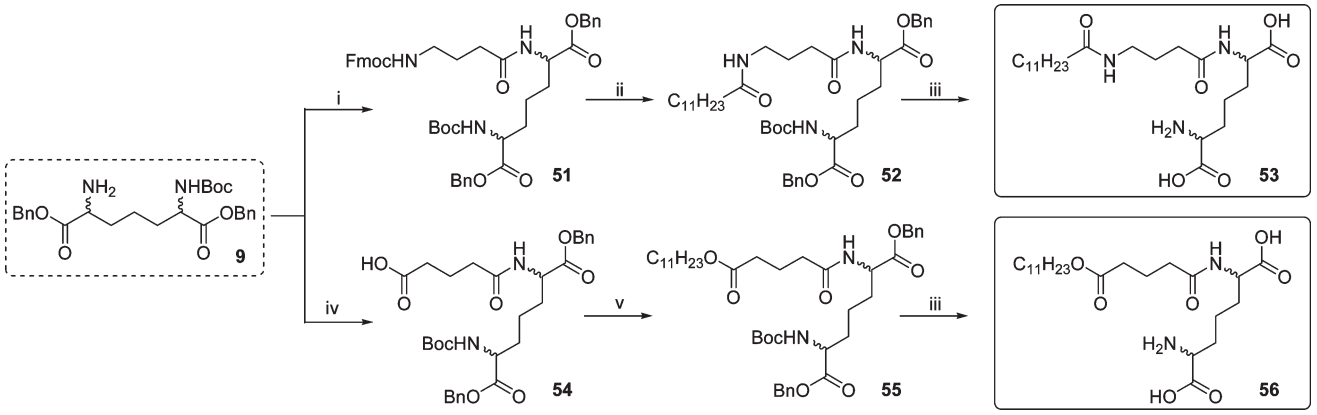
In order to examine the importance of the α-amino and carboxyl groups of D-Glu, γ-aminobutyric acid (des-carboxyl, **53**) or glutaric acid (des-amino, **56**) was coupled to the DAP derivative **9** as depicted in Scheme 5. Similarly, suitably protected monoaminopimelic acid **58**, D- and L-lysine derivatives (**62**−**65**, Scheme 6), and commercially available *N*-Boc-cadaverine and D, L-α-amino-ε-caprolactam were used to examine the roles of the amine and carboxyl groups of DAP (Scheme 7).

The *N*-acyl on the D-Glu residue appears to be optimal for Nod1-agonistic activity, since the transposition of the acyl group to the *N*′ position on DAP (**18**) or diacylation at *N* and *N*′ (**20**) resulted in substantial decrease in activity (Table 1). The substitution of the *N*-acyl (C_{12}) group with *N*-alkyl (**22**) led to virtually identical activity (27 pM versus 23 pM, Table 1). Compounds with C_8 or C_{16} *N*,*N*-dialkyl groups (**26**, **28**) displayed attenuated potency, while the C_{12} *N*,*N*-dialkyl compound **27** displayed a rather dramatic increase in potency, with residual partial activity evident even at very high dilutions (Table 1, Figure 1). Compound **27** was the most potent analogue identified in this study. The primary amines on both the DAP and D-Glu segments were found to be crucial; the guanidino analogues **32** and **43** and the aminoimidazolone analogue **30** exhibited diminished activity.

We next examined the importance of the free α-carboxyl group on D-Glu. The potencies of the undecanoyl ester of iE-DAP (**35**) and the ethyl ester of C_{12}-iE-DAP (**45**) were comparable to that of the reference compound C_{12}-iE-DAP (**16**). The undecanoyl ester of C_{12}-iE-DAP (**41**) and the thioester analogue **48** also retained modest activity. The 1-aminoundecane-derived amide analogue (**37**), however, was substantially less active. Augmented activity in **35** and **45** appears to be related to neither net charge nor bulk hydrophobicity, both of which could be reasonably expected to modulate transmembrane permeation and consequent presentation of the ligand to the intracellular Nod1 receptor. The net charge at physiological pH of **35** and **37** is zero, while that of **41** and **45** is −1. We surmised that the higher potency of **45** relative to **41** could be due to the more facile hydrolytic cleavage by intracellular esterases of the shorter ethyl ester of **45**, but our observation that the rate and kinetics of NF-κB induction for both compounds were very similar (data not shown) is not in agreement with our conjecture, and we do not yet understand the basis for the difference in

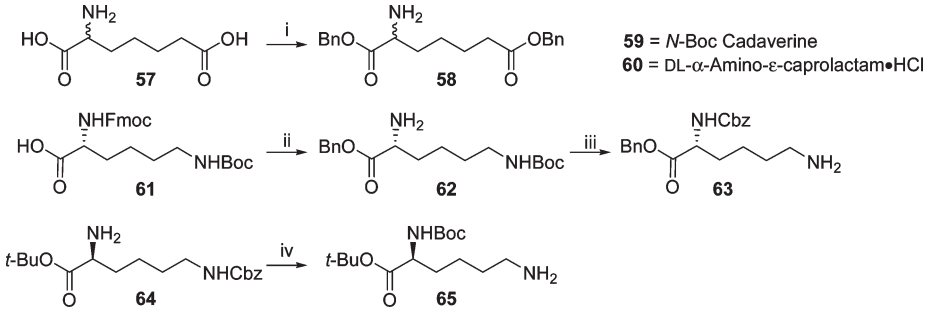
dx.doi.org/10.1021/jm101535e |*J. Med. Chem.* 2011, 54, 1490–1510

Scheme 5[a]



[a] Reagents: (i) Fmoc-GABA-OH, PS-carbodiimide, PS-DMAP, CH_2Cl_2; (ii) (a) 30% piperidine, CH_2Cl_2, (b) $C_{11}H_{23}COCl$, pyridine; (iii) (a) H_2, $Pd(OH)_2/C$, MeOH, 60 psi, (b) CF_3COOH; (iv) glutaric anhydride, CH_2Cl_2; (v) $C_{11}H_{23}OH$, HBTU, DMAP, TEA, DMF.

Scheme 6[a]



[a] Reagents: (i) BnOH, *p*-TSA, reflux; (ii) (a) BnOH, HBTU, DMAP, DMF, (b) 30% piperidine, CH_2Cl_2; (iii) (a) $PhCH_2OCOCl$, Na_2CO_3, dioxane, H_2O, (b) CF_3COOH; (iv) (a) $(Boc)_2O$, TEA, MeOH, (b) H_2, $Pd(OH)_2/C$, MeOH, 60 psi.

potencies of these analogues. Substitution of the *N*-acyl D-Glu fragment with either *N*-acyl-γ-aminobutyric acid (**53**, des-α-carboxyl analogue) or the undecanoyl ester of glutaric acid (**56**, des-α-amino analogue) resulted only in considerable loss in Nod1-agonistic activity. Taken together, the above SAR data suggest that considerable "plasticity" exists in the recognition of the D-Glu fragment. Indeed, a plot of the potencies of these analogues displays an unexpectedly diffuse, near-uniform distribution of EC_{50} values (Figure 1B), unlike the well-demarcated and discrete SAR that we had previously observed for TLR2-[13] and TLR7-active[12] compounds.

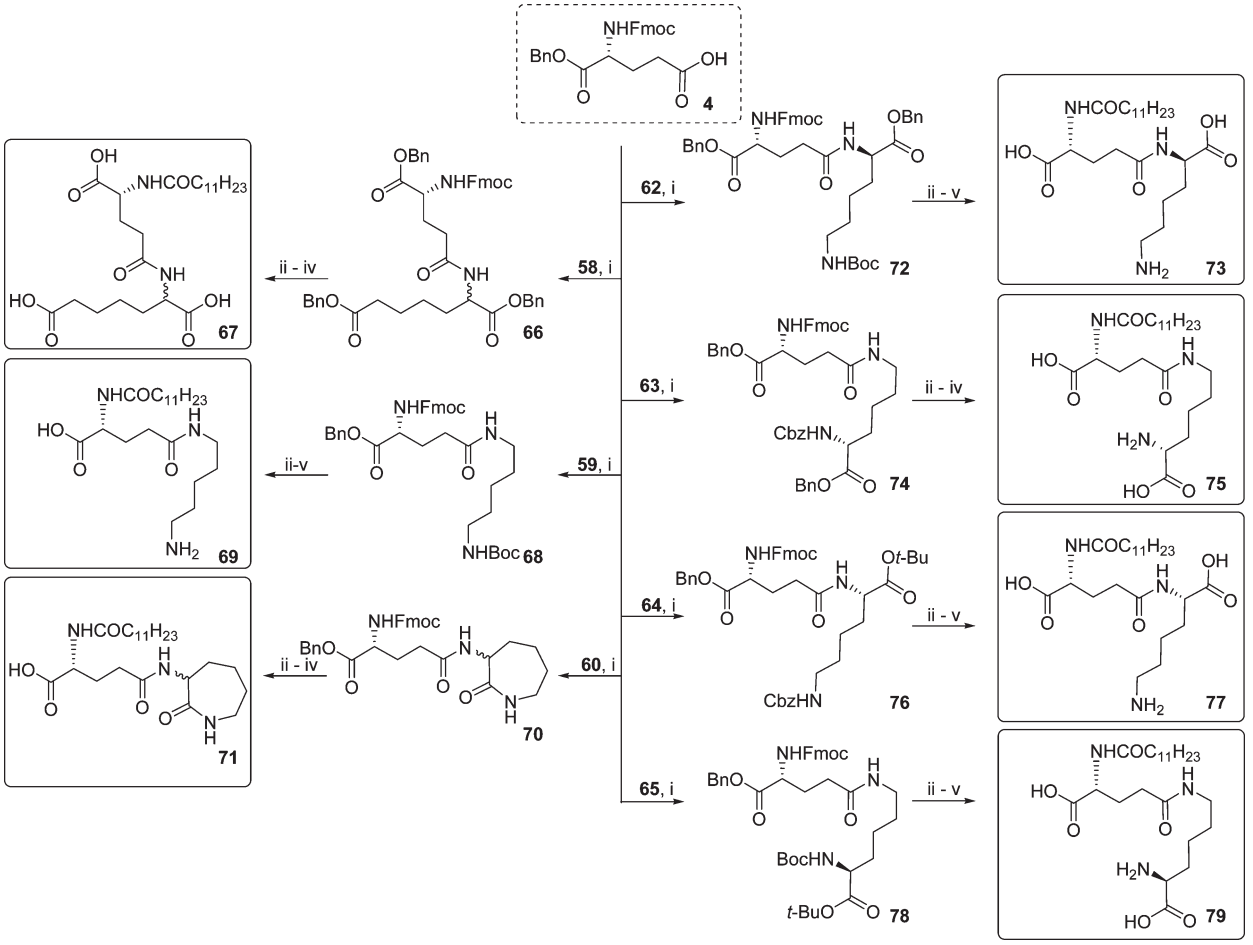
In contradistinction to the broad and diffuse SAR for D-Glu fragment, the DAP portion of the molecule is far more stringent in determining Nod1 agonism. Whereas esterification of carboxyl groups of DAP (**50**) is tolerated, substitution with monoaminopimelic acid (**67**, des-amino) or cadaverine (**69**, des-dicarboxyl) resulted in abrogation of activity. The *meso*-diaminopimelic acid-containing peptidoglycan is specific for Gram-negative bacteria, and the corresponding homologue in Gram-positive organisms is L-lysine coupled via its α-amine to γ-D-glutamyl residue (**77**), the activity of which is ∼10-fold less than that of **16**.[34,35] The D-lysine analogue **73** is bereft of any activity. The unnatural ε-amine-coupled lysine analogues were also evaluated. The D-lysine analogue **75** was found to be 4-fold more active than **79**, its L-lysine congener.

Primary assay readouts using cell-culture systems may not always accurately reflect in vivo behavior owing to a variety of reasons, including differential plasma protein binding (that we ourselves have observed and characterized),[36] and we therefore wished to confirm that **27** was indeed more potent than **16**, the reference Nod1 agonist, in ex vivo assays employing whole human blood. Although Nod1 agonists have previously been shown to enhance neutrophil recruitment in mice,[34] a recent report employing synthetic, nonacylated, γ-glutamyl-*meso*-diaminopimelic acid dipeptide indicated that Nod1-specific responses could not be elicited in isolated human neutrophils.[37] By use of an ex vivo system using fresh, whole human blood that we have established for immunoprofiling innate immune stimuli,[10,13,38] both p38 MAPK[39] and CD11b[40] were up-regulated in a dose-dependent manner, with **27** being more potent than **16** (Figure 2). Indeed, the nonacylated, γ-glutamyl-*meso*-diaminopimelic acid dipeptide was inactive in our primary and secondary screens (data not shown), emphasizing the requirement of an *N*-acyl functionality on glutamic acid.

Recent findings using murine models of immunization indicate that Nod1 engagement drives B and T cell immunity with a predominant Th2 polarization and additional Th17 priming.[32] However, mechanisms underlying the induction of strong Th2-biased immune responses have remained obscure. Given the significant differences between species-dependent recognition of

dx.doi.org/10.1021/jm101535e |*J. Med. Chem.* 2011, 54, 1490–1510

Scheme 7[a]



[a] Reagents: (i) PS-carbodiimide, PS-DMAP, TEA, CH_2Cl_2; (ii) 30% piperidine, CH_2Cl_2; (iii) $C_{11}H_{23}COCl$, pyridine; (iv) H_2, $Pd(OH)_2/C$, MeOH, 60 psi; (v) CF_3COOH.

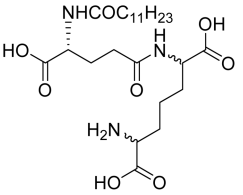
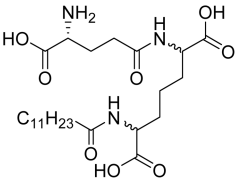
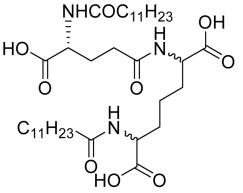
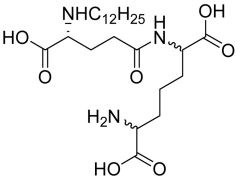
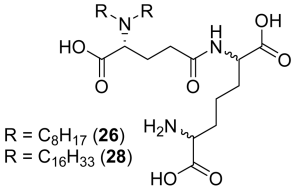
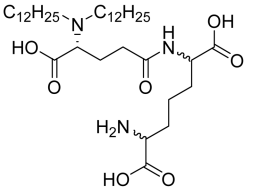
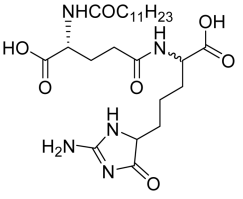
innate immune ligands,[11] we desired to evaluate whether surrogate markers of Th2-skewed responses could be observed in human ex vivo model systems, in the hope that these studies may also help uncover signaling events determining Th2 polarization. Transcriptomal profiling experiments using human blood revealed a very prominent up-regulation of class II helical cytokines by **27**, especially members of the IL-10 superfamily[41] including IL-19, IL-20, IL-22, and IL-24 (Table 2); these cytokines, especially IL-19,[42−45] have been shown to drive Th2-polarized responses. We have never before observed this particular constellation of cytokines in our earlier[10] (and ongoing) immunoprofiling studies, and we are excited in no small measure that quantifying the induction of IL-19 and related cytokines may have predictive value as biomarkers and surrogate signatures for Th2 induction in immunoprofiling vaccine adjuvants and may perhaps supplant the necessity of long and laborious immunization experiments that typically involve large numbers of animals. Also observed, as could be expected in the context of Th17 induction,[32] was the up-regulation of IL-17. Similar responses were observed with **16** but of lower magnitude, consistent with the lower potency of **16** (data not shown).

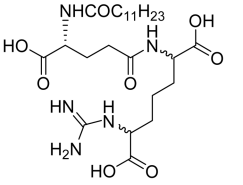
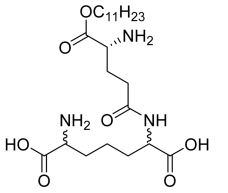
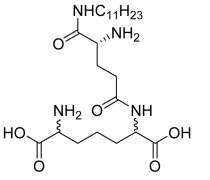
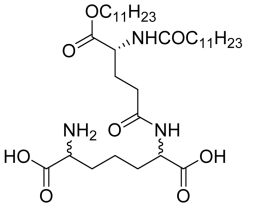
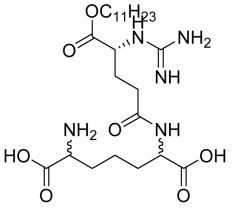
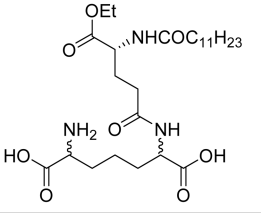
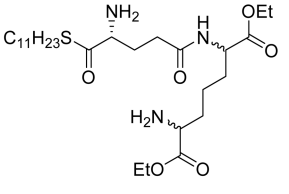
Further analyses of gene transcripts induced by **27** yielded the unexpected finding that exposure of human blood to **27** results in the activation of pathways involving signaling mediated by triggering receptor expressed on myeloid cells 1 (TREM-1, Figure 3). TREM-1 is a recently discovered receptor expressed on the cell surface of monocytes and neutrophils and plays an important role in amplifying myeloid cell-activated inflammatory responses.[46−48] Our observation of the up-regulation of TREM-1 signaling components may explain the hitherto unknown mechanism of synergy between Nod1 and TLR agonists.[32,34,49,50]

The overarching objective that led to our work on Nod1 ligands was not so much to synthesize higher potency compounds but rather to understand SAR that would permit us to covalently couple other innate immune ligands[12,13,33] to the iE-DAP scaffold in order to test the hypothesis that such hybrids may imbue Th1−Th2 balanced, yet effective vaccine adjuvants. The studies reported here indicate that various substitutions and modifications on the γ-D-glutamyl moiety are tolerated whereas the diaminopimelic acid fragment is far more stringent. The α-carboxylic acid of D-Glu appears to be optimal for coupling with other innate immune ligands, and work in this area is in progress. Secondary and tertiary screens, including transcriptomal profiling, have shed light on the possible mechanisms underlying the strong Th2 bias of Nod1 ligands and of synergy with other innate immune stimuli.

dx.doi.org/10.1021/jm101535e |*J. Med. Chem.* 2011, 54, 1490–1510

Table 1. EC_{50} Values of hNod1 Agonistic Activities of Analogues

Cmpd	Structure	EC_{50} (nM)
16		0.027
18		0.55
20		0.58
22		0.023
26 28	 R = C_8H_{17} (**26**) R = $C_{16}H_{33}$ (**28**)	**26**: 0.20 **28**: 0.12
27		0.0015
30		1.31

Cmpd	Structure	EC_{50} (nM)
32		0.622
35		0.066
37		0.826
41		0.134
43		0.238
45		0.069
48		0.181

dx.doi.org/10.1021/jm101535e |J. Med. Chem. 2011, 54, 1490–1510

Table 1. Continued

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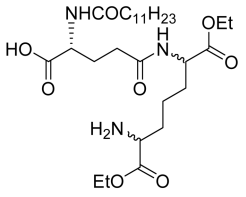
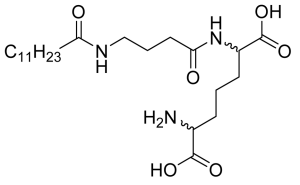
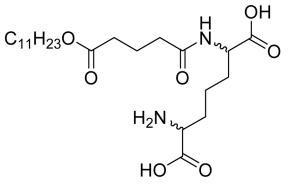
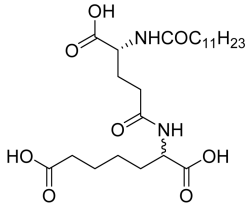
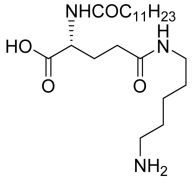
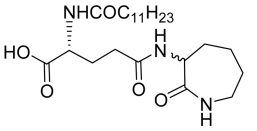
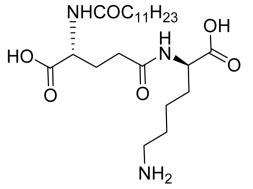
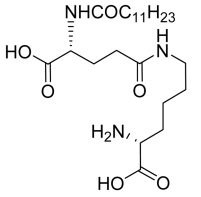
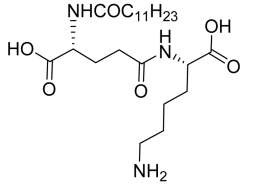
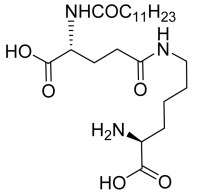
50		0.145
53		0.504
56		0.39
67		>100
69		>100
71		1.73
73		>100
75		0.37
77		0.57
79		0.96

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 an 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 (Teledyne-Isco, Lincoln, NE) 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 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 or H_2O−CH_3CN gradients (with 0.1% CF_3COOH in both mobile phases) and an Agilent ESI-TOF mass spectrometer (mass accuracy of 5 ppm) operating in the positive ion (or negative ion, as appropriate) acquisition mode. Total ion current from 150 to 3500 Da was measured.

Synthesis of Compound 2: (*R*)-1-Benzyl 5-*tert*-Butyl 2-(-(*tert*-butoxycarbonyl)amino)pentanedioate. To a solution of **1** (1 g, 3.29 mmol) in anhydrous DMF were added HBTU (1.38 g, 3.62 mmol), triethylamine (0.5 mL, 3.62 mmol), benzyl alcohol (682 μL, 6.59 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred for 4 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in ethyl acetate and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (10% EtOAc/hexanes) to obtain compound **2** (1.14 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39−7.31 (m, 5H), 5.16 (dt, J = 23.2, 11.5 Hz, 3H), 4.35 (dd, J = 13.0, 8.2 Hz, 1H), 2.35−2.22 (m, 2H), 2.13 (td, J = 13.3, 7.0 Hz, 1H), 1.92 (tt, J = 14.7, 7.5 Hz, 1H), 1.43 (s, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.09, 172.06, 155.39, 131.57, 118.82, 80.74, 79.93, 65.95, 53.11, 31.60, 28.30, 28.06, 27.89, 27.68. MS (ESI) calculated for $C_{21}H_{31}NO_6$, m/z 393.22, found 416.22 $(M + Na)^+$.

Synthesis of Compound 3: (*R*)-1-Allyl 5-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)pentanedioate. To a solution of **1** (1 g,

dx.doi.org/10.1021/jm101535e |*J. Med. Chem.* 2011, 54, 1490–1510

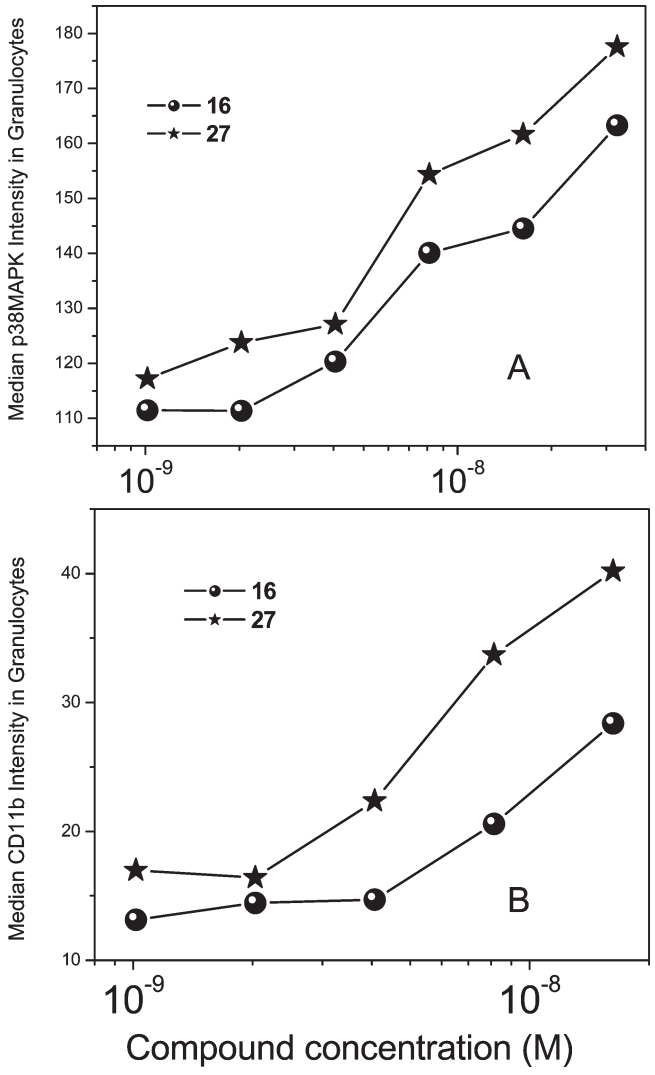


Figure 2. Dose-responses of p38 MAP kinase (A) and CD11b (B) induction in the granulocytic population in whole human blood by **16** and **27** determined by flow cytometry.

3.29 mmol) in anhydrous DMF were added HBTU (1.38 g, 3.62 mmol), triethylamine (0.5 mL, 3.62 mmol), allyl alcohol (450 μL, 6.59 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred for 4 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in ethyl acetate and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (10% EtOAc/hexanes) to obtain compound **3** (1.0 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.91 (ddt, J = 16.3, 10.6, 5.8 Hz, 1H), 5.34 (dd, J = 17.2, 1.3 Hz, 1H), 5.26 (dd, J = 10.4, 0.7 Hz, 1H), 5.11 (d, J = 8.0 Hz, 1H), 4.64−4.63 (m, 2H), 4.33 (dd, J = 13.2, 8.3 Hz, 1H), 2.38−2.26 (m, 2H), 2.17−2.11 (m, 1H), 1.96−1.88 (m, 1H), 1.45 (s, 9H), 1.44 (s, 9 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.05, 172.02, 155.35, 131.53, 118.78, 80.70, 79.89, 65.91, 53.07, 31.56, 28.26, 28.02, 27.85, 27.64. MS (ESI) calculated for $C_{17}H_{29}NO_6$, m/z 343.20, found 366.19 $(M + Na)^+$.

Synthesis of Compound 4: (*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(benzyloxy)-5-oxopentanoic Acid. The solution of **2** (1 g, 2.54 mmol) in trifluoroacetic acid was stirred for 1 h, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt. To the solution of trifluoroacetate salt in dioxane, the aqueous solution of sodium carbonate (810 mg, 7.64 mmol) was added at 10 °C. The reaction mixture was stirred at room temperature for 10 min, followed by the addition of FmocCl (722 mg, 2.79 mmol) solution in dioxane. The reaction mixture was stirred at room temperature for 16 h, followed by removal of the solvent under reduced pressure. The residue was dissolved in ethyl acetate and water, and the solution was acidified using 10% HCl until the pH was ~1. The aqueous layer was washed with ethyl acetate. The ethyl acetate was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (40% EtOAc/hexanes) to obtain compound **4** (1.0 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 7.4 Hz, 2H), 7.33 (qd, J = 15.0, 7.2 Hz, 9H), 5.47 (d, J = 8.2 Hz, 1H), 5.19−5.06 (m, 2H), 4.53−4.30 (m, 3H), 4.16 (dd, J = 15.6, 8.8 Hz, 1H), 2.48−2.12 (m, 3H), 1.94 (dt, J = 14.5, 7.7 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.59, 171.91, 156.27, 143.99, 143.84, 141.50, 141.49, 135.19, 128.88, 128.81, 128.56, 127.95, 127.30, 125.29, 125.26, 120.21, 120.20, 67.73, 67.34, 53.42, 47.32, 29.91, 27.64. MS (ESI) calculated for $C_{27}H_{25}NO_6$, m/z 459.16, found 482.17 $(M + Na)^+$.

Synthesis of Compound 5: (*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(allyloxy)-5-oxopentanoic Acid. The solution of compound **3** (1 g, 2.91 mmol) in trifluoroacetic acid was stirred for 1 h, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt. To the solution of trifluoroacetate salt in dioxane, the aqueous solution of sodium carbonate (926 mg, 8.74 mmol) was added at 10 °C. The reaction mixture was stirred at room temperature for 10 min, followed by the addition of FmocCl (830 mg, 3.2 mmol) solution in dioxane. The reaction mixture was stirred at room temperature for 16 h, followed by removal of the solvent under reduced pressure. The residue was dissolved in ethyl acetate and water, and the solution was acidified using 10% HCl until the pH was ~1. The aqueous layer was washed with ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (40% EtOAc/hexanes) to obtain compound **5** (1.1 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.1 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 5.90 (ddd, J = 22.5, 11.0, 5.7 Hz, 1H), 5.44 (d, J = 8.1 Hz, 1H), 5.30 (dd, J = 27.7, 13.7 Hz, 2H), 4.65 (d, J = 5.5 Hz, 2H), 4.58−4.35 (m, 3H), 4.21 (t, J = 6.8 Hz, 1H), 2.58−2.17 (m, 3H), 2.07−1.92 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.70, 172.54, 155.72, 155.57, 135.49, 128.74, 128.57, 128.43, 80.07, 67.19, 67.14, 53.27, 32.24, 28.47, 28.45, 21.48, 21.19, 21.08. MS (ESI) calculated for $C_{23}H_{23}NO_6$, m/z 409.15, found 432.14 $(M + Na)^+$.

Synthesis of Compound 7: Dibenzyl 2,6-Bis((*tert*-butoxycarbonyl)amino)heptanedioate. To a solution of compound **6** (1 g, 5.25 mmol) in water were added di-*tert*-butyl dicarbonate (4.58 g, 21.0 mmol) and triethylamine (2.92 mL, 21.0 mmol). The reaction mixture was stirred for 6 h, followed by removal of the solvent under reduced pressure to obtain the residue. To the solution of residue in anhydrous DMF were added HBTU (4.38 g, 11.55 mmol), triethylamine (1.6 mL, 11.55 mmol), benzyl alcohol (1.63 mL, 15.75 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred for 8 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in ethyl acetate and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (8% EtOAc/hexanes) to obtain compound **7** (2.48 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44−7.29 (m, 10H), 5.13 (dt, J = 12.4, 8.3 Hz, 6H), 4.29 (s, 2H), 1.85−1.71 (m, 2H), 1.68 (s, 2H), 1.49−1.31 (m, 20H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.70, 172.54, 155.72, 155.57, 135.49, 128.74, 128.57, 128.43, 80.07, 67.19,

Table 2. Interleukin Transcriptomal Responses in Human PBMCs Exposed to 20 μg/mL 27

probe set	fold change	regulation	gene symbol	gene title
220745_at	30.39	up	IL19	interleukin 19
205207_at	29.54	up	IL6	interleukin 6 (interferon, β2)
210118_s_at	14.34	up	IL1A	interleukin 1, α
220322_at	11.10	up	IL1F9	interleukin 1 family, member 9
216876_s_at	10.56	up	IL17A	interleukin 17A
216244_at	8.27	up	IL1RN	interleukin 1 receptor antagonist
212659_s_at	8.19	up	IL1RN	interleukin 1 receptor antagonist
216243_s_at	7.54	up	IL1RN	interleukin 1 receptor antagonist
207901_at	6.13	up	IL12B	interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)
207906_at	5.99	up	IL3	interleukin 3 (colony-stimulating factor, multiple)
212657_s_at	4.94	up	IL1RN	interleukin 1 receptor antagonist
39402_at	4.45	up	IL1B	interleukin 1, β
205067_at	4.14	up	IL1B	interleukin 1, β
224071_at	4.00	up	IL20	interleukin 20
221165_s_at	3.87	up	IL22	interleukin 22
207433_at	3.62	up	IL10	interleukin 10
211269_s_at	3.62	up	IL2RA	interleukin 2 receptor, α
206341_at	3.57	up	IL2RA	interleukin 2 receptor, α
206569_at	3.41	up	IL24	interleukin 24
227997_at	3.17	up	IL17RD	interleukin 17 receptor D
222223_s_at	3.15	up	IL1F5	interleukin 1 family, member 5 (δ)
211506_s_at	2.29	up	IL8	interleukin 8
224262_at	2.05	up	IL1F10	interleukin 1 family, member 10 (θ)
206295_at	2.03	up	IL18	interleukin 18 (interferon-γ-inducing factor)
1555431_a_at	4.05	down	IL31RA	interleukin 31 receptor A
220056_at	3.69	down	IL22RA1	interleukin 22 receptor, α1
207008_at	2.75	down	IL8RB	interleukin 8 receptor, β
220663_at	2.06	down	IL1RAPL1	interleukin 1 receptor accessory protein-like 1
205227_at	2.02	down	IL1RAP	interleukin 1 receptor accessory protein

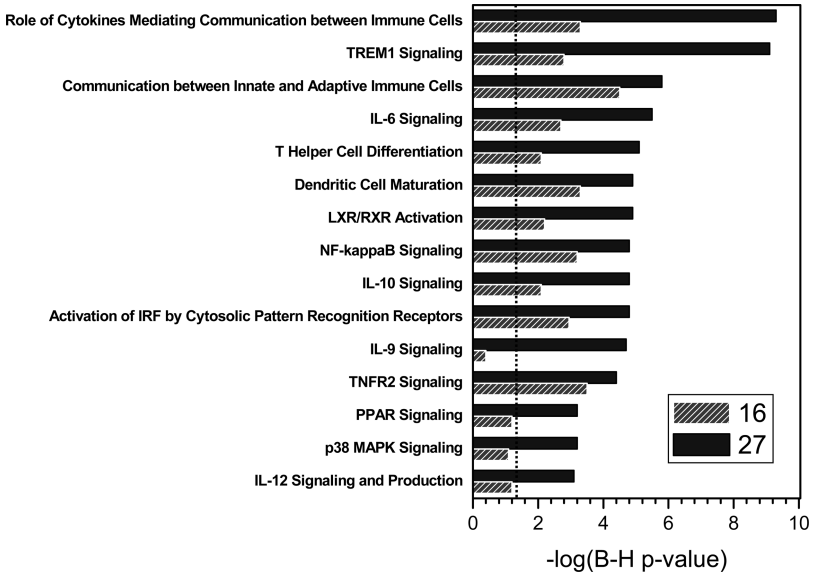


Figure 3. Pathway analysis of transcriptomal activation patterns in human PBMCs exposed to 20 μg/mL **16** and **27**. The dashed line corresponds to the threshold of −log (base 10) for $P = 0.05$ (after Benjamini−Hochberg multiple testing correction).

67.14, 53.27, 32.24, 28.47, 28.45, 21.48, 21.19, 21.08. MS (ESI) calculated for $C_{31}H_{42}N_2O_8$, m/z 570.29, found 593.27 $(M + Na)^+$.

Synthesis of Compound 8: Diethyl 2,6-Bis((*tert*-butoxycarbonyl)amino)heptanedioate. To a solution of compound

dx.doi.org/10.1021/jm101535e |*J. Med. Chem.* 2011, 54, 1490–1510

6 (1 g, 5.25 mmol) in water were added di-*tert*-butyl dicarbonate (4.58 g, 21.0 mmol) and triethylamine (2.92 mL, 21.0 mmol). The reaction mixture was stirred for 6 h, followed by removal of the solvent under reduced pressure to obtain the residue. To the solution of residue in anhydrous DMF were added HBTU (4.38 g, 11.55 mmol), triethylamine (1.6 mL, 11.55 mmol), ethyl alcohol (0.92 mL, 15.75 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred for 6 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in ethyl acetate and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (8% EtOAc/hexanes) to obtain compound **8** (2.0 g, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.17−4.99 (m, 2H), 4.30−4.08 (m, 6H), 1.84−1.74 (m, 2H), 1.72−1.55 (m, 4H), 1.48−1.31 (m, 18H), 1.25 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.24, 173.06, 156.07, 155.92, 80.33, 68.61, 61.80, 53.58, 39.17, 32.74, 32.67, 30.80, 29.38, 28.81, 28.79, 28.71, 24.19, 23.45, 21.82, 21.47, 14.64, 14.52. MS (ESI) calculated for $C_{21}H_{38}N_2O_8$, m/z 446.26, found 469.26 $(M + Na)^+$.

Synthesis of Compound 9: Dibenzyl 2-Amino-6-((*tert*-butoxycarbonyl)amino)heptanedioate. Compound **7** (2 g, 3.5 mmol) was dissolved in 10 mL of HCl−dioxane and stirred for 2 h. The solvent was then removed under vacuum to obtain the hydrochloride salt, which was then dissolved in methanol. Triethylamine was added to the solution until pH ∼7 was obtained, followed by gradual addition of di-*tert*-butyl dicarbonate (764 mg, 3.5 mmol). The reaction mixture was stirred for 30 min, followed by evaporation of the solvent under reduced pressure to obtain the crude product, which was then purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **9** (640 mg, 39%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45−7.28 (m, 10H), 5.23−5.09 (m, 4H), 5.03 (t, J = 7.3 Hz, 1H), 4.41−4.23 (m, 1H), 3.41 (td, J = 8.1, 5.2 Hz, 1H), 1.85−1.50 (m, 6H), 1.49−1.34 (m, 11H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.17, 176.14, 173.01, 155.83, 136.10, 135.84, 129.25, 129.09, 129.06, 128.88, 128.78, 80.36, 67.46, 67.16, 67.14, 54.70, 54.68, 53.80, 53.74, 34.73, 34.66, 32.77, 32.74, 28.77, 21.93. MS (ESI) calculated for $C_{26}H_{34}N_2O_6$, m/z 470.24, found 471.24 $(M + H)^+$.

Synthesis of Compound 10: Diethyl 2-Amino-6-((*tert*-butoxycarbonyl)amino)heptanedioate. Compound **8** (1 g, 2.24 mmol) was dissolved in 5 mL of HCl−dioxane and stirred for 2.5 h. The solvent was then removed under vacuum to obtain the hydrochloride salt, which was then dissolved in methanol. Triethylamine was added to the solution until pH ∼7 was obtained, followed by gradual addition of di-*tert*-butyl dicarbonate (490 mg, 2.24 mmol). The reaction mixture was stirred for 30 min, followed by evaporation of the solvent under reduced pressure to obtain the crude product, which was then purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **10** (271 mg, 35%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (dd, J = 31.4, 7.9 Hz, 1H), 4.28−4.16 (m, 5H), 3.96 (bs, 1H), 2.04−1.88 (m, 2H), 1.88−1.76 (m, 1H), 1.73−1.62 (m, 1H), 1.62−1.43 (m, 2H), 1.42 (s, 9H), 1.32−1.24 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.13, 176.08, 172.94, 155.59, 80.00, 61.50, 61.08, 54.40, 53.50, 53.44, 34.56, 34.53, 32.65, 32.62, 28.50, 21.73, 21.67, 14.42, 14.37. MS (ESI) calculated for $C_{16}H_{30}N_2O_6$, m/z 346.21, found 347.21 $(M + H)^+$.

Synthesis of Compound 11: (5*R*)-Tribenzyl 1-(9*H*-Fluoren-9-yl)-18,18-dimethyl-3,8,16-trioxo-2,17-dioxa-4,9,15-triazanonadecane-5,10,14-tricarboxylate. To a solution of **4** (500 mg, 1.08 mmol) in anhydrous dichloromethane were added **9** (522 mg, 1.11 mmol), polystyrene bound carbodiimide (967 mg, 1.19 mmol), and a catalytic amount of polystyrene bound DMAP. The reaction mixture was stirred at room temperature for 4 h, followed by filtration to remove the solid resin. The filtrate was evaporated under vacuum to obtain the residue which was then purified using column chromatography (35% EtOAc/hexanes) to obtain compound **11** (748 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.3 Hz, 2H), 7.39−7.30 (m, 19H), 6.59−6.49 (m, 0.50H), 6.28 (s, 0.50H), 5.71 (dd, J = 25.1, 7.7 Hz, 1H), 5.24−5.04 (m, 7H), 4.56 (d, J = 6.6 Hz, 1H), 4.50−4.33 (m, 3H), 4.33−4.15 (m, 2H), 2.31−2.16 (m, 2H), 2.03−1.90 (m, 2H), 1.85−1.75 (m, 2H), 1.70−1.51 (m, 2H), 1.40−1.37 (m, 11H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.65, 172.57, 172.16, 172.09, 171.97, 171.82, 156.53, 155.78, 144.10, 143.85, 141.51, 141.48, 135.55, 135.36, 128.85, 128.83, 128.81, 128.73, 128.64, 128.60, 128.53, 128.45, 127.92, 127.30, 125.36, 120.18, 80.12, 67.59, 67.41, 67.33, 67.24, 53.63, 53.53, 53.33, 52.21, 47.37, 47.34, 32.32, 32.22, 31.73, 28.93, 28.51, 21.54, 21.43, 21.28, 21.13. MS (ESI) calculated for $C_{53}H_{57}N_3O_{11}$, m/z 911.40, found 934.39 $(M + Na)^+$.

Synthesis of Compound 12: (5*R*)-5-Allyl 10,14-Dibenzyl-1-(9*H*-fluoren-9-yl)-18,18-dimethyl-3,8,16-trioxo-2,17-dioxa-4,9,15-triazanonadecane-5,10,14-tricarboxylate. To a solution of **5** (500 mg, 1.22 mmol) in anhydrous dichloromethane were added **9** (585 mg, 1.24 mmol), polystyrene bound carbodiimide (1.09 g, 1.34 mmol), and a catalytic amount of polystyrene bound DMAP. The reaction mixture was stirred at room temperature for 4 h, followed by filtration to remove the solid resin. The filtrate was evaporated under reduced pressure to obtain the residue which was then purified using column chromatography (35% EtOAc/hexanes) to obtain compound **12** (830 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 7.5 Hz, 2H), 7.63 (d, J = 6.6 Hz, 2H), 7.46−7.30 (m, 14H), 6.69−6.30 (m, 1H), 5.91 (dd, J = 19.3, 12.8 Hz, 1H), 5.72 (dd, J = 20.3, 7.6 Hz, 1H), 5.35 (d, J = 17.2 Hz, 1H), 5.27 (d, J = 10.4 Hz, 1H), 5.22−5.04 (m, 5H), 4.70−4.56 (m, 3H), 4.52−4.36 (m, 3H), 4.35−4.19 (m, 2H), 2.38−2.20 (m, 3H), 1.98 (dd, J = 19.2, 8.3 Hz, 1H), 1.91−1.56 (m, 4H), 1.44 (s, 11H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.66, 172.56, 172.20, 172.12, 171.99, 171.85, 156.56, 155.83, 144.09, 143.86, 141.51, 135.54, 135.45, 131.59, 128.83, 128.71, 128.64, 128.51, 127.94, 127.31, 125.35, 120.19, 119.40, 119.36, 119.32, 80.13, 67.43, 67.33, 67.23, 66.42, 53.61, 53.53, 53.33, 52.24, 47.37, 32.41, 32.34, 31.84, 31.75, 31.61, 29.00, 28.51, 21.46, 21.27, 21.14. MS (ESI) calculated for $C_{49}H_{55}N_3O_{11}$, m/z 861.38, found 884.33 $(M + Na)^+$.

Synthesis of Compound 13: (5*R*)-5-Benzyl 10,14-Diethyl-1-(9*H*-fluoren-9-yl)-18,18-dimethyl-3,8,16-trioxo-2,17-dioxa-4,9,15-triazanonadecane-5,10,14-tricarboxylate. To a solution of **4** (195 mg, 0.425 mmol) in anhydrous dichloromethane were added **10** (150 mg, 0.43 mmol), polystyrene bound carbodiimide (367 mg, 0.467 mmol), and a catalytic amount of polystyrene bound DMAP. The reaction mixture was stirred at room temperature for 4 h, followed by filtration to remove the solid resin. The filtrate was evaporated under reduced pressure to obtain the residue which was then purified using column chromatography (30% EtOAc/hexanes) to obtain compound **13** (232 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79−7.69 (m, 2H), 7.59 (dd, J = 21.4, 11.5 Hz, 2H), 7.55−7.26 (m, 9H), 6.55 (dd, J = 27.8, 7.3 Hz, 1H), 6.31 (dd, J = 18.6, 7.1 Hz, 1H), 5.75 (dd, J = 27.6, 7.7 Hz, 1H), 5.27−5.05 (m, 3H), 4.61−4.33 (m, 4H), 4.28−4.04 (m, 6H), 2.31−2.20 (m, 2H), 2.04−1.93 (m, 1H), 1.89−1.52 (m, 5H), 1.46−1.36 (m, 9H), 1.34−1.21 (m, 7H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.50, 144.11, 143.88, 141.52, 135.38, 131.11, 129.02, 128.87, 128.76, 128.66, 128.59, 127.93, 127.31, 125.37, 120.19, 80.07, 68.37, 67.61, 67.29, 61.75, 61.57, 53.29, 52.13, 47.36, 38.92, 32.48, 32.37, 31.74, 30.56, 29.13, 28.53, 23.94, 23.20, 21.44, 14.37, 14.28, 11.12. MS (ESI) calculated for $C_{43}H_{53}N_3O_{11}$, m/z 787.37, found 810.37 $(M + Na)^+$.

Synthesis of Compound 14: Dibenzyl 2-((*R*)-4-Amino-5-(benzyloxy)-5-oxopentanamido)-6-((*tert*-butoxycarbonyl)amino)heptanedioate. Compound **11** (500 mg, 0.55 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of 3 mL of piperidine. The reaction mixture was stirred for 15 min, followed by removal of the solvent under reduced pressure. The crude was purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain

compound **14** (347 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46−7.31 (m, 15H), 6.57 (dt, J = 36.1, 8.8 Hz, 1H), 5.25−5.05 (m, 7H), 4.59 (d, J = 5.6 Hz, 1H), 4.28 (s, 1H), 3.52 (dd, J = 8.2, 5.1 Hz, 1H), 2.36 (qt, J = 21.1, 6.8 Hz, 2H), 2.13 (dd, J = 12.5, 6.0 Hz, 1H), 1.93−1.50 (m, 7H), 1.49−1.32 (m, 11H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.03, 172.79, 172.67, 136.10, 135.85, 129.15, 129.12, 129.01, 128.94, 128.85, 128.76, 67.53, 67.32, 54.38, 54.01, 53.70, 52.40, 33.12, 33.00, 32.17, 30.48, 30.31, 28.82, 21.78, 21.59. MS (ESI) calculated for $C_{38}H_{47}N_3O_9$, m/z 689.33, found 690.33 $(M + H)^+$.

Synthesis of Compound 15: (15*R*)-Tribenzyl 2,2-Dimethyl-4,12,17-trioxo-3-oxa-5,11,16-triazaoctacosane-6,10,15-tricarboxylate. To a solution of **14** (200 mg, 0.29 mmol) in pyridine were added lauroyl chloride (82.6 μL, 0.34 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 6 h, followed by evaporation of the solvent under reduced pressure to obtain the residue, which was then dissolved in dichloromethane and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (50% EtOAc/hexanes) to obtain compound **15** (209 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42−7.27 (m, 15H), 6.98 (dd, J = 16.0, 7.8 Hz, 0.50H), 6.57−6.46 (m, 1.50H), 5.22−5.07 (m, 7H), 4.76−4.70 (m, 0.50H), 4.61−4.51 (m, 1.50H), 4.31−4.20 (m, 1H), 2.28−2.16 (m, 5H), 1.98−1.57 (m, 11H), 1.50−1.36 (m, 6H), 1.32−1.20 (m, 17H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.68, 172.61, 172.46, 172.38, 172.33, 172.24, 172.20, 172.12, 172.04, 135.57, 135.45, 135.40, 128.85, 128.84, 128.82, 128.71, 128.65, 128.62, 128.58, 128.53, 128.48, 128.47, 80.10, 67.53, 67.23, 53.39, 52.29, 51.86, 36.83, 36.77, 32.53, 32.40, 32.12, 31.58, 29.84, 29.82, 29.70, 29.56, 29.51, 29.18, 28.54, 25.86, 25.79, 22.90, 14.34. MS (ESI) calculated for $C_{50}H_{69}N_3O_{10}$, m/z 871.50, found 894.50 $(M + Na)^+$.

Synthesis of Compound 16: 2-Amino-6-((*R*)-4-carboxy-4-dodecanamidobutanamido)heptanedioic Acid. Compound **15** (50 mg, 57.3 μmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2$/C. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 mL of trifluoroacetic acid. The mixture was stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt of compound **16** (35 mg, quantitative yield). ^{1}H NMR (500 MHz, MeOD) δ 4.46−4.30 (m, 2H), 3.78−3.68 (m, 1H), 2.35 (t, J = 5.6 Hz, 2H), 2.27−2.10 (m, 3H), 1.88 (ddd, J = 24.9, 20.5, 16.8 Hz, 4H), 1.75−1.66 (m, 1H), 1.65−1.47 (m, 4H), 1.36−1.21 (m, 16H), 0.87 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.64, 175.50, 175.02, 173.29, 55.13, 54.97, 53.42, 53.16, 37.09, 36.99, 33.25, 33.04, 32.52, 32.45, 32.38, 31.64, 31.53, 31.47, 30.92, 30.82, 30.65, 30.50, 28.82, 28.58, 28.30, 27.14, 27.11, 23.89, 23.06, 22.71, 22.63, 14.59. MS (ESI) calculated for $C_{24}H_{43}N_3O_8$, m/z 501.31, found 502.33 $(M + H)^+$.

Synthesis of Compound 17: (5*R*)-Tribenzyl 1-(9*H*-Fluoren-9-yl)-3,8,16-trioxo-2-oxa-4,9,15-triazaheptacosane-5,10,14-tricarboxylate. Compound **11** (100 mg, 0.10 mmol) was dissolved in 5 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 trifluoroacetate salt. To the solution of trifluoroacetate salt in anhydrous dichloromethane were added lauroyl chloride (39 μL, 0.16 mmol), triethylamine (30 μL, 0.22 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred for 2 h, followed by complete removal of the solvent under reduced pressure. The residue was then dissolved in dichloromethane and washed with water. The organic solvent was dried over sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (40% EtOAc/hexanes) to obtain compound **17** (103 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83−7.70 (m, 2H), 7.68−7.52 (m, 2H), 7.46−7.27 (m, 19H), 6.27−6.06 (m, 1H), 5.93−5.74 (m, 1H), 5.30−5.04 (m, 6H), 4.67−4.28 (m, 5H), 4.28−4.18 (m, 1H), 2.33−2.26 (m, 2H), 2.22−2.13 (m, 2H), 2.01 (dd, J = 15.1, 7.4 Hz, 1H), 1.92−1.79 (m, 2H), 1.79−1.66 (m, 3H), 1.64−1.56 (m, 2H), 1.48−1.17 (m, 19H), 0.90 (t, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.48, 172.36, 172.06, 172.02, 156.61, 143.74, 141.50, 135.39, 128.84, 128.70, 128.47, 127.92, 127.30, 125.35, 120.17, 67.55, 67.41, 67.31, 53.83, 51.87, 51.64, 47.35, 36.69, 32.19, 32.11, 31.62, 29.83, 29.69, 29.54, 29.46, 25.81, 22.88, 21.67, 14.32. MS (ESI) calculated for $C_{60}H_{71}N_3O_{10}$, m/z 993.51, found 1016.53 $(M + Na)^+$.

Synthesis of Compound 18: 2-((*R*)-4-Amino-4-carboxybutanamido)-6-dodecanamido)heptanedioic Acid. Compound **17** (70 mg, 0.07 mmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2$/C. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain the crude product which was purified using column chromatography (50% MeOH/CH_2Cl_2) to obtain compound **18** (32 mg, 92%). ^{1}H NMR (500 MHz, MeOD) δ 4.34 (s, 2H), 3.78−3.69 (m, 2H), 2.50 (t, J = 17.3 Hz, 2H), 2.23 (t, J = 7.4 Hz, 2H), 2.17−2.06 (m, 2H), 1.86−1.66 (m, 5H), 1.64−1.55 (m, 2H), 1.50−1.41 (d, J = 15.2 Hz, 2H), 1.39−1.19 (m, 14H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.56, 53.81, 36.98, 33.24, 32.21, 30.92, 30.82, 30.64, 30.47, 27.13, 25.50, 23.90, 23.51, 14.59. MS (ESI) calculated for $C_{24}H_{43}N_3O_8$, m/z 501.31, found 502.32 $(M + H)^+$.

Synthesis of Compound 19: Dibenzyl 2-Amino-6-((*R*)-5-(benzyloxy)-4-dodecanamido-5-oxopentanamido)heptanedioate. Compound **15** (200 mg, 0.23 mmol) was dissolved in 10 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 trifluoroacetate salt of compound **19** (203 mg, quantitative yield). MS (ESI) calculated for $C_{45}H_{61}N_3O_8$, m/z 771.44, found 772.48 $(M + H)^+$.

Synthesis of Compound 20: 2-((*R*)-4-Carboxy-4-dodecanamidobutanamido)-6-dodecanamidoheptanedioic Acid. To a solution of **19** (50 mg, 56.4 μmol) in pyridine were added lauroyl chloride (16.1 μL, 67.7 μmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 6 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in dichloromethane and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product. The crude was purified using column chromatography (35% EtOAc/hexanes) to obtain the pure product, which was dissolved in methanol and subjected to catalytic hydrogenolysis using $Pd(OH)_2$/C at 60 psi hydrogen pressure for 2 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain compound **20** (34.3 mg, 89%). ^{1}H NMR (500 MHz, MeOD) δ 4.45−4.25 (m, 3H), 2.34 (t, J = 6.7 Hz, 2H), 2.28−2.07 (m, 5H), 1.98−1.79 (m, 3H), 1.70 (dd, J = 19.9, 14.8 Hz, 2H), 1.64−1.55 (m, 4H), 1.49−1.39 (m, 3H), 1.37−1.15 (m, 31H), 0.87 (t, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 176.64, 175.98, 175.72, 175.21, 53.63, 36.99, 33.35, 33.25, 32.27, 30.92, 30.83, 30.65, 30.49, 28.88, 28.73, 27.10, 23.89, 23.54, 14.59. MS (ESI) calculated for $C_{36}H_{65}N_3O_9$, m/z 683.47, found 682.49 $(M - H)^-$.

Synthesis of Compound 21: (15*R*)-Tribenzyl 2,2-Dimethyl-4,12-dioxo-3-oxa-5,11,16-triazaoctacosane-6,10,15-tricarboxylate. To a solution of **14** (190 mg, 0.28 mmol) in anhydrous dichloromethane (10 mL) and methanol (2 mL) were added dodecyl aldehyde (51.5 mg, 0.28 mmol), 3−4 drops of acetic acid, and macroporous resin bound sodium cyanoborohydride (150 mg, 0.33 mmol). The solution was stirred for 8 h, followed by filtration to remove the resin. The solvent was removed under vacuum to obtain the residue, which was purified using column chromatography (15% EtOAc/hexanes)

to obtain compound **21** (124 mg, 51%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43−7.27 (m, 15H), 5.25−5.09 (m, 6H), 4.50 (dd, J = 12.7, 7.7 Hz, 1H), 4.35−4.16 (m, 1H), 2.73−2.64 (m, 1H), 2.62−2.13 (m, 4H), 2.02 (t, J = 20.0 Hz, 1H), 1.91−1.72 (m, 3H), 1.71−1.50 (m, 4H), 1.41 (d, J = 12.6 Hz, 7H), 1.38−1.17 (m, 25H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.53, 172.11, 155.94, 155.65, 155.52, 135.36, 134.56, 130.91, 128.62, 128.43, 128.29, 79.94, 68.16, 67.97, 67.06, 60.40, 59.91, 53.22, 52.10, 47.94, 32.38, 32.20, 32.04, 31.95, 31.43, 30.99, 30.37, 29.64, 29.58, 29.36, 29.09, 28.92, 28.66, 28.34, 28.12, 27.43, 27.00, 23.76, 23.00, 22.72, 21.45, 21.24, 14.16. MS (ESI) calculated for $C_{50}H_{71}N_3O_9$, m/z 857.52, found 858.53 $(M + H)^+$.

Synthesis of Compound 22: 2-Amino-6-((*R*)-4-carboxy-4-(dodecylamino)butanamido)heptanedioic Acid. Compound **21** (50 mg, 58.3 μmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of the compound **22** (35 mg, quantitative yield in two steps). ^{1}H NMR (500 MHz, MeOD) δ 4.40 (d, J = 3.5 Hz, 1H), 3.88−3.56 (m, 3H), 3.21−3.07 (m, 1H), 3.06−2.94 (m, 2H), 2.53 (t, J = 6.7 Hz, 2H), 2.14 (d, J = 6.0 Hz, 2H), 1.91 (s, 3H), 1.79−1.43 (m, 6H), 1.43−1.20 (m, 15H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 175.33, 62.44, 62.15, 54.58, 53.37, 46.97, 46.32, 45.72, 45.40, 44.90, 33.10, 32.83, 32.34, 31.97, 31.13, 30.78, 30.68, 30.52, 30.24, 27.61, 27.46, 26.59, 26.28, 23.75, 23.09, 22.55, 22.34, 14.44. MS (ESI) calculated for $C_{24}H_{45}N_3O_7$, m/z 487.33, found 488.34 $(M + H)^+$.

Synthesis of Compound 23: (15*R*)-Tribenzyl 2,2-Dimethyl-16-octyl-4,12-dioxo-3-oxa-5,11,16-triazatetracosane-6,10,15-tricarboxylate. To a solution of **14** (100 mg, 0.15 mmol) in anhydrous dichloromethane (10 mL) and methanol (5 mL) were added octylaldehyde (56.5 μL, 0.36 mmol), 3−4 drops of acetic acid, and macroporous resin bound sodium cyanoborohydride (163.5 mg, 0.36 mmol). The reaction mixture was then stirred for 10 h, followed by filtration to remove the resin. The solvent was removed under vacuum to obtain compound **23** as a crude. MS (ESI) calculated for $C_{54}H_{79}N_3O_9$, m/z 913.58, found 914.60 $(M + H)^+$.

Synthesis of Compound 24: (15*R*)-Tribenzyl 16-Dodecyl-2,2-dimethyl-4,12-dioxo-3-oxa-5,11,16-triazaoctacosane-6,10,15-tricarboxylate. To the solution of **14** (100 mg, 0.15 mmol) in anhydrous dichloromethane (10 mL) and methanol (5 mL) were added dodecylaldehyde (66.8 mg, 0.36 mmol), 3−4 drops of acetic acid, and macroporous resin bound sodium cyanoborohydride (163.5 mg, 0.36 mmol). The solution was then stirred for 14 h, followed by filtration to remove the resin. The solvent was removed under vacuum to obtain the residue, which was purified using column chromatography (15% EtOAc/hexanes) to obtain compound **24** (105 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45−7.28 (m, 15H), 5.28 (dd, J = 12.0, 3.7 Hz, 1H), 5.23−5.01 (m, 6H), 4.44 (bs, 1H), 4.34−4.11 (m, 2H), 3.22−2.85 (m, 4H), 2.58−2.24 (m, 4H), 1.85−1.74 (m, 2H), 1.72−1.53 (m, 6H), 1.48−1.34 (m, 11H), 1.34−1.12 (m, 37H), 0.97−0.82 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.69, 172.23, 171.94, 171.40, 162.45, 135.64, 135.61, 134.64, 131.10, 129.21, 129.16, 129.03, 129.01, 128.92, 128.89, 128.82, 128.78, 128.62, 128.45, 128.38, 128.35, 80.10, 68.36, 68.11, 67.20, 63.20, 62.48, 53.51, 53.40, 52.72, 52.55, 38.92, 32.71, 32.12, 30.55, 29.82, 29.69, 29.63, 29.61, 29.54, 29.22, 29.20, 29.13, 28.51, 27.11, 24.94, 23.93, 23.20, 22.90, 21.88, 21.71, 14.34. MS (ESI) calculated for $C_{62}H_{95}N_3O_9$, m/z 1025.71, found 1026.73 $(M + H)^+$.

Synthesis of Compound 25: (15*R*)-Tribenzyl 16-Hexadecyl-2,2-dimethyl-4,12-dioxo-3-oxa-5,11,16-triazadotriacontane-6,10,15-tricarboxylate. To a solution of **14** (100 mg, 0.15 mmol) in anhydrous dichloromethane (10 mL) and methanol (5 mL) were added hexadecanal (86.4 mg, 0.36 mmol), 3−4 drops of acetic acid, and macroporous resin bound sodium cyanoborohydride (163.5 mg, 0.36 mmol). The solution was then stirred for 14 h, followed by filtration to remove the resin. The solvent was removed under vacuum to obtain the residue, which was purified using column chromatography (12% EtOAc/hexanes) to obtain compound **25** (112 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45−7.28 (m, 15H), 5.31−4.96 (m, 7H), 4.48 (d, J = 5.2 Hz, 1H), 4.26−4.18 (m, 1H), 3.24−2.06 (m, 9H), 1.77−1.55 (m, 8H), 1.46−1.33 (m, 11H), 1.33−1.07 (m, 53H), 0.88 (t, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.62, 172.24, 172.11, 171.63, 155.80, 155.69, 135.57, 131.10, 128.93, 128.83, 128.64, 128.57, 128.47, 128.41, 80.10, 68.36, 67.24, 67.18, 63.06, 62.51, 53.47, 53.37, 52.40, 52.14, 38.92, 32.82, 32.32, 32.14, 30.55, 29.92, 29.87, 29.86, 29.76, 29.74, 29.70, 29.58, 29.42, 29.36, 29.12, 28.51, 27.25, 25.07, 23.93, 23.19, 22.90, 21.78, 21.65, 14.34, 14.27. MS (ESI) calculated for $C_{70}H_{111}N_3O_9$, m/z 1137.83, found 1138.85 $(M + H)^+$.

Synthesis of Compound 26: 2-Amino-6-((*R*)-4-carboxy-4-(dioctylamino)butanamido)heptanedioic Acid. The crude of compound **23** was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 **26** (60 mg, 64%). ^{1}H NMR (500 MHz, 10% $CDCl_3$ in MeOD) δ 4.34 (dd, J = 9.2, 4.6 Hz, 1H), 3.91−3.79 (m, 2H), 3.21−3.00 (m, 4H), 2.51 (ddd, J = 29.4, 15.8, 6.3 Hz, 2H), 2.20−2.09 (m, 1H), 2.08−1.95 (m, 1H), 1.95−1.78 (m, 3H), 1.78−1.39 (m, 8H), 1.34−1.16 (m, 19H), 0.82 (t, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, 10% $CDCl_3$ in MeOD) δ 175.14, 174.73, 174.41, 171.93, 53.92, 53.35, 33.09, 32.93, 32.48, 31.97, 31.09, 30.27, 30.21, 30.19, 27.71, 25.66, 23.75, 23.42, 22.53, 14.55. MS (ESI) calculated for $C_{28}H_{53}N_3O_7$, m/z 543.39, found 544.41 $(M + H)^+$.

Synthesis of Compound 27: 2-Amino-6-((*R*)-4-carboxy-4-(didodecylamino)butanamido)heptanedioic Acid. Compound **24** (50 mg, 0.048 mmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of compound **27** (37 mg, quantitative yield). ^{1}H NMR (500 MHz, MeOD) δ 4.43−4.37 (m, 1H), 3.87 (dd, J = 29.3, 7.5 Hz, 2H), 3.20 (dd, J = 19.5, 10.2 Hz, 2H), 3.15−3.05 (m, 2H), 2.73−2.46 (m, 3H), 2.27−1.99 (m, 3H), 1.98−1.83 (m, 3H), 1.72 (s, 5H), 1.63−1.43 (m, 3H), 1.31 (d, J = 38.3 Hz, 33H), 0.88 (t, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 33.24, 30.92, 30.80, 30.69, 30.64, 30.34, 27.78, 23.90, 14.60. MS (ESI) calculated for $C_{36}H_{69}N_3O_7$, m/z 655.51, found 656.51 $(M + H)^+$.

Synthesis of Compound 28: 2-Amino-6-((*R*)-4-carboxy-4-(dihexadecylamino)butanamido)heptanedioic Acid. Compound **25** (50 mg, 0.043 mmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of the compound **28** (38 mg, quantitative yield). ^{1}H NMR (500 MHz, 20% $CDCl_3$ in MeOD) δ 4.48−4.20 (m, 2H), 3.86−3.73 (m, 3H), 3.29−3.04 (m, 4H), 2.72−2.44 (m, 3H), 2.31−1.80 (m, 7H),

1.80−1.48 (m, 8H), 1.46−1.12 (m, 46H), 0.89 (t, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, 20% $CDCl_3$ in MeOD) δ 31.40, 29.14, 29.11, 29.09, 29.04, 28.97, 28.94, 28.88, 28.82, 28.53, 26.03, 22.10, 13.07. MS (ESI) calculated for $C_{44}H_{85}N_3O_7$, m/z 767.64, found 768.65 $(M + H)^+$.

Synthesis of Compound 30: (2*R*)-5-((4-(2-Amino-4-oxo-4,5-dihydro-1*H*-imidazol-5-yl)-1-carboxybutyl)amino)-2-dodecanamido-5-oxopentanoic Acid. To a solution of **19** (100 mg, 0.13 mmol) in pyridine was added 1*H*-pyrazole-1-carboxamidine·HCl (55.2 mg, 0.37 mmol). The reaction mixture was heated in microwave at 65 °C for 1 h. The solvent was removed under vacuum, and the residue was dissolved in methanol, followed by the addition of $Pd(OH)_2$/C. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain the crude product which was purified using column chromatography (30% MeOH/CH_2Cl_2) to obtain compound **30** (38 mg, 58%). ^{1}H NMR (500 MHz, MeOD) δ 4.40−4.27 (m, 2H), 4.21−4.18 (m, 1H), 2.34 (t, J = 5.9 Hz, 2H), 2.24 (dd, J = 13.3, 6.9 Hz, 2H), 2.20−1.95 (m, 2H), 1.95−1.65 (m, 5H), 1.64−1.54 (m, 2H), 1.54−1.43 (m, 2H), 1.37−1.21 (m, 15H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.67, 176.56, 176.20, 175.20, 61.16, 54.19, 54.01, 53.67, 37.11, 37.03, 33.35, 33.25, 32.54, 32.46, 31.92, 31.80, 30.92, 30.81, 30.65, 30.51, 28.87, 27.09, 23.90, 22.56, 22.33, 22.19, 14.60. MS (ESI) calculated for $C_{25}H_{43}N_5O_7$, m/z 525.32, found 524.32 $(M - H)^-$.

Synthesis of Compound 32: 2-((*R*)-4-Carboxy-4-dodecanamidobutanamido)-6-guanidinoheptanedioic Acid. To a solution of **19** (50 mg, 0.06 mmol) in THF were added *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine·HCl (57.4 mg, 0.19 mmol) and pyridine (1 mL). The reaction mixture was then stirred at 50 °C for 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in methanol, followed by the addition of $Pd(OH)_2$/C. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 1 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain the crude, which was purified using column chromatography (8% MeOH/CH_2Cl_2). The product was dissolved in 5 mL of trifluoroacetic acid, and the reaction mixture was stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt of compound **32** (30 mg, 63%). ^{1}H NMR (500 MHz, MeOD) δ 4.34 (s, 2H), 4.00 (s, 1H), 2.38−2.28 (m, 2H), 2.24 (dd, J = 14.4, 7.0 Hz, 2H), 2.18−2.08 (m, 1H), 2.07−1.80 (m, 4H), 1.79−1.65 (m, 2H), 1.61−1.55 (m, 2H), 1.55−1.36 (m, 3H), 1.36−1.19 (m, 14H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, 5% $CDCl_3$ in MeOD) δ 176.46, 175.71, 175.05, 158.34, 57.03, 54.25, 37.11, 33.35, 33.20, 33.11, 32.83, 32.64, 30.88, 30.78, 30.62, 30.51, 29.28, 27.06, 23.86, 22.90, 22.58, 14.59. MS (ESI) calculated for $C_{25}H_{45}N_5O_8$, m/z 543.33, found 544.35 $(M + H)^+$.

Synthesis of Compound 33: (6*R*)-6-Allyl 11,15-Dibenzyl 2,2,19,19-tetramethyl-4,9,17-trioxo-3,18-dioxa-5,10,16-triazaicosane-6,11,15-tricarboxylate. Compound **12** (250 mg, 0.29 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of 3 mL of piperidine. The reaction mixture was stirred for 15 min, followed by removal of the solvent under reduced pressure. The residue was dissolved in dichloromethane, followed by the addition of di-*tert*-butyl dicarbonate (95 mg, 0.43 mmol) and triethylamine (80 μL, 0.58 mmol). The reaction mixture was stirred at room temperature for 1 h, followed by removal of the solvent under reduced pressure. The crude was purified using column chromatography (30% EtOAc/hexanes) to obtain compound **33** (147 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41−7.29 (m, 10H), 5.97−5.82 (m, 1H), 5.30 (ddd, J = 37.2, 23.6, 5.8 Hz, 3H), 5.21−5.06 (m, 5H), 4.68−4.51 (m, 3H), 4.38−4.20 (m, 2H), 2.31 (dd, J = 14.5, 7.3 Hz, 2H), 2.25−2.13 (m, 1H), 1.99−1.54 (m, 6H), 1.43 (s, J = 2.9 Hz, 20H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.67, 172.58, 172.32, 172.18, 171.85, 156.06, 155.83, 135.55, 135.53, 135.47, 132.32, 132.24, 131.68, 128.83, 128.81, 128.69, 128.64, 128.61, 128.51, 128.46, 119.23, 119.13, 80.34, 80.17, 80.08, 77.48, 77.23, 76.98, 67.37, 67.27, 67.22, 67.18, 66.26, 53.34, 53.16, 53.04, 52.25, 52.18, 32.47, 32.24, 31.85, 31.76, 31.64, 29.88, 29.26, 29.01, 28.86, 28.50. MS (ESI) calculated for $C_{39}H_{53}N_3O_{11}$, m/z 739.37, found 762.37 $(M + Na)^+$.

Synthesis of Compound 34: (6*R*)-11,15-Dibenzyl 6-Undecyl 2,2,19,19-tetramethyl-4,9,17-trioxo-3,18-dioxa-5,10,16-triazaicosane-6,11,15-tricarboxylate. To a solution of compound **33** (80 mg, 0.10 mmol) in ethanol/water (9:1) was added Wilkinson's catalyst (10 mg, 0.01 mmol). The reaction mixture was refluxed for 3 h at 90 °C, followed by the removal of the solvent under reduced pressure. The crude was purified using column chromatography (5% MeOH/CH_2Cl_2) and dried under vacuum. To the solution of the afforded product (52 mg, 0.07 mmol) in anhydrous DMF were added 1-undecanol (30 μL, 0.15 mmol), HBTU (33.7 mg, 0.09 mmol), triethylamine (20 μL, 0.15 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 14 h, followed by removal of the solvent under reduced pressure. The residue was dissolved in ethyl acetate and washed with water. The organic fraction was dried over anhydrous sodium sulfate, filtered, concentrated, and purified using column chromatography (35% EtOAc/hexanes) to obtain compound **34** (44.4 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40−7.30 (m, 10H), 5.27 (dd, J = 22.5, 8.4 Hz, 1H), 5.20−4.96 (m, 5H), 4.65−4.49 (m, 1H), 4.41−4.20 (m, 2H), 4.16−4.07 (m, 2H), 2.30 (t, J = 6.8 Hz, 2H), 2.24−2.13 (m, 1H), 1.93−1.72 (m, 3H), 1.67−1.59 (m, 3H), 1.43 (d, J = 2.7 Hz, 20H), 1.35−1.18 (m, 18H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.93, 170.59, 170.19, 154.13, 133.94, 127.20, 127.03, 126.85, 115.67, 78.69, 78.48, 65.63, 64.37, 51.77, 51.70, 51.47, 51.42, 50.64, 50.59, 30.87, 30.48, 30.17, 28.18, 28.07, 27.91, 27.82, 27.09, 26.90, 24.36, 21.27, 12.95. MS (ESI) calculated for $C_{47}H_{71}N_3O_{11}$, m/z 853.51, found 876.47 $(M + Na)^+$.

Synthesis of Compound 35: 2-Amino-6-((*R*)-4-amino-5-oxo-5-(undecyloxy)pentanamido)heptanedioic Acid. Compound **34** (40 mg, 46.9 μmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2$/C. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of compound **35** (32.8 mg, quantitative yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.40 (dd, J = 9.2, 4.8 Hz, 1H), 4.32−4.22 (m, 2H), 4.11 (dd, J = 11.8, 6.0 Hz, 1H), 3.82−3.74 (m, 1H), 2.53 (t, J = 6.9 Hz, 2H), 2.23 (dt, J = 13.3, 6.7 Hz, 1H), 2.18−2.09 (m, 1H), 1.90 (ddd, J = 21.0, 13.9, 5.3 Hz, 3H), 1.80−1.67 (m, 3H), 1.64−1.46 (m, 2H), 1.43−1.22 (m, 16H), 0.91 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.90, 172.93, 70.30, 57.13, 56.15, 56.04, 55.92, 55.86, 35.59, 34.66, 34.59, 33.84, 33.76, 33.26, 33.17, 32.99, 32.88, 32.08, 29.78, 29.71, 29.39, 26.26, 25.15, 24.95, 16.95. MS (ESI) calculated for $C_{23}H_{43}N_3O_7$, m/z 473.31, found 474.31 $(M + H)^+$.

Synthesis of Compound 36: Dibenzyl 2-((*tert*-Butoxycarbonyl)amino)-6-((*R*)-4-((*tert*-butoxycarbonyl)amino)-5-oxo-5-(undecylamino)pentanamido)heptanedioate. To a solution of **33** (240 mg, 0.32 mmol) in ethanol/water (9:1) was added Wilkinson's catalyst (30 mg, 0.03 mmol). The reaction mixture was refluxed for 3 h at 90 °C, followed by removal of the solvent under reduced pressure. The crude was purified using column chromatography (5% MeOH/CH_2Cl_2). To the solution of afforded product (193 mg, 0.27 mmol) in anhydrous dichloromethane were added undecylamine (118 μL, 0.55 mmol), HBTU (157 mg, 0.41 mmol), triethylamine (77 μL, 0.55 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 14 h, followed by removal of the solvent under reduced pressure. The residue was dissolved in ethyl

acetate and washed with water. The organic fraction was dried over anhydrous sodium sulfate, filtered, concentrated, and purified using column chromatography (25% EtOAc/hexanes) to obtain compound **36** (188 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44−7.27 (m, 10H), 6.65−6.49 (m, 1H), 5.63 (d, J = 8.1 Hz, 1H), 5.62−5.52 (m, 1H), 5.23−4.92 (m, 5H), 4.60−4.45 (m, 2H), 4.27 (d, J = 8.2 Hz, 1H), 4.09 (d, J = 6.7 Hz, 1H), 3.31−3.14 (m, 2H), 2.38−2.22 (m, 1H), 2.00−1.53 (m, 5H), 1.49−1.32 (m, 23H), 1.31−1.21 (m, 16H), 0.90−0.84 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.07, 173.52, 172.99, 172.68, 171.76, 171.40, 157.18, 156.56, 135.51, 135.42, 128.88, 128.81, 128.74, 128.66, 128.59, 128.50, 128.38, 80.44, 80.17, 67.43, 67.28, 67.19, 53.47, 53.21, 52.65, 52.28, 39.89, 39.73, 32.98, 32.75, 32.47, 32.11, 31.96, 31.51, 30.67, 29.82, 29.76, 29.54, 28.53, 27.12, 22.89, 22.03, 21.87, 21.48, 21.35, 14.34. MS (ESI) calculated for $C_{47}H_{72}N_4O_{10}$, m/z 852.52, found 875.53 $(M + Na)^+$.

Synthesis of Compound 37: 2-Amino-6-((*R*)-4-amino-5-oxo-5-(undecylamino)pentanamido)heptanedioic Acid. Compound **36** (40 mg, 46.9 μmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of compound **37** (32.8 mg, quantitative yield). ^{1}H NMR (500 MHz, MeOD) δ 4.47−4.32 (m, 1H), 3.92−3.79 (m, 1H), 3.64−3.54 (m, 1H), 3.28−3.13 (m, 2H), 2.56−2.34 (m, 2H), 2.19−2.01 (m, 2H), 2.00−1.60 (m, 4H), 1.58−1.41 (m, 4H), 1.30 (d, J = 21.4 Hz, 16H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 175.94, 175.78, 174.26, 174.03, 169.72, 55.49, 53.98, 53.77, 53.47, 40.75, 33.08, 32.29, 31.95, 31.82, 31.51, 31.31, 30.75, 30.46, 30.32, 28.74, 28.49, 28.04, 23.74, 22.62, 22.07, 14.44. MS (ESI) calculated for $C_{23}H_{44}N_4O_6$, m/z 472.33, found 473.33 $(M + H)^+$.

Synthesis of Compound 38: (5*R*)-10,14-Dibenzyl 5-Undecyl 1-(9*H*-fluoren-9-yl)-18,18-dimethyl-3,8,16-trioxo-2,17-dioxa-4,9,15-triazanonadecane-5,10,14-tricarboxylate. To a solution of **12** (100 mg, 0.11 mmol) in ethanol/water (9:1) was added Wilkinson's catalyst (10 mg, 0.01 mmol). The reaction mixture was refluxed for 3 h at 90 °C, followed by removal of the solvent under reduced pressure. The crude was purified using column chromatography (5% $MeOH/CH_2Cl_2$) and dried under vacuum. To the solution of the afforded product (70 mg, 85.2 μmol) in anhydrous DMF were added 1-undecanol (35 μL, 170 μmol), HBTU (38.8 mg, 102.2 μmol), and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 14 h, followed by removal of the solvent under reduced pressure. The crude was dissolved in ethyl acetate and washed with water. The organic fraction was dried over anhydrous sodium sulfate, filtered, concentrated, and purified using column chromatography (15% EtOAc/hexanes) to obtain the compound **38** (63 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 6.4 Hz, 2H), 7.50−7.26 (m, 14H), 6.64 (s, 0.50H), 6.37 (s, 0.50H), 5.67 (dd, J = 32.4, 7.9 Hz, 1H), 5.21−4.99 (m, 5H), 4.62−4.53 (m, 1H), 4.47−4.31 (m, 3H), 4.30−4.19 (m, 2H), 4.13 (td, J = 6.5, 2.6 Hz, 2H), 2.34−2.16 (m, 3H), 1.99−1.89 (m, 1H), 1.87−1.74 (m, 2H), 1.72−1.56 (m, 6H), 1.39 (d, J = 20.0 Hz, 10H), 1.33−1.20 (m, 15H), 0.90 (dt, J = 13.9, 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.77, 172.70, 172.64, 144.54, 144.29, 141.96, 135.98, 129.25, 129.14, 129.06, 128.95, 128.88, 128.36, 127.73, 125.79, 120.62, 80.57, 67.85, 67.75, 67.67, 66.58, 53.76, 52.66, 47.80, 32.91, 32.54, 32.19, 30.23, 30.13, 29.96, 29.85, 29.59, 29.13, 28.94, 26.41, 23.32, 21.58, 14.77. MS (ESI) calculated for $C_{57}H_{73}N_3O_{11}$, m/z 975.52, found 998.46 $(M + Na)^+$.

Synthesis of Compound 39: (5*R*)-10,14-Dibenzyl 5-Ethyl-1-(9*H*-fluoren-9-yl)-18,18-dimethyl-3,8,16-trioxo-2,17-dioxa-4,9,15-triazanonadecane-5,10,14-tricarboxylate. To a solution of **12** (100 mg, 0.11 mmol) in ethanol/water (9:1) was added Wilkinson's catalyst (10 mg, 0.01 mmol). The reaction mixture was refluxed for 3 h at 90 °C, followed by removal of the solvent under reduced pressure. The crude was purified using column chromatography (5% $MeOH/CH_2Cl_2$) and dried under vacuum. To the solution of the afforded product (75 mg, 91.3 μmol) in anhydrous DMF were added ethyl alcohol (11 μL, 182 μmol), HBTU (41.6 mg, 109.5 μmol), and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 14 h, followed by removal of the solvent under reduced pressure. The residue was dissolved in ethyl acetate and washed with water. The organic fraction was dried over anhydrous sodium sulfate, filtered, concentrated, and purified using column chromatography (15% EtOAc/hexanes) to obtain compound **39** (58.9 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79−7.69 (m, 2H), 7.57−7.52 (m, 2H), 7.57−7.26 (m, 14H), 6.61 (d, J = 6.9 Hz, 0.50H), 6.37 (t, J = 6.9 Hz, 0.50H), 5.65 (dd, J = 29.3, 7.3 Hz, 1H), 5.23−5.00 (m, 5H), 4.64−4.54 (m, 1H), 4.45−4.37 (m, 2H), 4.29−4.15 (m, 4H), 2.36−2.17 (m, 3H), 1.98−1.88 (m, 1H), 1.87−1.74 (m, 2H), 1.73−1.62 (m, 2H), 1.48−1.33 (m, 10H), 1.33−1.23 (m, 5H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.67, 172.18, 171.99, 156.58, 155.85, 144.11, 143.88, 141.52, 135.55, 135.46, 131.10, 129.00, 128.83, 128.71, 128.64, 128.53, 127.95, 127.31, 125.36, 120.20, 80.12, 68.37, 67.43, 67.24, 61.98, 53.55, 53.35, 52.22, 47.39, 38.92, 32.45, 31.79, 30.56, 29.12, 28.82, 28.52, 23.94, 23.20, 21.47, 21.29, 21.14, 14.36, 14.29, 11.17. MS (ESI) calculated for $C_{48}H_{55}N_3O_{11}$, m/z 849.38, found 872.37 $(M + Na)^+$.

Synthesis of Compound 40: (15*R*)-10,6-Dibenzyl 15-Undecyl-2,2-dimethyl-4,12,17-trioxo-3-oxa-5,11,16-triazaoctacosane-6,10,15-tricarboxylate. Compound **38** (50 mg, 0.05 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of 3 mL of piperidine. The reaction mixture was stirred for 15 min, followed by removal of the solvent under reduced pressure. The residue (0.05 mmol) was dissolved in pyridine, followed by the addition of lauroyl chloride (14.6 μL, 0.06 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 4 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in dichloromethane and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (18% EtOAc/hexanes) to obtain compound **40** (36.4 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74−7.29 (m, 10H), 7.18−7.06 (m, 1H), 6.59−6.41 (m, 2H), 5.31−5.08 (m, 5H), 4.73−4.63 (m, 1H), 4.54 (ddd, J = 12.1, 10.0, 5.6 Hz, 2H), 4.30−4.20 (m, 1H), 4.15−4.07 (m, 2H), 2.38−2.14 (m, 5H), 1.97−1.54 (m, 17H), 1.41 (d, J = 19.6 Hz, 7H), 1.34−1.19 (m, 26H), 0.98−0.81 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.71, 172.50, 172.32, 172.18, 135.62, 128.86, 128.82, 128.65, 128.52, 128.46, 80.11, 67.31, 67.24, 66.15, 53.36, 52.27, 51.86, 36.87, 32.12, 29.82, 29.72, 29.56, 29.52, 29.44, 28.70, 28.55, 25.99, 25.90, 22.90, 14.34. MS (ESI) calculated for $C_{54}H_{85}N_3O_{10}$, m/z 935.62, found 958.59 $(M + Na)^+$.

Synthesis of Compound 41: 2-Amino-6-((*R*)-4-dodecanamido-5-oxo-5-(undecyloxy)pentanamido)heptanedioic Acid. Compound **40** (30 mg, 32.0 μmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of compound **41** (24.6 mg, quantitative yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.51−4.26 (m, 2H), 4.21 (ddd, J = 19.6, 10.9, 5.9 Hz, 1H), 4.15−3.95 (m, 2H), 2.51−2.13 (m, 4H), 2.12−1.48 (m, 21H), 1.48−1.38 (m, 2H), 1.37−1.05 (m, 21H), 0.96−0.80 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.30, 68.63, 50.65, 50.48, 50.31, 50.14, 49.97, 39.16,

32.38, 30.79, 30.15, 29.86, 29.83, 29.37, 26.21, 24.17, 23.44, 23.14, 14.53, 14.51, 11.41. MS (ESI) calculated for $C_{35}H_{65}N_3O_8$, m/z 655.48, found 654.51 $(M - H^+)^-$.

Synthesis of Compound 43: 2-Amino-6-((*R*)-4-guanidino-5-oxo-5-(undecyloxy)pentanamido)heptanedioic Acid. Compound **38** (50 mg, 0.05 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of 3 mL of piperidine. The reaction mixture was stirred for 15 min, followed by removal of the solvent under reduced pressure. The crude mixture was purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain the free primary amine derivative, which was dissolved in pyridine, followed by the addition of 1*H*-pyrazole-1-carboxamidine·HCl (22.2 mg, 0.15 mmol). The sealed reaction mixture was then heated in microwave at 65 °C for 1 h. The solvent was removed under vacuum, and the residue was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 4 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain the crude product which was purified using column chromatography (30% MeOH/CH_2Cl_2) to obtain the free carboxylic acid derivative, which was dissolved in 5 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 trifluoroacetate salt of compound **43** (20.3 mg, 47% in four steps). ^{1}H NMR (500 MHz, MeOD) δ 4.43−4.08 (m, 5H), 3.58 (bs, 1H), 2.46−2.27 (m, 3H), 2.12−1.90 (m, 2H), 1.84 (bs, 3H), 1.66 (dd, J = 13.7, 6.9 Hz, 2H), 1.53−1.41 (m, 2H), 1.40−1.19 (m, 15H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 67.16, 33.09, 30.76, 30.68, 30.49, 30.39, 29.66, 26.97, 23.75, 14.46. MS (ESI) calculated for $C_{24}H_{45}N_5O_7$, m/z 515.33, found 516.31 $(M + H)^+$.

Synthesis of Compound 44: (15*R*)-10,6-Dibenzyl 15-Ethyl-2,2-dimethyl-4,12,17-trioxo-3-oxa-5,11,16-triazaoctacosane-6,10,15-tricarboxylate. Compound **39** (100 mg, 0.11 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of 3 mL of piperidine. The reaction mixture was stirred for 15 min, followed by removal of the solvent under reduced pressure to obtain the residue which was dissolved in pyridine, followed by the addition of lauroyl chloride (41.9 μL, 0.18 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 4 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in dichloromethane and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (35% EtOAc/hexanes) to obtain compound **44** (65.4 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73−7.27 (m, 10H), 7.06 (dd, J = 17.7, 8.8 Hz, 1H), 6.65−6.53 (m, 1H), 6.47−6.36 (m, 1H), 5.20−5.05 (m, 4H), 4.53−4.50 (m, 3H), 4.33−4.09 (m, 4H), 2.35−2.11 (m, 5H), 1.93−1.69 (m, 4H), 1.68−1.65 (m, 3H), 1.47−1.33 (m, 9H), 1.32−1.17 (m, 18H), 0.92−0.83 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.42, 172.26, 135.58, 131.11, 129.02, 128.82, 128.65, 128.52, 128.47, 80.10, 68.37, 67.24, 61.95, 53.43, 52.32, 51.83, 38.93, 36.85, 32.12, 31.64, 30.56, 29.83, 29.71, 29.56, 29.51, 29.13, 28.55, 25.89, 23.94, 23.20, 22.91, 14.34, 14.28, 11.18. MS (ESI) calculated for $C_{45}H_{67}N_3O_{10}$, m/z 809.48, found 832.51 $(M + Na)^+$.

Synthesis of Compound 45: 2-Amino-6-((*R*)-4-dodecanamido-5-ethoxy-5-oxopentanamido)heptanedioic Acid. Compound **44** (30 mg, 37.0 μmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of compound **45** (23.4 mg, quantitative yield). ^{1}H NMR (500 MHz, MeOD) δ 4.35−4.26 (m, 2H), 4.08 (qd, J = 7.1, 3.1 Hz, 2H), 3.73 (d, J = 11.0 Hz, 1H), 2.32−2.24 (m, 2H), 2.20−2.01 (m, 3H), 1.92−1.74 (m, 4H), 1.74−1.31 (m, 6H), 1.30−1.07 (m, 18H), 0.81 (dt, J = 13.9, 4.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 173.46, 62.57, 53.58, 53.23, 37.02, 36.92, 33.23, 33.13, 32.83, 31.46, 30.91, 30.82, 30.63, 30.46, 30.44, 28.53, 28.23, 27.18, 27.11, 23.89, 14.65, 14.59. MS (ESI) calculated for $C_{26}H_{47}N_3O_8$, m/z 529.33, found 528.36 $(M - H)^-$.

Synthesis of Compound 46: Diethyl 2-((*R*)-4-Amino-5-(benzyloxy)-5-oxopentanamido)-6-((*tert*-butoxycarbonyl)amino)heptanedioate. Compound **13** (100 mg, 0.12 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of 3 mL of piperidine. The reaction mixture was stirred for 15 min, followed by removal of the solvent under reduced pressure to obtain the crude product which was purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **46** (66 mg, 92%). MS (ESI) calculated for $C_{28}H_{43}N_3O_9$, m/z 565.30, found 588.30 $(M + Na)^+$.

Synthesis of Compound 48: Diethyl 2-Amino-6-((*R*)-4-amino-5-oxo-5-(undecylthio)pentanamido)heptanedioate. To a solution of compound **46** (50 mg, 0.08 mmol) in dichloromethane were added di-*tert*-butyl dicarbonate (28.9 mg, 0.13 mmol) and triethylamine (24.6 μL, 0.17 mmol). The reaction mixture was stirred for 30 min, followed by complete removal of the solvent under reduced pressure. The residue was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was then subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in anhydrous DMF, followed by the addition of 1-undecanethiol (40 μL, 0.17 mmol), HBTU (40.2 mg, 0.10 mmol), triethylamine (24.7 μL, 0.17 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 14 h, followed by removal of the solvent under reduced pressure. The residue was dissolved in ethyl acetate and washed with water. The organic fraction was dried over anhydrous sodium sulfate, filtered, concentrated, and purified using column chromatography (30% EtOAc/hexanes) to obtain compound **47** (41 mg, 63%). Compound **47** (40 mg, 0.05 mmol) was dissolved in 5 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 trifluoroacetate salt of compound **48** (41 mg, quantitative yield). ^{1}H NMR (500 MHz, 20% $CDCl_3$ in MeOD) δ 4.32−4.26 (m, 1H), 4.26−4.13 (m, 3H), 4.09 (q, J = 7.1 Hz, 2H), 3.91 (dt, J = 2.0, 6.5 Hz, 1H), 2.95 (t, J = 7.3 Hz, 2H), 2.50−2.35 (m, 2H), 2.28−1.69 (m, 6H), 1.69−1.59 (m, 1H), 1.57−1.35 (m, 4H), 1.34−1.11 (m, 21H), 0.80 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, 20% $CDCl_3$ in MeOD) δ 197.50, 174.15, 63.80, 62.71, 62.66, 59.98, 53.86, 53.83, 53.78, 53.74, 33.18, 31.98, 31.79, 31.65, 31.19, 31.16, 30.84, 30.71, 30.58, 30.30, 29.94, 28.52, 28.44, 23.85, 22.77, 22.63, 14.64, 14.59, 14.56. MS (ESI) calculated for $C_{27}H_{51}N_3O_6S$, m/z 545.35, found 568.36 $(M + Na)^+$.

Synthesis of Compound 50: (2*R*)-5-((6-Amino-1,7-dethoxy-1,7-dioxoheptan-2-yl)amino)-2-dodecanamido-5-oxopentanoic Acid. To a solution of **46** (36 mg, 0.06 mmol) in pyridine were added lauroyl chloride (22.6 μL, 0.09 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 4 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in dichloromethane and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (18% EtOAc/hexanes) to obtain compound **49** (39.0 mg, 82%). Compound **49** (30 mg, 0.04 mmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was then dissolved in 5 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 trifluoroacetate salt of

compound **50** (26.4 mg, quantitative yield in two steps). ^{1}H NMR (500 MHz, MeOD) δ 4.45−4.37 (m, 2H), 4.29 (q, J = 7.1 Hz, 2H), 4.16 (q, J = 6.6 Hz, 2H), 4.05−3.97 (m, 1H), 2.34 (d, J = 2.6 Hz, 2H), 2.28−2.11 (m, 3H), 2.01−1.80 (m, 4H), 1.78−1.40 (m, 6H), 1.40−1.19 (m, 21H), 0.90 (dt, J = 13.6, 4.7 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.49, 175.06, 174.90, 173.29, 170.44, 63.70, 62.49, 53.88, 53.80, 53.33, 53.13, 52.83, 49.52, 49.35, 49.18, 49.01, 48.84, 48.67, 48.50, 36.96, 36.87, 33.10, 32.79, 32.09, 32.04, 31.95, 31.02, 30.89, 30.78, 30.68, 30.51, 30.38, 28.84, 28.47, 27.01, 26.97, 23.76, 22.65, 22.42, 22.32, 14.44. MS (ESI) calculated for $C_{28}H_{51}N_3O_8$, m/z 557.37, found 558.40 $(M + H)^+$.

Synthesis of Compound 51: Dibenzyl 2-(4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)butanamido)-6-((*tert*-butoxycarbonyl)amino)heptanedioate. To a solution of **9** (221 mg, 0.47 mmol) in anhydrous dichloromethane were added 4-Fmoc-aminobutyric acid (150 mg, 0.46 mmol), polystyrene bound carbodiimide (382.11 mg, 0.47 mmol), and a catalytic amount of polystyrene bound DMAP. The reaction mixture was stirred at room temperature for 8 h, followed by filtration to remove the solid resin. The filtrate was evaporated under vacuum to obtain the residue which was then purified using column chromatography (50% EtOAc/hexanes) to obtain compound **51** (230 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.4 Hz, 2H), 7.44−7.25 (m, 14H), 6.47 (d, J = 7.2 Hz, 1H), 5.21−5.00 (m, 6H), 4.61−4.50 (m, 1H), 4.46−4.33 (m, 2H), 4.31−4.15 (m, 2H), 3.29−3.11 (m, 2H), 2.26−2.12 (m, 2H), 1.76 (dd, J = 19.9, 6.1 Hz, 4H), 1.70−1.52 (m, 2H), 1.38 (d, J = 13.3 Hz, 11H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.70, 172.59, 172.45, 172.29, 157.10, 155.76, 144.15, 141.53, 135.54, 128.85, 128.82, 128.72, 128.64, 128.57, 128.45, 127.88, 127.25, 125.28, 120.16, 80.19, 67.42, 67.34, 67.24, 66.76, 53.34, 52.18, 47.49, 40.21, 33.33, 32.45, 32.30, 31.65, 28.53, 26.21, 21.59, 21.28. MS (ESI) calculated for $C_{45}H_{51}N_3O_9$, m/z 777.36, found 800.33 $(M + Na)^+$.

Synthesis of Compound 52: Dibenzyl 2-((*tert*-Butoxycarbonyl)amino)-6-(4-dodecanamidobutanamido)heptanedioate. Compound **51** (140 mg, 0.18 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of 3 mL of piperidine. The reaction mixture was stirred for 15 min, followed by removal of the solvent under reduced pressure to obtain the residue which was dissolved in pyridine, followed by the addition of lauroyl chloride (64.0 μL, 0.27 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 4 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in dichloromethane and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (30% EtOAc/hexanes) to obtain compound **52** (100 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41−7.29 (m, 10H), 6.84−6.76 (m, 1H), 5.97−5.88 (m, 1H), 5.26−5.09 (m, 5H), 4.59−4.50 (m, 1H), 4.26 (d, J = 8.5 Hz, 1H), 3.40−3.30 (m, 1H), 3.25−3.17 (m, 1H), 2.29−2.22 (m, 2H), 2.17−2.14 (m, 2H), 1.87−1.76 (m, 4H), 1.66 (s, 4H), 1.63−1.58 (m, 2H), 1.42 (d, J = 14.4 Hz, 9H), 1.33−1.20 (m, 16H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.14, 173.13, 172.73, 172.62, 172.43, 172.27, 155.92, 155.80, 135.57, 128.84, 128.69, 128.65, 128.56, 128.46, 80.12, 67.27, 53.39, 52.34, 38.80, 37.10, 33.62, 32.30, 32.12, 31.57, 29.84, 29.82, 29.72, 29.57, 29.55, 28.55, 26.06, 25.93, 22.90, 21.66, 21.34, 14.34. MS (ESI) calculated for $C_{42}H_{63}N_3O_8$, m/z 737.46, found 760.41 $(M + Na)^+$.

Synthesis of Compound 53: 2-Amino-6-(4-dodecanamidobutanamido)heptanedioic Acid. Compound **52** (50 mg, 0.06 mmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2$/C. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of compound **53** (38 mg, quantitative yield). ^{1}H NMR (500 MHz, MeOD) δ 4.39 (dd, J = 9.3, 4.7 Hz, 1H), 3.80−3.73 (m, 1H), 3.20 (t, J = 7.0 Hz, 2H), 2.32−2.24 (m, 2H), 2.21−2.14 (m, 2H), 2.00−1.68 (m, 7H), 1.66−1.42 (m, 5H), 1.36−1.24 (m, 14H), 0.90 (dd, J = 13.1, 6.2 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.66, 175.79, 175.46, 173.04, 54.83, 53.43, 39.81, 37.36, 34.18, 33.23, 32.45, 32.39, 31.61, 31.48, 30.90, 30.80, 30.63, 30.52, 27.24, 26.85, 23.89, 23.07, 22.75, 14.58. MS (ESI) calculated for $C_{23}H_{43}N_3O_6$, m/z 457.32, found 458.39 $(M + H)^+$.

Synthesis of Compound 54: 5-((1,7-Bis(benzyloxy)-6-(-(*tert*-butoxycarbonyl)amino)-1,7-dioxoheptan-2-yl)amino)-5-oxopentanoic Acid. To a solution of **9** (100 mg, 0.21 mmol) in anhydrous dichloromethane was added glutaric anhydride (25.5 mg, 0.22 mmol). The reaction mixture was stirred at room temperature for 1 h, followed by removal of the solvent under reduced pressure. The residue was then purified using column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **54** (118 mg, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73−7.29 (m, 10H), 6.87−6.34 (m, 1H), 5.22−5.08 (m, 4H), 4.60 (bs, 1H), 4.32−3.92 (m, 1H), 2.54−2.17 (m, 4H), 2.13−1.54 (m, 6H), 1.52−1.20 (m, 13H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.46, 172.80, 172.69, 172.41, 135.41, 131.11, 129.01, 128.86, 128.84, 128.74, 128.69, 128.54, 80.46, 68.37, 67.51, 67.43, 67.15, 53.35, 52.10, 51.82, 38.92, 35.67, 35.08, 33.61, 33.49, 32.99, 32.44, 31.80, 31.66, 31.00, 30.55, 29.91, 29.13, 28.53, 28.31, 23.93, 23.20, 21.55, 21.21, 20.61, 19.92. MS (ESI) calculated for $C_{31}H_{40}N_2O_9$, m/z 584.27, found 607.26 $(M + Na)^+$.

Synthesis of Compound 55: Dibenzyl 2-((*tert*-Butoxycarbonyl)amino)-6-(5-oxo-5-(undecyloxy)pentanamido)heptanedioate. To a solution of **54** (90 mg, 0.15 mmol) in anhydrous DMF were added 1-undecanol (63 μL, 0.30 mmol), HBTU (70 mg, 0.18 mmol), triethylamine (43 μL, 0.30 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 12 h, followed by removal of the solvent under reduced pressure. The residue was dissolved in ethyl acetate and washed with water. The organic fraction was dried over anhydrous sodium sulfate, filtered, concentrated, and purified using column chromatography (10% EtOAc/hexanes) to obtain compound **55** (86.4 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40−7.29 (m, 10H), 6.15 (d, J = 6.3 Hz, 1H), 5.20−5.02 (m, 5H), 4.62−4.54 (m, 1H), 4.32−4.19 (m, 1H), 4.05 (t, J = 6.8 Hz, 2H), 2.40−2.32 (m, 2H), 2.30−2.22 (m, 2H), 2.00−1.90 (m, 2H), 1.87−1.57 (m, 7H), 1.43 (s, 9H), 1.33−1.19 (m, 17H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.49, 173.48, 172.70, 172.57, 172.42, 172.27, 172.26, 155.85, 155.70, 135.55, 135.42, 128.86, 128.82, 128.73, 128.66, 128.55, 128.47, 80.20, 80.09, 67.43, 67.35, 67.25, 64.90, 53.31, 52.03, 51.99, 35.39, 33.47, 32.49, 32.26, 32.11, 31.96, 31.84, 29.80, 29.79, 29.73, 29.54, 29.47, 28.82, 28.52, 26.11, 22.89, 21.48, 21.20, 20.97, 14.33. MS (ESI) calculated for $C_{42}H_{62}N_2O_9$, m/z 738.45, found 761.43 $(M + Na)^+$.

Synthesis of Compound 56: 2-Amino-6-(5-oxo-5-(undecyloxy)pentanamido)heptanedioic Acid. Compound **55** (35 mg, 0.04 mmol) was dissolved in methanol, followed by the addition of $Pd(OH)_2$/C. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain the residue, which was dissolved in 5 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 trifluoroacetate salt of the compound **56** (26 mg, quantitative yield). ^{1}H NMR (500 MHz, MeOD) δ 4.34 (dd, J = 8.7, 5.0 Hz, 1H), 4.02 (t, J = 6.7 Hz, 2H), 3.82−3.73 (m, 1H), 2.34 (t, J = 7.5 Hz, 2H), 2.27 (t, J = 7.2 Hz, 2H), 1.97−1.80 (m, 5H), 1.74−1.63 (m, 1H), 1.62−1.42 (m, 4H), 1.29−1.25 (m, 16H), 0.86 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 175.63, 175.60, 175.35, 175.32, 175.12, 172.76, 65.83, 54.68, 54.54, 53.40, 53.36, 35.86, 34.37, 33.19, 32.40, 32.32, 31.51, 31.39, 30.85,

30.84, 30.78, 30.59, 30.51, 29.86, 27.16, 23.86, 22.99, 22.73, 22.27, 14.60. MS (ESI) calculated for $C_{23}H_{42}N_2O_7$, m/z 458.30, found 459.32 $(M + H)^+$.

General Procedure for the Syntheses of Compounds 66−79. Synthesis of Compound 66: Dibenzyl 2-((*R*)-4-(((-(9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(benzyloxy)-5-oxopentanamido)heptanedioate. To a solution of **4** (100 mg, 0.21 mmol) in dichloromethane were added **58** (78.8 mg, 0.22 mmol), polystyrene bound carbodiimide (170 mg, 0.22 mmol), triethylamine (60 μL, 0.43 mmol), and a catalytic amount of polystyrene bound DMAP. The reaction mixture was stirred at room temperature for 8 h, followed by filtration to remove the solid resin. The filtrate was evaporated under vacuum to obtain the residue which was then purified using column chromatography (55% EtOAc/hexanes) to obtain the compound **66** (154 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.0 Hz, 2H), 7.41−7.24 (m, 19H), 6.47 (d, J = 7.8 Hz, 0.50H), 6.19 (d, J = 7.8 Hz, 0.50H), 5.67 (dd, J = 20.4, 8.0 Hz, 1H), 5.20−5.03 (m, 6H), 4.59 (ddd, J = 13.0, 7.5, 5.3 Hz, 1H), 4.49−4.33 (m, 3H), 4.18 (t, J = 6.8 Hz, 1H), 2.27−2.19 (m, 5H), 2.02−1.77 (m, 3H), 1.63−1.61 (m, 1H), 1.38−1.16 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.17, 171.06, 170.13, 170.04, 169.40, 153.91, 141.79, 141.42, 139.22, 133.04, 126.56, 126.55, 126.53, 126.51, 126.46, 126.31, 126.24, 126.21, 126.13, 126.09, 125.63, 125.00, 123.03, 117.90, 65.30, 65.07, 64.95, 64.08, 51.35, 50.01, 45.06, 31.74, 29.88, 26.66, 26.14, 22.55, 22.27. MS (ESI) calculated for $C_{48}H_{48}N_2O_9$, m/z 796.34, found 819.31 $(M + Na)^+$.

Synthesis of Compound 67: 2-((*R*)-4-Carboxy-4-dodecanamidobutanamido)heptanedioic Acid. Compound **66** (147 mg, 0.18 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of 3 mL of piperidine. The reaction mixture was stirred for 15 min, followed by removal of the solvent under reduced pressure to obtain the residue, which was dissolved in pyridine, followed by the addition of lauroyl chloride (64.0 μL, 0.27 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 4 h, followed by evaporation of the solvent under reduced pressure. The residue was then dissolved in dichloromethane and washed with water. The organic solvent was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to obtain the crude product which was purified using column chromatography (25% EtOAc/hexanes) to obtain the protected intermediate of compound **67** (100 mg, 73%). The protected intermediate (50 mg, 0. 06 mmol) was then dissolved in methanol, followed by the addition of $Pd(OH)_2/C$. The solution was subjected to catalytic hydrogenolysis at 60 psi hydrogen pressure for 2 h, followed by filtration through a Celite bed. The solvent was removed under reduced pressure to obtain compound **67** (29 mg, quantitative yield). ^{1}H NMR (500 MHz, MeOD) δ 4.42−4.28 (m, 2H), 2.33 (t, J = 6.8 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 2.24−2.18 (m, 2H), 2.18−2.10 (m, 1H), 1.93 (dt, J = 13.8, 6.3 Hz, 1H), 1.85−1.82 (m, 1H), 1.72−1.54 (m, 5H), 1.41 (dd, J = 14.5, 7.4 Hz, 3H), 1.30−1.26 (m, 15H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 177.59, 176.63, 176.63, 175.03, 54.04, 53.66, 37.03, 34.85, 33.36, 33.24, 32.50, 32.45, 30.91, 30.81, 30.64, 30.48, 28.97, 28.79, 27.07, 26.66, 25.78, 23.90, 14.60. MS (ESI) calculated for $C_{24}H_{42}N_2O_8$, m/z 486.29, found 485.29 $(M - H)^-$.

In the case of *N*-Boc protected intermediates (compounds **68**, **72**, **76**, and **78**), the compounds after hydrogenolysis were dissolved in trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salts of compound **69**, **73**, **77**, and **79**.

Synthesis of Compound 68: (*R*)-Benzyl 2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-((5-((*tert*-butoxycarbonyl)amino)pentyl)amino)-5-oxopentanoate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.71 (dt, J = 7.3, 3.6 Hz, 1H), 7.61 (t, J = 6.8 Hz, 2H), 7.54 (dd, J = 5.7, 3.3 Hz, 1H), 7.40 (t, J = 7.5 Hz, 2H), 7.37−7.30 (m, 5H), 5.18 (q, J = 12.1 Hz, 2H), 4.46−4.32 (m, 3H), 4.25−4.17 (m, 2H), 3.42 (dd, J = 5.5, 3.9 Hz, 1H), 3.18 (ddd, J = 20.5, 13.5, 7.0 Hz, 2H), 3.07 (s, 2H), 2.20−2.18 (m, 3H), 1.52−1.38 (m, 10H), 1.37−1.27 (m, 7H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.20, 141.65, 135.35, 131.45, 129.30, 129.14, 129.03, 128.87, 128.24, 127.59, 125.59, 120.48, 79.88, 68.70, 67.86, 50.32, 50.15, 49.98, 47.61, 39.76, 39.20, 30.83, 30.05, 29.41, 28.88, 24.34, 24.21, 23.47. MS (ESI) calculated for $C_{37}H_{45}N_3O_7$, m/z 643.33, found 666.34 $(M + Na)^+$.

Synthesis of Compound 69: (*R*)-5-((5-Aminopentyl)amino)-2-dodecanamido-5-oxopentanoic Acid. ^{1}H NMR (400 MHz, MeOD) δ 4.21 (dd, J = 8.4, 4.9 Hz, 1H), 3.30−3.18 (m, 2H), 2.92 (t, J = 6.7 Hz, 2H), 2.27−2.20 (m, 4H), 2.08 (td, J = 13.5, 6.5 Hz, 1H), 1.94 (td, J = 13.6, 7.3 Hz, 1H), 1.72−1.39 (m, 8H), 1.37−1.20 (m, 16H), 0.88 (dd, J = 12.0, 5.4 Hz, 3H). ^{13}C NMR (101 MHz, 10% $CDCl_3$ in MeOD) δ 174.03, 53.83, 38.93, 37.98, 36.02, 32.27, 31.46, 29.16, 29.04, 28.87, 27.91, 26.29, 25.40, 22.64, 22.17, 13.26. MS (ESI) calculated for $C_{22}H_{43}N_3O_4$, m/z 413.33, found 414.37 $(M + H)^+$.

Synthesis of Compound 70: (2*R*)-Benzyl 2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-oxo-5-((2-oxoazepan-3-yl)amino)pentanoate. ^{1}H NMR (500 MHz, $CDCl_3$, ethyl acetate) δ 7.76 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.4 Hz, 2H), 7.35 (qd, J = 14.7, 7.3 Hz, 9H), 6.98−6.90 (m, 1H), 6.06 (bs, 1H), 5.88−5.81 (m, 1H), 5.23−5.13 (m, 2H), 4.54−4.48 (m, 1H), 4.46−4.33 (m, 3H), 4.21 (t, J = 7.1 Hz, 1H), 3.30−3.18 (m, 2H), 2.37−2.18 (m, 3H), 1.99−1.97 (m, 1H), 1.84−1.76 (dd, J = 14.6, 9.4 Hz, 1H), 1.62 (bs, 3H), 1.49−1.35 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.57, 172.14, 171.16, 156.34, 144.15, 143.99, 141.49, 135.45, 128.85, 128.71, 128.54, 127.90, 127.30, 125.43, 120.17, 67.51, 67.29, 53.98, 53.91, 52.46, 47.38, 42.38, 32.37, 32.32, 31.76, 31.70, 29.05, 28.10, 27.98, 27.93. MS (ESI) calculated for $C_{33}H_{35}N_3O_6$, m/z 569.25, found 592.23 $(M + Na)^+$.

Synthesis of Compound 71: (2*R*)-2-Dodecanamido-5-oxo-5-((2-oxoazepan-3-yl)amino)pentanoic Acid. ^{1}H NMR (500 MHz, MeOD) δ 4.54 (dd, J = 11.4, 1.5 Hz, 1H), 4.41−4.31 (m, 1H), 3.30−3.13 (m, 3H), 2.34 (tt, J = 6.4, 5.0 Hz, 2H), 2.27−2.11 (m, 3H), 2.02−1.72 (m, 5H), 1.65−1.49 (m, 3H), 1.43−1.22 (m, 16H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 177.24, 176.35, 176.30, 174.12, 174.02, 53.35, 49.46, 49.29, 42.47, 36.94, 33.37, 33.27, 33.10, 32.21, 32.16, 30.78, 30.77, 30.67, 30.51, 30.50, 30.36, 29.94, 29.16, 28.87, 28.80, 26.92, 23.75, 14.45. MS (ESI) calculated for $C_{23}H_{41}N_3O_5$, m/z 439.30, found 462.31 $(M + Na)^+$.

Synthesis of Compound 72: (*R*)-Benzyl 2-((*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(benzyloxy)-5-oxopentanamido)-6-((*tert*-butoxycarbonyl)amino)hexanoate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.2 Hz, 2H), 7.45−7.28 (m, 14H), 6.32 (d, J = 6.9 Hz, 1H), 5.71 (d, J = 8.0 Hz, 1H), 5.22−5.08 (m, 4H), 4.59 (t, J = 10.9 Hz, 2H), 4.40 (d, J = 6.9 Hz, 3H), 4.20 (t, J = 6.9 Hz, 1H), 3.02 (bs, 2H), 2.24 (s, 3H), 1.95 (dd, J = 15.4, 7.9 Hz, 1H), 1.83 (ddd, J = 15.7, 10.6, 5.3 Hz, 1H), 1.67 (d, J = 8.6 Hz, 1H), 1.41 (s, 11H), 1.36−1.23 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.37, 172.01, 171.81, 156.51, 156.28, 144.10, 143.86, 141.53, 135.48, 135.36, 128.87, 128.84, 128.78, 128.73, 128.63, 128.56, 127.94, 127.31, 125.34, 120.20, 120.18, 79.31, 67.61, 67.36, 67.27, 53.63, 52.36, 47.37, 40.17, 32.26, 32.02, 29.71, 28.62, 22.50. MS (ESI) calculated for $C_{45}H_{51}N_3O_9$, m/z 777.36, found 800.35 $(M + Na)^+$.

Synthesis of Compound 73: (*R*)-6-Amino-2-((*R*)-4-carboxy-4-dodecanamidobutanamido)hexanoic Acid. ^{1}H NMR (500 MHz, MeOD) δ 4.31 (dd, J = 9.1, 4.4 Hz, 1H), 4.26 (dd, J = 9.6, 4.3 Hz, 1H), 2.89−2.79 (m, 2H), 2.26 (t, J = 7.2 Hz, 2H), 2.21−2.08 (m, 3H), 1.88−1.75 (m, 2H), 1.68−1.48 (m, 5H), 1.47−1.08 (m, 18H), 0.80 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.55, 174.81, 53.42, 53.24, 40.56, 36.97, 33.10, 33.04, 32.34, 30.79, 30.77, 30.68, 30.52, 30.50, 30.38, 28.64, 27.95, 27.00, 23.78, 23.75, 14.45. MS (ESI) calculated for $C_{23}H_{43}N_3O_6$, m/z 457.32, found 458.33 $(M + H)^+$.

Synthesis of Compound 74: (*R*)-Benzyl 6-((*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(benzyloxy)-5-oxopentanamido)-2-(((benzyloxy)carbonyl)amino)hexanoate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.61−7.52 (m, 2H), 7.38−7.25 (m, 19H), 5.80 (bs, 1H), 5.71 (d, J = 7.8 Hz, 1H), 5.45−5.41 (m, 1H), 5.19−5.09 (m, 4H), 5.06 (s, 2H), 4.44−4.30 (m, 4H), 4.16 (t, J = 6.8 Hz, 1H), 3.14 (dt, J = 13.8, 6.8 Hz, 2H), 2.17−2.11 (m, 3H), 1.93 (dd, J = 17.2, 11.2 Hz, 1H), 1.80 (dt, J = 10.2, 7.3 Hz, 1H), 1.70−1.62 (m, 1H), 1.49−1.38 (m, 2H), 1.33−1.24 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.41, 172.02, 156.59, 156.24, 144.10, 143.81, 141.53, 141.48, 136.40, 135.46, 135.37, 128.86, 128.84, 128.74, 128.72, 128.62, 128.53, 128.41, 128.30, 127.94, 127.29, 125.35, 125.31, 120.20, 67.58, 67.37, 67.28, 67.20, 53.88, 53.75, 47.34, 39.29, 32.59, 32.34, 29.00, 28.82, 22.53. MS (ESI) calculated for $C_{48}H_{49}N_3O_9$, m/z 811.35, found 834.34 $(M + Na)^+$.

Synthesis of Compound 75: (*R*)-2-Amino-6-((*R*)-4-carboxy-4-dodecanamidobutanamido)hexanoic Acid. ^{1}H NMR (500 MHz, MeOD) δ 4.34 (dd, J = 9.1, 4.9 Hz, 1H), 3.80 (t, J = 6.1 Hz, 1H), 3.22 (dt, J = 13.3, 6.7 Hz, 1H), 3.13 (dt, J = 13.5, 6.7 Hz, 1H), 2.30−2.12 (m, 5H), 1.97−1.79 (m, 3H), 1.64−1.56 (m, 2H), 1.52 (dd, J = 13.8, 6.7 Hz, 2H), 1.49−1.39 (m, 2H), 1.30−1.26 (d, J = 17.6 Hz, 16H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.53, 174.85, 54.57, 53.08, 39.85, 36.89, 33.27, 33.10, 31.40, 30.78, 30.68, 30.52, 30.50, 30.34, 30.00, 28.69, 26.96, 23.76, 23.40, 14.46. MS (ESI) calculated for $C_{23}H_{43}N_3O_6$, m/z 457.32, found 456.33 $(M - H)^-$.

Synthesis of Compound 76: (*S*)-*tert*-Butyl 2-((*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(benzyloxy)-5-oxopentanamido)-6-(((benzyloxy)carbonyl)amino)hexanoate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.61−7.54 (m, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.35−7.25 (m, 12H), 6.30 (dd, J = 84.8, 7.6 Hz, 1H), 5.74 (dd, J = 17.5, 7.9 Hz, 1H), 5.14 (t, J = 12.2 Hz, 2H), 5.08−5.01 (m, 2H), 4.88 (bs, 1H), 4.42−4.30 (ddt, J = 31.5, 17.9, 7.6 Hz, 4H), 4.17 (t, J = 6.8 Hz, 1H), 3.14 (dd, J = 12.7, 6.4 Hz, 2H), 2.31−2.13 (m, 3H), 1.98 (dd, J = 14.5, 7.7 Hz, 1H), 1.76 (ddd, J = 15.5, 10.2, 5.3 Hz, 1H), 1.64−1.61 (m, 1H), 1.51−1.44 (m, 2H), 1.44−1.42 (m, 9H), 1.37−1.27 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.11, 171.84, 171.77, 156.72, 156.51, 144.12, 143.85, 141.50, 136.79, 135.36, 128.85, 128.73, 128.70, 128.57, 128.28, 127.92, 127.30, 125.35, 120.19, 120.17, 82.33, 67.57, 67.31, 66.76, 53.67, 52.76, 47.34, 40.77, 32.41, 32.16, 29.58, 28.70, 28.19, 22.29. MS (ESI) calculated for $C_{45}H_{51}N_3O_9$, m/z 777.36, found 800.36 $(M + Na)^+$.

Synthesis of Compound 77: (*S*)-6-Amino-2-((*R*)-4-carboxy-4-dodecanamidobutanamido)hexanoic Acid. ^{1}H NMR (500 MHz, MeOD) δ 4.35 (bs, 2H), 2.91 (t, J = 7.4 Hz, 2H), 2.32 (t, J = 6.7 Hz, 2H), 2.22 (t, J = 7.5 Hz, 2H), 2.17−2.08 (m, 1H), 1.96−1.87 (m, 2H), 1.77−1.55 (m, 5H), 1.48 (dd, J = 15.2, 7.6 Hz, 2H), 1.30−1.27 (m, 16H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.53, 175.19, 53.81, 40.68, 37.07, 33.41, 33.23, 32.47, 32.36, 30.92, 30.81, 30.65, 30.50, 29.17, 28.86, 28.12, 27.10, 23.95, 23.89, 14.60. MS (ESI) calculated for $C_{23}H_{43}N_3O_6$, m/z 457.32, found 458.32 $(M + H)^+$.

Synthesis of Compound 78: (*S*)-*tert*-Butyl 6-((*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(benzyloxy)-5-oxopentanamido)-2-((*tert*-butoxycarbonyl)amino)hexanoate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.61−7.55 (m, 2H), 7.41−7.25 (m, 9H), 5.89 (bs, 1H), 5.78 (d, J = 7.9 Hz, 1H), 5.16 (q, J = 12.2 Hz, 2H), 5.07 (d, J = 8.1 Hz, 1H), 4.37 (ddd, J = 28.8, 10.6, 7.1 Hz, 3H), 4.19 (t, J = 7.0 Hz, 1H), 3.19 (ddt, J = 25.6, 13.1, 6.5 Hz, 2H), 2.18 (t, J = 10.6 Hz, 2H), 2.01−1.92 (m, 1H), 1.78−1.62 (m, 3H), 1.58 (ddd, J = 11.4, 9.3, 5.7 Hz, 1H), 1.53−1.45 (m, 2H), 1.41 (d, J = 8.0 Hz, 18H), 1.38−1.27 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.29, 170.24, 170.19, 154.78, 153.95, 142.30, 142.03, 139.72, 139.67, 133.58, 127.06, 126.95, 126.81, 126.13, 126.12, 125.48, 123.57, 123.54, 118.40, 118.38, 80.27, 78.07, 65.77, 65.53, 52.05, 52.00, 45.54, 37.78, 31.12, 30.81, 27.32, 26.97, 26.73, 26.40, 20.95. MS (ESI) calculated for $C_{42}H_{53}N_3O_9$, m/z 743.38, found 766.36 $(M + Na)^+$.

Synthesis of Compound 79: (*S*)-2-Amino-6-((*R*)-4-carboxy-4-dodecanamidobutanamido)hexanoic Acid. ^{1}H NMR (500 MHz, MeOD) δ 4.35 (dd, J = 8.9, 4.9 Hz, 1H), 3.74 (t, J = 5.9 Hz, 1H), 3.24−3.12 (m, 2H), 2.30−2.13 (m, 5H), 1.95−1.80 (m, 3H), 1.66−1.37 (m, 7H), 1.31−1.27 (m, 15H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 176.28, 175.26, 174.74, 173.09, 54.56, 53.07, 39.70, 36.88, 33.15, 32.90, 31.17, 30.61, 30.59, 30.50, 30.35, 30.31, 30.23, 30.16, 29.73, 28.66, 26.78, 23.59, 23.11, 14.54. MS (ESI) calculated for $C_{23}H_{43}N_3O_6$, m/z 457.32, found 458.35 $(M + H)^+$.

NF-κB Induction. The induction of NF-κB was quantified using HEK-Blue cells as previously described by us.[10,51] HEK293 cells stably transfected with human Nod1 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/AP-1 promoters is inducible by Nod1 agonists, and extracellular sAP in the supernatant is proportional to Nod1-mediated 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 in subsequently graded concentrations of compounds. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in the HEK-detection medium as supplied by the vendor) at 620 nm.

Experiments Involving Human Blood. Human blood was obtained from healthy adults by antecubital venipuncture in accordance with University of Kansas Human Subjects Experimentation protocols (Protocol No. HSCL 12397).

Phosflow Flow Cytometric Assay for p38MAPK. Assays were performed as described by us previously.[10,38,52] Briefly, 1 mL aliquots of fresh whole blood anticoagulated with heparin were incubated with 25 μL of an equal volume of graded concentrations of compounds diluted in saline for 15 min at 37 °C. Erythrocytes were lysed and leukocytes were fixed in one step by mixing 200 μL of the samples in 4 mL of prewarmed whole blood lyse/fix buffer (Becton-Dickinson Biosciences, San Jose, CA). After the cells at 500 g were washed for 8 min in buffer, the cells were permeabilized in ice-cold methanol for 30 min, washed twice in phosphate-buffered saline, and transferred to a Millipore MultiScreen BV 1.2 μm filter plate and stained with either phycoerythrin (PE)-conjugated mouse anti-p38MAPK (pT180/pY182, BD Biosciences) mAb or a matched PE-labeled mouse IgG_1 κ isotype control mAb for 60 min. The cells were washed twice in the plate by aspiration according to protocols supplied by the vendor. Cytometry was performed using a BD FACSArray instrument in the single-color mode for PE acquisition on 20 000 gated events. Postacquisition analyses were performed using FlowJo, version 7.0, software (Treestar, Ashland, OR).

CD11b Flow Cytometric Assay. Assays were performed as described by us previously.[10,38,52] Briefly, 1 mL aliquots of fresh anticoagulated whole blood were incubated with 25 μL of graded dilutions of the compounds for 1 h at 37 °C. Negative (saline) controls were included in each experiment. Samples were placed on ice for 15 min before an amount of 20 μL of anti-CD11b/Mac-1 antibody (Becton-Dickinson) was added to each sample tube and allowed to incubate on ice for 30 min. This 0 °C incubation step prevented internalization of antibody and ensured staining of only extracellularly expressed CD11b. Erythrocytes were lysed, and leukocytes were fixed in one step by mixing 200 μL of the samples in 4 mL of prewarmed whole blood lyse/fix buffer (Becton-Dickinson Biosciences, San Jose, CA). After the cells were washed twice at 200 g for 5 min in CBA buffer, the cells were transferred to a 96-well plate. Flow cytometry was performed using a BD FACSArray instrument in the single-color mode for PE acquisition on 20 000

gated events. Postacquisition analyses were performed using FlowJo, version 7.0, software.

Transcriptomal Profiling in Whole Human Blood. Assays were performed as described by us previously.[10] Briefly, peripheral blood mononuclear cells (PBMCs) isolated from fresh heparin-anticoagulated human blood were stimulated with 20 μg/mL of the compounds for 2 h, and total RNA was extracted from treated and negative control PBMC samples with QIAamp RNA Blood Mini Kit (Qiagen). An amount of 4 μg of each of the RNA samples was used for transcriptomal profiling, employing the human genome GeneChip U133 Plus 2.0 oligonucleotide array (Affymetrix, Santa Clara, CA). Established standard protocols at the KU Genomics Facility were performed on cRNA target preparation, array hybridization, washing, staining, and image scanning. The microarray data were collected using the Affymetrix GeneChip Command Console software (AGCC) and then subjected to quality assessment before further analyses. QC criteria included low background, low noise, detection of positive controls, and a 5′/3′ ratio of <3.0. To facilitate direct comparison of gene expression data between different samples, the GeneChip data were first subjected to preprocessing, which included scaling of data from all chips to a target intensity value of 500 (in Affymetrix Expression Console software) and further normalization in GeneSpring GX (Agilent Technologies, Santa Clara, CA). Prior to identification of target genes, genes that were detected as nonexpressed in all samples, i.e., those with absence (A) cells, were filtered out. Genes whose expression was changed by our compounds by at least 2-fold (compared to the negative control) were identified to be differentially expressed. Pathway analyses of gene expression were performed using IPA (Ingenuity Systems, Redwood, CA).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^{1}H NMR, ^{13}C NMR, and MS results of all compounds and LC−MS data of final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was supported by NIH/NIAID Contract HHSN272200900033C.

■ ABBREVIATIONS USED

AP-1, activator protein-1; CD, cluster of differentiation; DAP, diaminopimelic acid; DMAP, 4-dimethylaminopyridine; EC_{50}, half-maximal effective concentration; ESI-TOF, electrospray ionization time of flight; FmocCl, fluorenylmethyloxycarbonyl chloride; Glu, glutamic acid; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HEK, human embryonic kidney; iE-DAP, γ-D-glutamyldiaminopimelic acid; IL, interleukin; NF-κB, nuclear factor κB; Nod1, nucleotide oligomerization domain 1; Nod2, nucleotide oligomerization domain 2; NLR, Nod-like receptor; p38MAPK, p38 mitogen activated protein kinase; PBMCs, peripheral blood mononuclear cells; PE, phycoerythrin; PRRs, pattern recognition receptors; RIG-I, retinoic acid inducible gene I; RNA, ribonucleic acid; sAP, secreted alkaline phosphatase; SAR, structure−activity relationship; Th1, helper T lymphocyte type 1; Th2, helper T lymphocyte type 2; TLR, Toll-like receptor; TREM-1, triggering receptor expressed on myeloid cells 1

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