Design, Synthesis, and Preliminary Pharmacological Evaluation of *N*-Acyl-3-aminoglutarimides as Broad-Spectrum Chemokine Inhibitors in Vitro and Anti-inflammatory Agents in Vivo

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A series of N-substituted 3-aminoglutarimides have been synthesized and tested for inhibitory activity against a range of chemokines in vitro and for suppression of lipopolysaccharide-induced inflammation in vivo. The results show that they represent the first class of small molecules with broad-spectrum chemokine inhibitory effects. Among the compounds studied, **10** (NR58,4) was the most potent, being active at doses between 5 and 15 nM in vitro and at 0.3 mg kg^{-1} in vivo.

Introduction

Chemokines are small protein (8−12 kDa) signaling molecules[1] involved in the regulation of the immune system both during physiological host defense[2] and in pathological inflammation.[3] Neutralizing antibodies and genetic deletion strategies have been successfully employed to demonstrate that reduced chemokine signaling can reduce or abolish pathological inflammation.[3] For example, when mice are treated with the nonspecific pro-inflammatory stimulus of bacterial lipopolysaccharide (LPS), neutralizing antibodies against a range of chemokines act synergistically to reduce inflammation.[4,5] These studies clearly demonstrate that the different chemokines (more than 50 have now been described) can act in parallel to promote inflammation.

Because inappropriate chemokine activity has been suggested to participate in a wide range of human diseases, including asthma, atherosclerosis, cancer, and autoimmune disorders,[3] there has been a large number of attempts to identify chemokine inhibitors suitable for use in vivo.[6−11] The chemokines signal through interaction with a range of chemokine receptors, all of which are members of the intensively studied heptahelical G-protein-coupled receptor (GPCR) superfamily.[1] As a result, most attempts to identify chemokine inhibitors have relied on screening strategies similar to those that have been successful for a wide range of other GPCR ligands. These studies have yielded a number of classes of compounds that act as receptor antagonists for one or several of the chemokine receptors.[6−11] Of these compounds, most show structural similarity to antagonists of unrelated GPCR families. For example, several 4-hydroxypiperazine derivatives have been shown to act as chemokine inhibitors and closely resemble the dopamine receptor inhibitor domperinone. Unfortunately, only a few of the chemokine receptor antagonists described to date have been shown to have specificity over other members of the GPCR superfamily. For many, it remains possible that they bind to a range of different GPCR targets, limiting their application as specific chemokine inhibitors.[6]

With few exceptions, the chemokine inhibitors published to date inhibit the function of chemokines binding to one or a small subset of related chemokine receptors.[9−11] In circumstances where an inflammatory response is dependent on the parallel action of a range of chemokines signaling through a number of different receptors, these selective inhibitors may be largely ineffective. For example, neutralizing antibodies against individual chemokines often have little or no effect on models of inflammation in vivo.[12]

In marked contrast, we recently described a short peptide sequence derived from the human chemokine monocyte chemoattractant protein-1 (MCP-1) that is able to simultaneously inhibit the activity of a wide range of chemokines in vitro.[13] This peptide (EICADPKQKWVQ (single letter amino acid code) termed peptide 3), has no effect on leukocyte migration induced by a range of other pro-inflammatory signals.[13] Thus, peptide 3 is the first example of a broad-spectrum chemokine inhibitor. Consistent with its effects in vitro, we have recently demonstrated that a modified peptide of related structure (NR58-3.14.3) has anti-inflammatory properties in vivo; NR58-3.14.3 abolished leukocyte recruitment in response to intradermal LPS injection[14] and reduced the pathological inflammation in animal models of asthma, stroke,[15] and atherosclerosis.[16]

There are generally significant limitations on the use of synthetic oligopeptides both experimentally and therapeutically in vivo. For example, most peptides (including peptide 3 and the related NR58-3.14.3) have poor oral bioavailability and short plasma half-lives, often being secreted at the glomerular filtration rate.[17] We have therefore performed a structure−activity relationship (SAR) analysis to identify the structural motifs essential for broad-spectrum chemokine inhibitory activity within the peptide 3 molecule and designed a series of non-peptide mimetics that possess broad-spectrum chemokine inhibitory activity in the nanomolar concentration range.

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Table 1. Effect of Peptide 3 Sequence Variants on Leukocyte Migration Induced by MCP-1[a]

peptide	ED_{50} versus MCP-1 (μM)
EICADPKQKWVQ	8 ± 3
CADPKQKWVQ	8 ± 5
EICADP	50 ± 17
KQKWVQ	6 ± 3
KQK	12 ± 4
WVQ	4 ± 2
VWQ	inactive
WQV	inactive
GVQ	inactive
VVQ	>100
YVQ	25 ± 7
WVN	>100
WVR	inactive
WVG	inactive

[a] THP-1 cells were induced to migrate in response to 25ng/mL MCP-1 (see text) in the presence of various concentrations of the peptides shown above (single-letter amino acid code). The concentration that reduced migration by 50% (ED_{50}) is shown, and in each case is the mean ± sem for three separate determinations. Peptides that did not statistically significantly reduce migration at 100 μM (the highest concentration tested) are marked "inactive". Peptides that reduced migration significantly, but by less than 50%, at 100 μM are marked ">100".

Peptide SAR

We have already performed a limited mutagenesis study on the full-length peptide 3 sequence in which variant amino acids from different chemokines were substituted into the MCP-1-derived peptide 3 sequence. All of these substitutions had only small effects on the inhibitory potency of the peptide (ranging from 2 to 10 μM, with Leu_4Ile_{11}-peptide 3[3−13] being the most potent), and none of the substitutions affected the broad chemokine specificity of the molecule.[13] We have therefore performed a further SAR with less conservative changes to the molecule. The 12 amino acid peptide 3 sequence was divided first into two hexapeptides, and each was tested for the ability to inhibit migration induced by MCP-1 (Table 1). Both hexapeptides derived from peptide 3 inhibited MCP-1-induced leukocyte migration in a ChemoTx migration assay,[13] although only the C-terminal hexapeptide retained the same potency as the original dodecapeptide. Surprisingly, when the C-terminal hexapeptide was further divided into two tripeptides, both inhibited MCP-1-induced migration with a potency similar to that of the original dodecapeptide. Thus, the tripeptides KQK and WVQ appear to be structurally distinct chemokine inhibitors.

An important feature of the peptide 3 dodecapeptide was the ability to inhibit all chemokines tested with similar potency. We therefore tested whether the tripeptides retained this attribute. WVQ inhibited all the chemokines tested (MCP-1, MIP-1α, RANTES, IL-8, and SDF-1α) with similar potency (2−10 μM) and was therefore indistinguishable from the original peptide 3 sequence in these experiments. In marked contrast, the KQK tripeptide inhibited MCP-1-induced migration (ED_{50} = 12 μM) but had no effect on migration induced by MIP-1α, RANTES, IL-8, or SDF-1α. It seems likely, therefore, that the two tripeptides are inhibiting chemokine-induced signaling by distinct mechanisms.

Since our aim was to design a non-peptide chemokine inhibitor that retained the broad specificity of peptide 3, the KQK tripeptide was not studied further. Further variations of the WVQ tripeptide were synthesized and characterized to determine what structural features of this peptide were likely to be important for broad-spectrum chemokine inhibitory activity. None of dipeptides WV, WQ, and VQ had any detectable activity as chemokine inhibitors. Similarly the scrambled tripeptides VQW and VWQ were inactive (Table 1). The tripeptides with an N-terminal glutamine (Q) residue were not synthesized because of potential contamination with cyclized pyroglutamate-containing peptides. These experiments confirmed that the chemokine inhibitory activity of WVQ tripeptide required the presence of each amino acid constituent in an appropriate spatial arrangement.

We next synthesized a series of peptides with substitutions of each of the three amino acids in turn. Substitution of the tryptophan (W) with glycine (G) or valine (V) completely abolished the activity of the tripeptide, but tripeptides containing the aromatic amino acids tyrosine (Y) or phenylalanine (F) had activity similar to that of the parent tripeptide (Table 1). This suggests that an aromatic group, or at least a bulky hydrophobic group, at the P1 position is necessary for broad-spectrum chemokine inhibitory activity.

Replacement of valine (V) at the P2 position with glycine (G) slightly increased the potency of the tripeptide (by approximately 3-fold). It is possible that this results from increased conformational flexibility of the tripeptide. We therefore substituted the P2 position with a range of amino acids with alkyl side chains of varying length, using leucine, isoleucine, ethylglycine, alanine, D-alanine, and D-valine in addition to glycine and valine. A plot of potency against the number of carbon atoms in the side chain revealed a negative correlation (Figure 1) with the WGQ tripeptide as the most potent.

All the substitutions we tested at the P3 position resulted in complete loss of chemokine inhibitory activity (Table 1). The conservative substitution replacing glutamine (Q) with asparagine (N), as well as with glycine (G) and arginine (R), abolished the activity. On the basis of the data in Table 1, we concluded that the broad-spectrum chemokine inhibitory activity of WVQ-related tripeptides depended on the presence of a large hydrophobic or aromatic group at an appropriate distance from a glutamine-like primary amide.

As an alternative approach for identifying the structural features in peptide 3 that are essential for broad-spectrum chemokine inhibition, we synthesized a panel of 231 peptides in which each amino acid in Leu_4Ile_{11}-Cys_{13}-peptide 3[3−12] was substituted with the other 19 naturally occurring amino acids. This sequence, with the addition of a C-terminal cysteine residue, was chosen for the substitution analysis because it is the most potent linear analogue of peptide 3 described to date.[13] This panel of peptides, all in the linear, fully reduced state, was then tested for ability to inhibit IL-8-induced migration of human neutrophils. Most of the variant peptides retained the activity of the parent sequence (green squares in Figure 2). However, a number of substitutions decreased the activity of the peptide considerably (red squares in Figure 2; darker red indicating greater loss of activity). Consistent with the results from the deletion analysis, substitution of the amino acids in the C-terminus of the molecule reduced the activity to a much greater extent than

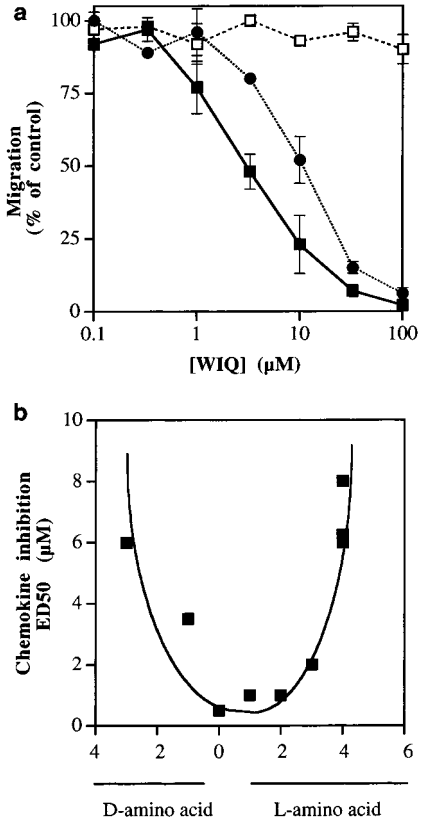


Figure 1. Effect of W*x*Q tripeptides on leukocyte migration. (a) THP-1 cells were induced to migrate in response to 25 ng/mL MCP-1 (filled squares), 3.125 ng/mL SDF-1α (filled circles), or 100 nM fMLP as a non-chemokine chemoattractant (open squares) in the presence of various concentrations of the tripeptide WIQ. The number of cells migrating under each condition is reported as a proportion of the number of cells that migrated in the absence of WIQ (mean ± sem of triplicate wells). (b) The concentration of various W*x*Q tripeptides that inhibited MCP-1-induced migration of THP-1 cells by 50% (ED_{50}) was determined for x = L-isoleucine, L-allo-isoleucine, L-valine, L-ethylglycine, L-alanine, glycine, D-alanine, and D-valine. The ED_{50} was plotted against the number of carbon atoms in the simple alkyl side chains of the amino acid at the P2 position (x).

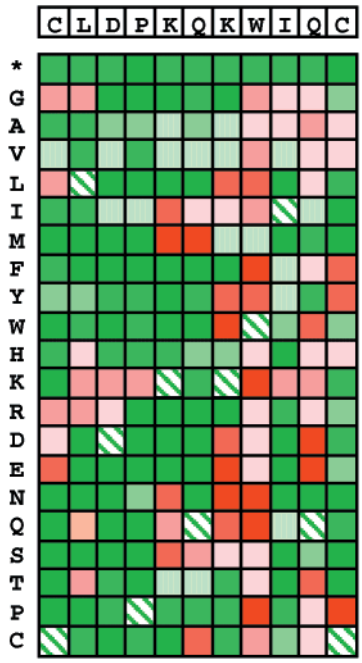


Figure 2. Summary of the structure−activity relationship for a replacement net analysis of $Leu_4Ile_{11}Cys_{13}$-peptide 3[3−12]. $Leu_4Ile_{11}Cys_{13}$-peptide 3[3−12] was synthesized on a parallel peptide synthesizer in 231 combinations (including controls) in which each amino acid in turn was substituted with the other 19 amino acids or with the cognate D-amino acid (*). Each of the 231 variants was then tested for the ability to inhibit IL-8-induced migration of freshly prepared human peripheral blood polymorphonuclear cells (predominantly neutrophils). Peptides with activity similar to that of the parent sequence are shown as green squares (lighter green indicating slight loss of activity). Peptides with more than 10-fold loss of activity are shown as red squares (darker red indicating greater loss of activity such that a dark red square indicates no inhibitory activity even at 100 μM). The parental sequence was separately synthesized 11 times in the panel and was fully active in each case (indicated by the hatched green squares).

substitutions in the N-terminal region. Every substitution at position 10 (tryptophan) significantly, and in many cases completely, reduced the activity of the peptide, while most (18/19) substitutions at position 12 (glutamine) reduced the activity substantially.

Both the deletion analysis (Table 1) and the substitution analysis (Figure 2) strongly indicate that the motif W*x*Q is responsible for the broad-spectrum chemokine inhibitory activity in peptide 3, although the substantially increased potency of the retroinverso analogue NR58-3.14.3 compared with that of peptide 3 suggests that the context of the W*x*Q is also important.[14]

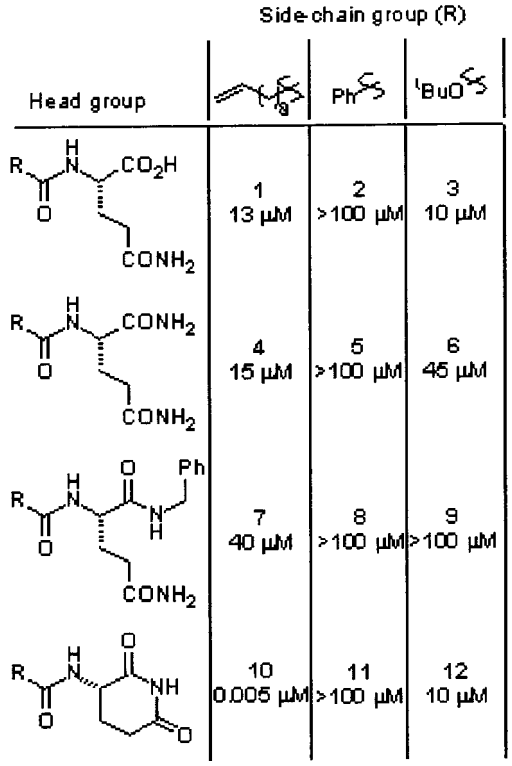
Design and Synthesis of Non-peptide Broad-Spectrum Chemokine Inhibitors

As a first step toward defining the constituent pharmacophores needed to yield a broad-spectrum chemokine inhibitor, we selected four candidate glutamine mimetics (including glutamine itself) shown in Table 2 and three candidate large hydrophobic groups (as tryptophan mimetics) and synthesized the 12 combinations (Table 2).

The syntheses of glutamine derivatives **1**, **2**, and **4**−**20** were performed using standard peptide coupling reagents and conditions. Initially L-glutamine was reacted with undec-10-enoyl chloride and benzoyl chloride in THF and aqueous KOH. The N-acylated products **1** and **2**, along with commercially available Boc-glutamine **3**, were then each coupled with ammonia and benzylamine to give the bis primary amides **4**−**6** and the benzylamides **7**−**9**, respectively (Scheme 1). The acids were also activated using DCC and *N*-hydroxysuccinimide without external nucleophiles to produce the acylaminoglutarimides **10**−**12** by intramolecular nucleophilic attack of the primary amide nitrogen.

Further acylaminoglutarimides were made by deprotection and acylation of the amino group of Boc-aminoglutarimide **12** (Scheme 2). Coupling of simple saturated carboxylic acids produced linear nine-, seven-, and five-carbon-side-chain derivatives **13**−**15**, as well as a branched eight-carbon-side-chain analogue **16**. Further functionalization of undec-10-enoylaminoglutarimide **10** gave three further analogues (Scheme 3); the terminal olefin was reduced to give the saturated eleven-carbon-side-chain compound **17**, and the same functional group was oxidatively cleaved to give 10-carbon-side-chain acid glutarimide **18**. The ring nitrogen

Table 2. Effect of WxQ Mimetics on Leukocyte Migration Induced by MCP-1[a]



[a] THP-1 cells were induced to migrate in response to 25 ng/mL MCP-1 in the presence of various concentrations of the compounds shown (from DMSO stock; 1% final concentration). The concentration that reduced migration by 50% (ED_{50}) is shown. Compounds that reduced migration statistically significantly, but by less than 50% at 100 μM (the highest concentration tested), are shown as ">100 μM".

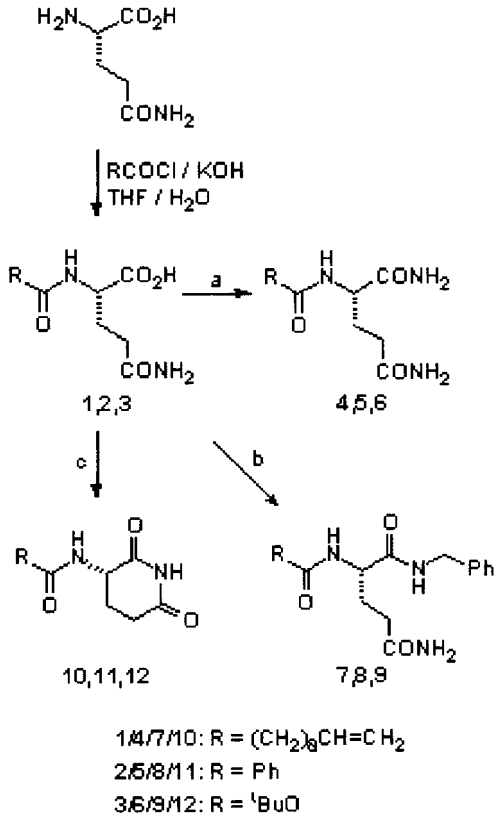
of the parent glutarimide was also methylated to produce compound **19**.

The structurally related acylaminolactam **20** was synthesized from L-ornithine (Scheme 4). Acid-catalyzed formation of the ornithine methyl ester bishydrochloride was followed by treatment with 2 equiv of sodium methoxide to give the free bisamino ester, which cyclized in situ to give the amino lactam. This rather unstable compound was immediately acylated with the undec-10-enoyl chloride to give the aminolactam derivative **20**.

In Vitro Activity of Non-peptide Analogues

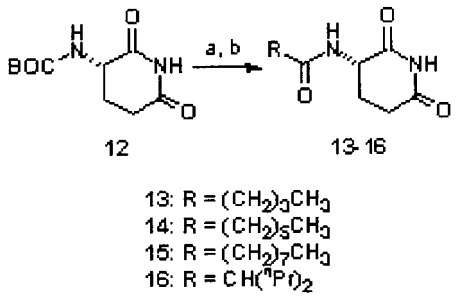
The ability of each of these molecules to inhibit MCP-1-induced migration of THP-1 cells was then tested. Two of the series (using Boc and benzoyl groups as the hydrophobic pharmacophore) were poorly active irrespective of the glutamine mimetic employed (Table 2). In contrast, all four members of the series employing an undec-10-enoyl group as the hydrophobic pharmacophore were active as chemokine inhibitors (Table 2). Of this series, compound **1**, using glutamine itself, had potency similar to that of the WVQ tripeptide (13 μM). The C-terminal primary amide of the same molecule (**4**) had similar potency, but the C-terminal *N*-benzylamide (**7**) was approximately 3-fold less active. In contrast, replacement of the glutamine with 3-aminoglutarimide (**10**) resulted in a very significant increase in potency. Compound **10** (also termed NR58,4) was a potent

Scheme 1[a]



[a] (a) BOP, Et_3N, DMF, NH_3. (b) BOP, Et_3N, DMF, $PhCH_2NH_2$. (c) DCC, NHS, DMF, Δ.

Scheme 2[a]

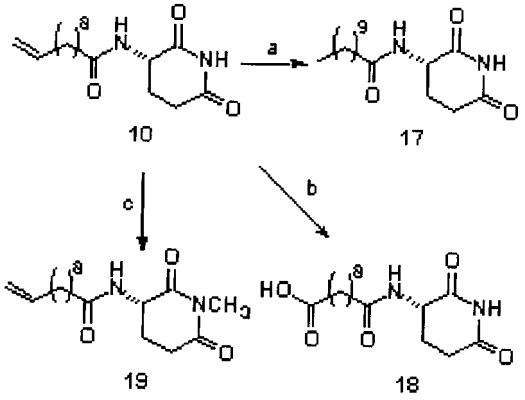


[a] (a) TFA, CH_2Cl_2. (b) BOP, Et_3N, DMF, RCO_2H.

inhibitor of MCP-1-induced leukocyte migration (ED_{50} = 5nM). NR58,4 retains the key properties of peptide 3, inhibiting migration induced by a range of different chemokines with similar ED_{50} values (Figure 3) while having no effect on migration induced by nonchemokines such as C5a (Figure 3). Thus, NR58,4 has properties and potency similar to those of the retroinverso peptide analogue NR58-3.14.3 (ED_{50} = 3nM) as a broad-spectrum chemokine inhibitor.

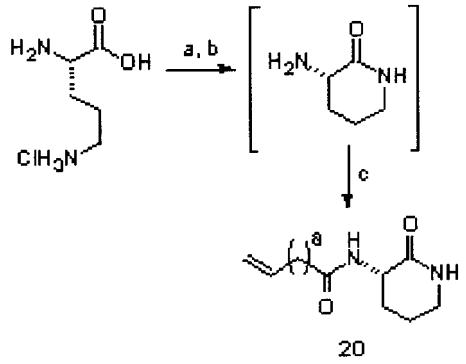
We next performed a more detailed SAR analysis based on the NR58,4 structure (**10**) shown in Table 3. Modifications were made to the undec-10-enoyl group as the hydrophobic pharmacophore, in each case retaining the 3-aminoglutarimide group. Loss of the terminal double bond had very little effect on the activity of the molecule, as did shortening the alkyl chain (such that the molecule with a 5-carbon chain **15**, the shortest tested, was approximately 4-fold less active than NR58,4). In marked contrast, replacement of the undec-10-enoyl group with a branched alkyl chain (compound **16**)

Scheme 3[a]



[a] (a) H_2, Pd/C, EtOAc. (b) $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O. (c) CsOH, CH_3I, DMF.

Scheme 4[a]



[a] (a) CH_3OH, HCl. (b) CH_3ONa, CH_3OH. (c) $CH_2{=}CH(CH_2)_2COCl$, Et_3N, $CHCl_3$.

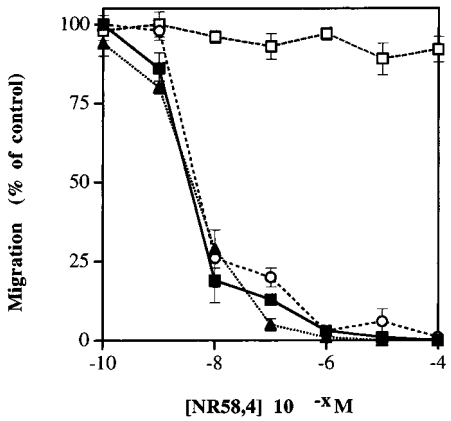
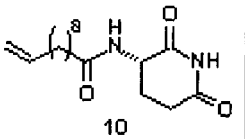
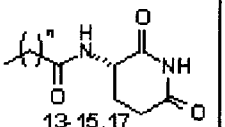
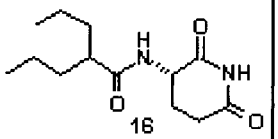
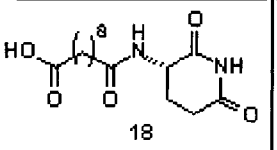
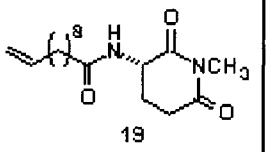
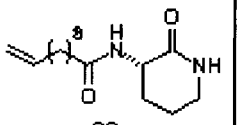


Figure 3. Effect of NR58,4 (compound **10**) on leukocyte migration. THP-1 cells (for MCP-1 and SDF-1α experiments) or freshly prepared human polymorphonuclear cells (for IL-8 and C5a experiments) were induced to migrate in response to the chemokines MCP-1 (filled square), SDF-1α (filled triangle), IL-8 (open circle), or the non-chemokine control C5a (open square) in the presence of various concentrations of NR58,4 (**10**). The vehicle (DMSO) was present at 1% final concentration in all wells (including the controls). The number of leukocytes that migrated is shown as the mean ± sem of triplicate wells, expressed as a percentage of migration occurring in the absence of compound.

dramatically reduced the potency of the molecule (by more than 100-fold), suggesting that there may be a steric requirement for a linear alkyl group at this site in the molecule. Interestingly, however, introduction of a charged carboxyl group at the end of a long alkyl chain

Table 3. Effect of NR58,4 Analogues on Leukocyte Migration Induced by MCP-1[a]

Compound	Activity
 10	5 nM
 13-15,17	17 n=9 10 nM 15 n=7 10 nM 14 n=5 20 nM 13 n=3 33 nM
 16	660 nM
 18	66 nM
 19	33 nM
 20	100 nM

[a] THP-1 cells were induced to migrate in response to 25 ng/mL MCP-1 in the presence of various concentrations of the compounds shown (from DMSO stock; 1% final concentration). The concentration that reduced migration by 50% (ED_{50}) is shown. All the compounds shown reduced migration by >90% at 100 μM.

(compound **18**) reduced activity by only 10-fold compared with NR58,4 (**10**).

Several compounds were also synthesized in which the undec-10-enoyl group was retained, but changes were made to the 3-aminoglutarimide moiety. N-Methylation of the glutarimide ring (**19**) resulted in some loss of activity compared with NR58,4 (**10**), although this compound was still an active broad-spectrum chemokine inhibitor (Table 2). However, the 6-deoxo derivative of NR58,4 (*N*-undec-10-enoyl-3-aminotetrahydropyridin-2-one; **20**) was approximately 20-fold less potent than NR58,4 (**10**) (Table 2). Thus, of all the compounds studied here, *N*-undec-10-enoyl-3-aminogluarimide (NR58,4; **10**) is the most potent broad-spectrum chemokine inhibitor in vitro.

Pharmacology

Intraperitoneal injection of bacterial lipopolysaccharide (LPS) leads to local recruitment of leukocytes and increased systemic levels of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α). This approach has been widely used as a crude model of systemic inflammation in vivo. Although it is unlikely to have

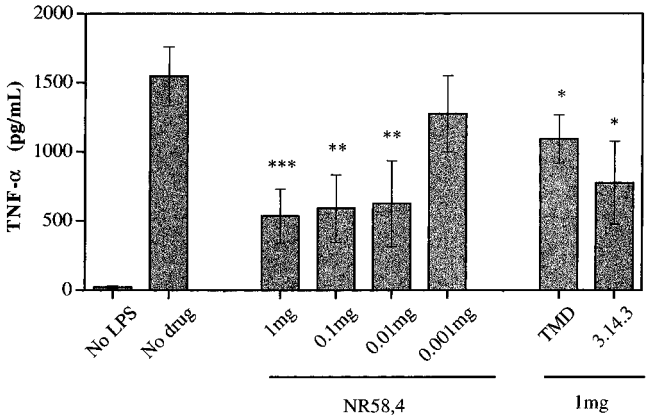


Figure 4. Effect of NR58,4 (compound **10**) on LPS-induced TNF-α production in vivo. Mice (n = 6 per group) were pretreated with NR58,4 (at various doses), thalidomide (TMD), or NR58-3.14.3 by subcutaneous injection. After 45 min, the mice were injected with 750 μg of bacterial lipopolysaccharide (LPS) via the intraperitoneal route. After a further 3 h, the mice were sacrificed and the level of TNF-α in serum was measured by ELISA. The mean (±sem) serum TNF-α for each group of mice is shown (***, $p < 0.005$; **, $p < 0.01$; *, $p < 0.05$ student's unpaired t test versus mice receiving LPS but no pretreatment).

any relevance as a model of human sepsis, nevertheless it represents a rapid screen for anti-inflammatory activity. As a result, we examined the effects of NR58,4 (compound **10**) on LPS-stimulated TNF-α production in this model. Adult female CD-1 mice were given a single dose of either the free acid of NR58,4 dissolved in DMSO vehicle or the potassium salt in saline solution. After 45 min, the animals were given an intraperitoneal injection containing 750 μg of LPS (from *E. coli* O111: B4) in saline. Three hours after injection of LPS, the mice were sacrificed and the level of TNF-α, a serum marker of systemic inflammation, was measured by enzyme-linked immunosorbent assay (ELISA). In mice that received saline alone with no LPS treatment, TNF-α levels were very low (<25 pg/mL). However, injection of LPS resulted in a substantial increase in TNF-α within 3 h (1525 ± 225 pg/mL). Pretreatment with NR58,4 (either as a free acid or as the potassium salt) resulted in a marked reduction in serum TNF-α levels compared to animals pretreated with vehicle alone (Figure 4). In contrast, the substantially less active branched chain analogue **16** had no effect on serum TNF-α even at the highest dose (data not shown).

In a further experiment, various doses of NR58,4 as the potassium salt were given to mice 45 min prior to injection with LPS. Doses as low as 0.3 mg/kg (~1 μmol/kg) significantly reduced serum TNF-α levels (65% reduction; $p < 0.05$ student's unpaired t test). Taken together, these studies demonstrate that NR58,4 has potent anti-inflammatory effects in vivo.

Discussion

Using the ability to inhibit migration of the human myelomonocytic cell line THP-1 in response to various chemokines, we have begun to identify the essential structural motifs present in peptide 3 which are necessary for its broad-spectrum chemokine inhibitory properties. Both deletion analysis and substitution analysis of the peptide demonstrated that the majority of the inhibitory activity resided in the C-terminal tripeptide W*x*Q. On the basis of this analysis, we designed a series of simple compounds coupling various hydrophobic groups to glutamine mimetics and showed that *N*-acyl-3-aminoglutarimides were the most active broad-spectrum chemokine inhibitors in this series. One of these compounds (NR58,4; **10**) was the most potent, inhibiting migration induced by a range of chemokines in the concentration range 5−15 nM in vitro and suppressing LPS-induced TNF-α at doses down to 0.3 mg/kg in vivo.

Importantly, the *N*-acyl-3-aminoglutarimides retained the characteristic property of peptide 3: the ability to inhibit all chemokines tested with very similar potency. This observation strongly suggests that neither peptide 3 nor the *N*-acyl-3-aminoglutarimides are functioning as simple receptor anatagonists. It is very unlikely that a range of structurally distinct molecules would bind to and inhibit a range of different (albeit related) chemokine receptors with similar affinity. Consistent with this hypothesis, we have recently demonstrated that peptide 3 does not bind to CCR1, CCR2, CCR4, CCR5, CXCR1, CXCR2, or CXCR4 overexpressing CHO cells to any greater extent than to the parental CHO cells (unpublished data). It is likely, therefore, that both peptide 3 and *N*-acyl-3-aminoglutarimides mediate chemokine inhibition by a mechanism, as yet unidentified, that is distinct from that of simple receptor antagonists. These compounds are currently being tested against a range of signaling pathways in an attempt to determine the precise molecular target for broad-spectrum chemokine inhibitors.

An important, but unresolved, consideration is the stereochemistry of the active *N*-acyl-3-aminoglutarimides we have studied. The chiral starting material for the synthesis of NR58,4 **10** is L-glutamine, and it is likely that the synthesis of the 3-aminoglutarimide proceeds with retention of stereochemistry to ultimately yield the *N*-acyl-L-3-aminoglutarimides. However, on the basis of experience with other derivatized aminoglutarimides (such as thalidomide), it is likely that the proton at the chiral center is sufficiently acidic to allow exchange to occur in a protic solvent at an appreciable rate leading to racemization. Since the biological activity of these molecules, both in vitro and in vivo, is assessed in aqueous medium, we have deliberately avoided assigning the steroeochemistry of the active forms because we cannot exclude the possibility that formation of a small amount of *N*-acyl-D-3-aminoglutarimide through racemization is required for biological activity.

The 3-aminoglutarimide group is not a common constituent of biologically active compounds, with one notable exception: thalidomide. Although originally used as a sedative,[18] it was later discovered to be a selective inhibitor of TNF-α biosynthesis[19] and hence to have anti-inflammatory properties.[20] It is these anti-inflammatory properties that led to the continued clinical use of thalidomide, most notably to reduce the impact of leprosy infection.[21] At present, however, the molecular basis for suppression of TNF-α production by thalidomide remains unknown. Our studies of simpler *N*-acyl derivatives of 3-aminoglutarimide suggest that inhibition of chemokine activity is a plausible mechanism for the reduction of TNF-α biosynthesis following

thalidomide treatment, since other chemokine inhibitors are known to reduce TNF-α biosynthesis.[14] Consistent with this suggestion, we find that thalidomide does inhibit MCP-1-induced THP-1 migration (ED_{50} = 60 μM), although it is at least 10 000 times less potent than NR58,4 **10**. Because the well-publicized teratogenic side effects of thalidomide are likely to result from oxidation of the aryl ring[22,23] (substitution of all 4 aryl protons with fluorine[24] abolishes the teratogenic properties of the molecule), it is unlikely that NR58,4 **10** will have teratogenic properties similar to those of thalidomide. However, it will be important to rigorously exclude any possible teratogenic effects of *N*-acyl-3-aminoglutarimides prior to consideration of their use as anti-inflammatory agents in humans.

The injection of NR58,4 **10** into mice reduced TNF-α production in response to endotoxin injection to an extent similar to that of the modified peptide NR58-3.14.3,[14] suggesting that both molecules have similar anti-inflammatory properties in vivo. At high doses of NR58,4 (above 50 mg/kg), we observed anaesthetic-like effects with the mice becoming comatose for 4−6 h before recovering with apparently no chronic toxic effects. However, at lower doses (which still have maximal anti-inflammatory properties) we did not observe any acute toxicity, consistent with our previous observation using NR58-3.14.3 that, at least in the short term, broad-spectrum chemokine inhibition may be well tolerated.[14−16] However, NR58,4 is at least 5-fold more potent than NR58-3.14.3 in vivo (despite having similar potencies in vitro), retaining activity at 100 nmol/kg (whereas NR58-3.14.3 is inactive below 5 μmol/kg in the LPS endotoxemia model). This difference is likely to result from the pharmacodynamic profiles of the two molecules; NR58-3.14.3 is cleared from plasma at the glomerular filtration rate (plasma $t_{1/2}$ = 20 min; ref 17), whereas the hydrophobic NR58,4 may have a much longer plasma residence time. The cheap, simple syntheses of *N*-acyl-3-aminoglutarimides such as NR58,4 **10**, together with any improvements in pharmacodynamics, are likely to render these small molecules the broad-spectrum chemokine inhibitors of choice over NR58-3.14.3 for further investigation of the pharmacology of this class of drugs in vivo.

Experimental Section

General Experimental Procedures. All solvents were distilled before use. THF was freshly distilled from lithium aluminum hydride, while CH_2Cl_2 was freshly distilled from calcium hydride. Triphenylmethane was used as an indicator for THF. Methanol was freshly distilled from sodium methoxide. DMF and DMSO were dried by stirring over and distilling from calcium hydride (at reduced pressure when necessary) and were stored over activated 4 Å molecular sieves. All reactions were carried out with oven-dried glassware, and all reactions in nonaqueous solutions were carried out under an atmosphere of argon.

Flash column chromatography was carried out using Merck Kieselgel 60 (230−400 mesh). Thin-layer chromatography was carried out on commercially available precoated plates (Merck Kieselgel $60F_{254}$). Proton and carbon NMR spectra were recorded on Bruker DRX400 or DPX400 Fourier transform spectrometers using an internal deuterium lock with DMSO-d_6 as solvent. Chemical shifts are quoted in parts per million downfield of tetramethylsilane, and values of coupling constants (*J*) are given in hertz and are quoted to the nearest 0.5 Hz (or nearest 0.1 Hz for $J < 2$ Hz). Carbon NMR spectra were recorded with broad-band proton decoupling, and assignments were aided by distortionless enhancement by polarization transfer (DEPT) analysis. Melting points were measured on a Stuart Scientific melting point apparatus (SMP 1) and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1600 (FT-IR) spectrophotometer. Mass spectra were recorded on a Kratos (MS890) single-beam spectrometer or a micromass platform with a HP1050 LC system. Microanalyses were carried out by the staff of the University Chemical Laboratory using Carlo Erba 1106 or Perkin-Elmer 240 automatic analyzers.

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter (using the sodium D line; 589 nm). Specific rotations are given in units of 10^{-1} deg dm^2 g^{-1}.

(*S*)-*N*-α-Undec-10-enoylglutamine 1. L-(*S*)-Glutamine (14.62 g, 0.1 mol) and KOH (11.2 g, 0.2 mol) were dissolved in water (200 mL) at 20 °C. A solution of undec-10-enoyl chloride (20.3 g, 0.1 mol) in THF (200 mL) was then added over 10 min. The reaction mixture was stirred for 16 h at 20 °C and was then reduced in vacuo to a volume of approximately 250 mL. Concentrated HCl (ca. 12 M) was added dropwise until the solution was pH 1. The white precipitate was collected by filtration, then washed with water (100 mL) and diethyl ether (100 mL). The solid was then dissolved in refluxing toluene (500 mL), and the excess water present was removed by distillation at ambient pressure using a Dean−Stark trap. The solution was then allowed to cool to 20 °C. After 16 h, the white solid precipitate was collected via filtration and recrystallized from EtOAc. This solid was dried in vacuo to give (*S*)-*N*-α-undec-10-enoylglutamine as a white powder (9.36 g, 30%): mp 112−113 °C; $[\alpha]^{20}_D$ (*c* 1.13, MeOH) −4.5; ν_{max}/cm^{-1} 1732 (C=O, acid), 1685, 1670, 1642, 1621 (C=O, amide), 1545, 1523 (N−H, amide). Anal. ($C_{16}H_{28}N_2O_4$) C, H, N: calcd C 61.5, H 9.0, N 9.0; found C 61.3, H 9.0, N 8.6. ^{1}H NMR: δ_H 12.40 (1H, br s, OH), 8.00 (1H, d, *J* 7.5, $C_{10}H_{19}CONH$), 7.25 (1H, br s, $CONH_2$), 6.72 (1H, br s, $CONH_2$), 5.77 (1H, ddt, *J* 17.0, 10.5 and 6.5, $CH{=}CH_2$), 4.97 (1H, dq, *J* 17.0 and 1.5, $CH{=}CH_2$), 4.91 (1H, ddt, *J* 10.0, 2.5 and 1.2, $CH{=}CH_2$), 4.11 (1H, ddd, *J* 9.0, 8.0 and 5.0, NCH), 2.12−2.05 (4H, m, CH_2CONH and CH_2CONH_2), 1.98 (1H, br q, *J* 6.5, $CH_2CH{=}CH_2$), 1.90 (1H, dtd, *J* 14.0, 8.0, and 5.0, $CH_2CH_2CONH_2$), 1.71 (1H, dtd, *J* 14.0, 9.0, and 6.5, $CH_2CH_2CONH_2$), 1.46 (2H, br qn, *J* 7.0, CH_2), 1.32 (2H, br qn, *J* 7.0, CH_2), 1.28−1.18 (8H, m, $CH_2 \times 4$). ^{13}C NMR: δ_C 174.0, 173.9, 172.8 (C=O), 115.0 ($CH{=}CH_2$), 129.3 ($CH{=}CH_2$), 51.9 (NCH), 35.5, 33.6, 31.8, 29.2 (×2), 29.0, 28.9, 28.7, 27.3, 25.6 (CH_2). HRMS (+ESI) $C_{16}H_{28}N_2O_4Na$: calcd 335.1947; found 335.1935.

(*S*)-*N*-α-Benzoylglutamine 2. Benzoyl chloride (7.03 g, 50 mmol) dissolved in THF (200 mL) was added slowly to a stirred solution containing water (200 mL), L-(*S*)-glutamine (7.30 g, 50 mmol), KOH (2.80 g, 50 mmol), and K_2CO_3 (6.90 g, 50 mmol). The reaction was then stirred at ambient temperature for 15 h. Concentrated HCl (ca. 12 M) was added dropwise until the solution was pH 1, and the solvents were then removed to dryness under reduced pressure with the aid of the addition of toluene. The resulting solid was recrystallized from toluene/MeOH to give (*S*)-*N*-α-benzoylglutamine as a white solid (5.25 g, 42%): mp 146−147 °C; $[\alpha]^{20}_D$ (*c* 0.95, MeOH) −9.1; ν_{max}/cm^{-1} 1710 (C=O, acid), 1655, 1634, 1608 (C=O, amide), 1538 (N−H, amide). Anal. ($C_{12}H_{14}N_2O_4$) C, H, N: calcd C 57.5, H 5.6, N 11.2; found C 57.1, H 5.5, N 11.1. ^{1}H NMR: δ_H 12.50 (1H, br s, OH), 8.70 (1H, d, *J* 7.5, PhCONH), 7.85 (2H, br d, *J* 7.0, *ortho*-Ph), 7.55−7.48 (1H, m, *para*-Ph), 7.47−7.42 (2H, m, *meta*-Ph), 7.33 (1H, br s, $CONH_2$), 6.80 (1H, br s, $CONH_2$), 4.32 (1H, ddd, *J* 12.0, 7.5, and 4.0, NCH), 2.06 (2H, br t, *J* 7.0, CH_2CONH_2), 2.12−2.01 (1H, m, $CH_2CH_2CONH_2$), 1.98−1.86 (1H, m, $CH_2CH_2CONH_2$). ^{13}C NMR: δ_C 174.0, 173.6, 166.5 (C=O), 134.1 (*ipso*-Ph), 131.5, 128.4, 127.5 (*ortho*-, *meta*-, and *para*-Ph), 52.6 (NCH), 31.7, 26.3 (CH_2). HRMS (+ESI) $C_{12}H_{14}N_2O_4Na$: calcd 273.0851; found 273.0850.

General Procedure for Synthesis of (*S*)-*N*-α-Acylglutamineamides 4−6 (Method A). The appropriate (*S*)-*N*-α-acylglutamine (1 mmol) was dissolved in DMF (5 mL).

Triethylamine (0.28 cm^3, 2 mmol) and BOP (442 mg, 1 mmol) were added, and the reaction mixture was stirred for 1 h. The reaction mixture was then added to liquid ammonia (20 mL) at −40 °C, and the resulting mixture was allowed to warm to ambient temperature over 15 h.

(*S*)-*N*-α-Undec-10-enoylglutamineamide 4. Method A was used, and the crude precipitated product was removed from the reaction mixture via filtration, washed with water (5 mL) and EtOAc (5 mL), and dried in vacuo. The crude product was recrystallized from MeOH to give (*S*)-*N*-α-undec-10-enoylglutamineamide as a white solid (136 mg, 41%): mp 170−171 °C; $[\alpha]^{20}_D$ (*c* 0.91, DMSO) −50.5; ν_{max}/cm^{-1} 1655, 1637 (C═O, amide), 1540 (N−H, amide). Anal. ($C_{16}H_{29}N_3O_3$) C, H, N: calcd C 61.7, H 9.4, N 13.5; found C 61.7, H 9.4, N 13.5. ^{1}H NMR: δ_H 7.80 (1H, d, *J* 8.0, $C_{10}H_{19}CONH$), 7.26 (2H, br s, $CONH_2$), 6.96 (1H, br s, $CONH_2$), 6.72 (1H, br s, $CONH_2$), 5.79 (1H, ddt, *J* 17.0, 10.0, and 6.5, $CH{=}CH_2$), 4.99 (1H, dq, *J* 17.0 and 1.5, $CH{=}CH_2$), 4.93 (1H, ddt, *J* 10.0, 3.0, and 1.0, $CH{=}CH_2$), 4.14 (1H, td, *J* 8.5 and 5.5, NCH), 2.11 (2H, t, *J* 7.5, CH_2CO), 2.06 (2H, t, *J* 8.0, CH_2CO), 2.00 (2H, br q, *J* 7.0, $CH_2{-}CH{=}CH_2$), 1.91−1.79 (1H, m, $CH_2CH_2CONH_2$), 1.74−1.62 (1H, m, $CH_2CH_2CONH_2$), 1.47 (2H, br qn, *J* 6.5, CH_2), 1.34 (2H, br qn, *J* 6.5, CH_2), 1.29−1.20 (8H, m, $CH_2 \times 4$). ^{13}C NMR: δ_C 173.7, 173.6, 172.1 (C═O), 138.8 ($CH{=}CH_2$), 114.7 ($CH{=}CH_2$), 52.0 (NCH), 35.3, 33.3, 31.7, 28.8 (×2), 28.7, 28.6, 28.3, 28.0, 25.3 (CH_2). HRMS (+ESI) $C_{16}H_{29}N_3O_3Na$: calcd 334.2107; found 334.2099.

(*S*)-*N*-α-Benzoylglutamineamide 5. Method A was used, and the crude precipitated product was removed from the reaction via filtration, washed with water (5 mL) and EtOAc (5 mL), and dried in vacuo. The crude product was recrystallized from MeOH to give (*S*)-*N*-α-benzoylglutamineamide as a white solid (132 mg, 53%): mp 210−211 °C; $[\alpha]^{20}_D$ (*c* 0.63, DMSO) −90.6; ν_{max}/cm^{-1} 1659, 1631 (C═O, amide), 1521 (N−H, amide). Anal. ($C_{12}H_{15}N_3O_3$) C, H, N: calcd C 57.8, H 6.1, N 16.9; found C 57.4, H 6.0, N 16.6. ^{1}H NMR: δ_H 8.47 (1H, d, *J* 7.5, PhCO*NH*), 7.86 (2H, br d, *J* 8.0, *ortho*-Ph), 7.53−7.48 (1H, m, *para*-Ph), 7.47−7.41 (2H, m, *meta*-Ph), 7.40 (1H, br s, $CONH_2$), 7.34 (1H, br s, $CONH_2$), 7.00 (1H, br s, $CONH_2$), 6.78 (1H, br s, $CONH_2$), 4.30 (1H, ddd, *J* 9.0, 8.0, and 5.0, NCH), 2.23−2.10 (2H, m, CH_2CONH_2), 2.03−1.82 (2H, m, $CH_2CH_2{-}CONH_2$). ^{13}C NMR: δ_C 174.7, 174.1, 166.8 (C═O), 134.5 (*ipso*-Ph), 131.8, 128.6, 127.9 (*ortho*-, *meta*-, and *para*-Ph), 53.7 (NCH), 32.1, 27.7 (CH_2). HRMS (+ESI) $C_{12}H_{15}N_3O_3Na$: calcd 272.1011; found 272.1008.

(*S*)-*N*-α-*tert*-Butoxycarbonylglutamineamide 6. Method A was used, and the crude reaction was partitioned between water (30 mL) and EtOAc (3 × 50 mL). The combined organic extracts were washed with water (2 × 50 mL), dried (Na_2SO_4), and reduced in vacuo. The residue was recrystallized from EtOAc to give (*S*)-*N*-α-*tert*-butoxycarbonylglutamineamide as a white solid (91 mg, 37%): mp 116−117 °C; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −1.4; ν_{max}/cm^{-1} 1685 (C═O, carbamate), 1645 (C═O, amide), 1521 (N−H, amide). Anal. ($C_{10}H_{19}N_3O_4$) C, H, N: calcd C 49.0, H 7.8, N 17.1; found C 48.7, H 7.7, N 17.0. ^{1}H NMR: δ_H 7.26 (1H, s, NH), 7.22 (1H, s, NH), 6.96 (1H, s, NH), 6.78−6.69 (2H, m, NH × 2), 3.78 (1H, td, *J* 8.5 and 5.5, NCH), 2.12−1.98 (2H, m, CH_2CONH_2), 1.85−1.72 (1H, m, $CH_2CH_2CONH_2$), 1.69−1.58 (1H, m, $CH_2CH_2CONH_2$), 1.34 (9H, s, $CH_3 \times 3$). ^{13}C NMR: δ_C 175.0, 174.9, 156.3 (C═O), 79.0 ($OCMe_3$), 54.9 (NCH), 32.6 (CH_2), 29.2 ($CH_3 \times 3$), 28.8 (CH_2). HRMS (+ESI) $C_{10}H_{19}N_3O_4Na$: calcd 268.1273; found 268.1270.

General Procedure for Synthesis of (*S*)-*N*-α-Acylglutaminebenzylamides 7−9 (Method B). The appropriate (*S*)-*N*-α-acylglutamine (1 mmol) was dissolved in DMF (5 mL). Triethylamine (0.28 cm^3, 2 mmol), BOP (442 mg, 1 mmol), and $PhCH_2NH_2$ (214 mg, 2 mmol) were added, and the reaction mixture was stirred for 15 h.

(*S*)-*N*-α-Undec-10-enoylglutaminebenzylamide 7. Method B was used, and the crude precipitated product was removed from the reaction via filtration, washed with water (5 mL) and EtOAc (5 mL), and dried in vacuo. The crude product was recrystallized from EtOAc/MeOH to give (*S*)-*N*-α-undec-10-enoylglutamineamide as a white solid (204 mg, 51%): mp 173−174 °C; $[\alpha]^{20}_D$ (*c* 0.50, DMSO) −117.2; ν_{max}/cm^{-1} 1662, 1627 (C═O, amide), 1538 (N−H, amide). Anal. ($C_{23}H_{35}N_3O_3$) C, H, N: calcd C 68.8, H 8.8, N 10.5; found C 68.6, H 8.7, N, 10.4. ^{1}H NMR: δ_H 8.31 (1H, t, *J* 6.0, $NHCH_2Ph$), 7.91 (1H, d, *J* 8.0, $C_{10}H_{19}CONH$), 7.30−7.16 (6H, m, Ph and NH_2), 6.71 (1H, br s, $CONH_2$), 5.76 (1H, ddt, *J* 17.0, 10.0, and 6.5, $CH{=}CH_2$), 4.96 (1H, dq, *J* 17.0 and 1.5, $CH{=}CH_2$), 4.91 (1H, ddt, *J* 10.0, 2.0, and 1.0, $CH{=}CH_2$), 4.30−4.17 (3H, m, NCH and NCH_2-Ph), 2.13−2.03 (4H, m, CH_2CO), 1.97 (2H, br q, *J* 7.0, $CH_2{-}CH{=}CH_2$), 1.91−1.81 (1H, m, $CH_2CH_2CONH_2$), 1.75−1.64 (1H, m, $CH_2CH_2CONH_2$), 1.45 (2H, br qn, *J* 7.0, CH_2), 1.31 (2H, br qn, *J* 7.0, CH_2), 1.26−1.16 (8H, m, $CH_2 \times 4$). ^{13}C NMR: δ_C 174.1, 172.8, 172.0 (C═O), 139.8 (*ipso*-Ph), 139.3 ($CH{=}CH_2$), 128.6, 127.4, 127.1 (*ortho*-, *meta*-, and *para*-Ph), 115.1 ($CH{=}CH_2$), 52.8 (NCH), 42.4, 35.7, 33.6, 32.0, 29.2 (×2), 29.1, 28.9, 28.7, 28.3, 25.6 (CH_2). HRMS (+ESI) $C_{23}H_{35}N_3O_3Na$: calcd 424.2576; found 424.2580.

(*S*)-*N*-α-Benzoylglutaminebenzylamide 8. Method B was used, and the crude precipitated product was removed from the reaction via filtration, washed with water (5 mL) and EtOAc (5 mL), and dried in vacuo. The crude product was recrystallized from H_2O/MeOH to give (*S*)-*N*-α-benzoylglutaminebenzylamide as a white solid (88 mg, 26%): mp 212−213 °C; $[\alpha]^{20}_D$ (*c* 1.00, DMSO) −25.8; ν_{max}/cm^{-1} 1655, 1622 (C═O, amide), 1534 (N−H, amide). Anal. ($C_{19}H_{21}N_3O_3 + \_H_2O$) C, H, N: calcd C 65.5, H 6.4, N 12.1; found C 65.4, H 6.1, N 12.0. ^{1}H NMR: δ_H 8.61 (1H, d, *J* 7.5, PhCO*NH*), 8.45 (1H, t, *J* 6.0, $NHCH_2Ph$), 7.88 (2H, br d, *J* 8.0, *ortho*-PhCO), 7.51 (1H, t, *J* 8.0, Ph), 7.45 (2H, t, *J* 7.5, Ph), 7.33 (1H, br s, $CONH_2$), 7.31−7.16 (5H, m, Ph), 6.83 (1H, br s, $CONH_2$), 4.39 (1H, ddd, *J* 9.5, 7.5, and 5.0, NCH), 4.27 (2H, d, *J* 6.0, CH_2Ph), 2.25−2.10 (2H, m, CH_2CONH_2), 2.07−1.85 (2H, m, $CH_2CH_2CONH_2$). ^{13}C NMR: δ_C 174.2, 171.7, 166.5 (C═O), 139.6, 134.2 (*ipso*-Ph), 129−126 (m, *ortho*-, *meta*-, and *para*-Ph), 53.8 (NCH), 42.1, 32.1, 27.7 (CH_2). HRMS (+ESI) $C_{19}H_{21}N_3O_3Na$: calcd 362.1481; found 362.1476.

(*S*)-*N*-α-*tert*-Butoxycarbonylglutaminebenzylamide 9. Method B was used, and the crude reaction was partitioned between water (30 mL) and EtOAc (3 × 50 mL). The combined organic extracts were washed with 0.1 M HCl (2 × 50 mL), dried (Na_2SO_4), and reduced in vacuo. The residue was recrystallized from EtOAc to give (*S*)-*N*-α-*tert*-butoxycarbonylglutaminebenzylamide as a white solid (74 mg, 22%): mp 151−152 °C; $[\alpha]^{20}_D$ (*c* 0.66, MeOH) −15.3; ν_{max}/cm^{-1} 1694 (C═O, carbamate), 1657 (C═O, amide), 1534 (N−H, amide). Anal. ($C_{17}H_{25}N_3O_4$) C, H, N: calcd C 60.9, H 7.5, N 12.5; found C 60.5, H 7.4, N 12.5. ^{1}H NMR: δ_H 8.30 (1H, t, *J* 6.0, $NHCH_2{-}Ph$), 7.30−7.16 (6H, m, Ph and NH_2), 6.91 (1H, d *J* 8.0, N*H*CH), 6.67 (1H, s, NH_2), 4.28 (1H, dd, *J* 15.5 and 6.0, CH_2Ph), 4.23 (1H, dd, *J* 15.5 and 6.0, CH_2Ph), 3.89 (1H, td, *J* 8.5 and 5.5, NCH), 2.15−2.00 (2H, m, CH_2CONH_2), 1.90−1.77 (1H, m, $CH_2{-}CH_2CONH_2$), 1.75−1.61 (1H, m, $CH_2CH_2CONH_2$), 1.36 (9H, s, $CH_3 \times 3$). ^{13}C NMR: δ_C 175.0, 173.2, 156.5 (C═O), 140.6 (*ipso*-Ph), 129.4, 128.1, 127.9 (*ortho*-, *meta*-, and *para*-Ph), 79.3 ($OCMe_3$), 55.6 (NCH), 43.1 (CH_2Ph), 32.8 (CH_2), 29.4 ($CH_3 \times 3$), 28.8 (CH_2). HRMS (+ESI) $C_{17}H_{25}N_3O_4Na$: calcd 358.1743; found 358.1730.

General Procedure for the Synthesis of 3-Acylaminoglutarimides (Method C). The appropriate *N*-α-acylated glutamine (10 mmol) was dissolved in DMF (20 mL) along with *N*-hydroxysuccinimide (1.15 g, 10 mmol) and 1,3-dicyclohexylcarbodiimide (2.06 g, 10 mmol). The reaction mixture was heated to 80 °C for 6 h and then allowed to cool. The DMF was removed under reduced pressure, and EtOAc (100 mL) was added to the residue. The resulting suspension was filtered, retaining the filtrate. Then the solid was extracted with additional EtOAc (100 mL), and the combined filtrate solution was reduced in vacuo.

(*S*)-3-(Undec-10-enoylamino)glutarimide 10. Method C was used, and the residue was subjected to silica column chromatography (diethyl ether) and then recrystallized from EtOAc to give (*S*)-3-(undec-10-enoylamino)glutarimide as a white solid (1.79 g, 61%): mp 124−125 °C; $[\alpha]^{20}_D$ (*c* 1.07, MeOH) −13.9; ν_{max}/cm^{-1} 1720 (C═O, imide), 1653 (C═O,

amide), 1538 (N−H, amide). Anal. ($C_{16}H_{26}N_2O_3$) C, H, N: calcd C 65.3, H 8.9, N 9.5; found C 65.3, H 8.9, N 9.5. ^{1}H NMR: δ_H 10.74 (1H, br s, CONHCO), 8.08 (1H, d, *J* 8.5, $C_{10}H_{19}CONH$), 5.75 (1H, ddt, *J* 17.0, 10.0, and 6.5, $CH{=}CH_2$), 4.96 (1H, dq, *J* 17.0 and 1.5, $CH{=}CH_2$), 4.90 (1H, ddt, *J* 10.0, 2.0, and 1.0, $CH{=}CH_2$), 4.49 (1H, q, *J* 8.5, NCH), 2.74−2.62 (1H, m, CH_2-CONHCO), 2.46 (1H, dt *J* 17.0 and 4.0, $CH_2CONHCO$), 2.09 (2H, t, *J* 7.5, $C_9H_{17}CH_2CONH$), 1.98 (2H, br q, *J* 7.5, $CH_2CH{=}CH_2$), 1.92−1.82 (2H, m, $NCHCH_2CH_2CONH$), 1.47 (2H, br qn, *J* 7.0, CH_2), 1.29 (2H, br qn, *J* 6.0, CH_2), 1.28−1.18 (8H, m, $CH_2 \times 4$). ^{13}C NMR: δ_C 173.0, 172.3, 172.3 (C═O), 138.9 ($CH{=}CH_2$), 114.7 ($CH{=}CH_2$), 49.0 (NCH), 35.3, 33.3, 31.0, 28.8 (×2), 28.6, 28.5, 28.3, 25.3, 24.5 (CH_2). HRMS (+ESI) $C_{16}H_{26}N_2O_3$-Na: calcd 317.1841; found 317.1827.

(*S*)-3-(Benzoylamino)glutarimide 11. Method C was used, and the residue was subjected to silica column chromatography (diethyl ether) and then recrystallized from EtOAc to give (*S*)-3-(benzoylamino)glutarimide as a white solid (626 mg, 27%): mp 180 °C (dec); $[\alpha]^{20}_D$ (*c* 0.60, MeOH) −55.1; ν_{max}/cm^{-1} 1702 (C═O, imide), 1642 (C═O, amide), 1528 (N−H, amide). Anal. ($C_{12}H_{12}N_2O_3$) C, H, N: calcd C 62.1, H 5.2, N 12.1; found C 61.6, H 5.2, N 11.8. ^{1}H NMR: δ_H 10.85 (1H, s, CONHCO), 8.75 (1H, d, *J* 8.5, PhCONH), 7.89−7.84 (2H, m, *ortho*-Ph), 7.57−7.53 (1H, m, *para*-Ph), 7.52−7.46 (2H, m, *meta*-Ph), 4.78 (1H, ddd, *J* 12.5, 8.5, and 5.5, NCH), 2.80 (1H, ddd, *J* 17.5, 13.5, and 5.5, CH_2CONH), 2.55 (1H, dt, *J* 17.5 and 3.5, CH_2CONH), 2.13 (1H, qd, *J* 13.0 and 4.5, CH_2CH_2-CONH), 1.98 (1H, dtd, *J* 13.0, 5.5, and 3.0, $CH_2CH_2CONH_2$). ^{13}C NMR: δ_C 173.1, 172.3, 166.2 (C═O), 134.0 (*ipso*-Ph), 131.6, 128.5, 127.4 (*ortho*-, *meta*-, and *para*-Ph), 49.6 (NCH), 31.1, 24.3 (CH_2). HRMS (+ESI) $C_{12}H_{12}N_2O_3Na$: calcd 255.0746; found 255.0755.

(*S*)-3-(*tert*-Butoxycarbonylamino)glutarimide 12. Method C was used, and the residue was subjected to silica column chromatography (diethyl ether) and then recrystallized from EtOAc to give (*S*)-3-(*tert*-butoxycarbonylamino)glutarimide as a white solid (1.30 g, 57%): mp 152−153 °C; $[\alpha]^{20}_D$ (*c* 0.63, MeOH) −55.2; ν_{max}/cm^{-1} 1723 (C═O, imide), 1685 (C═O, carbamate), 1538 (N−H, carbamate). Anal. ($C_{10}H_{16}N_2O_4$) C, H, N: calcd C 52.6, H 7.1, N 12.3; found C 53.1, H 7.1, N, 12.1. ^{1}H NMR: δ_H 10.72 (1H, s, CONHCO), 7.11 (1H, d, *J* 8.5, NHCH), 4.25−4.13 (1H, m, NCH), 2.68 (1H, ddd, *J* 18.0, 8.5, and 6.5, CH_2CONH), 2.45 (1H, dt, *J* 17.5 and 3.5, CH_2CONH), 2.01−1.82 (2H, m, CH_2CH_2CONH), 1.36 (9H, s, $CH_3 \times 3$). ^{13}C NMR: δ_C 175.0, 174.5, 157.4 (C═O), 80.1 ($OCMe_3$), 52.3 (NCH), 32.9 (CH_2), 30.1 ($CH_3 \times 3$), 25.7 (CH_2). HRMS (+ESI) $C_{10}H_{16}N_2O_4Na$: calcd 251.1008; found 251.1012.

General Procedure for the Synthesis of 3-Acylaminoglutarimides (Method D). (*S*)-3-(*tert*-Butoxycarbonylamino)-glutarimide **12** (228 mg, 1 mmol) was dissolved in CH_2Cl_2 (3 mL), and trifluoroacetic acid (1 mL) was added. The reaction was stirred for 1 h and then reduced in vacuo to give a thick oil. The residue was redissolved in DMF (2 mL), and to this solution was added BOP (442 mg, 1 mmol), triethylamine (1 mL), and the appropriate carboxylic acid to be incorporated as a side chain (1 mmol). The reaction mixture was stirred at ambient temperature for 15 h and was then partitioned between water (20 mL) and EtOAc (3 × 50 mL). The combined organic layers were washed with 0.1 M HCl, dried (Na_2SO_4), and reduced in vacuo to give the crude product. This residue was subjected to silica column chromatography (EtOAc) and then recrystallized (EtOAc) to give the 3-acylaminoglutarimide.

(*S*)-3-(Nonanoylamino)glutarimide 13. Method D was used (164 mg, 61%): mp 121−122 °C; $[\alpha]^{20}_D$ (*c* 1.00, MeOH) −32.9; ν_{max}/cm^{-1} 1716 (C═O, imide), 1647 (C═O, amide), 1551 (N−H, amide). Anal. ($C_{14}H_{24}N_2O_3$) C, H, N: calcd C 62.7, H 9.0, N 10.4; found C 63.0, H 8.9, N 10.5. ^{1}H NMR: δ_H 10.71 (1H, br s, CONHCO), 8.09 (1H, d, *J* 8.0, $C_8H_{17}CONH$), 4.50 (1H, q, *J* 8.0, NCH), 2.75−2.62 (1H, m, $CH_2CONHCO$), 2.46 (1H, dt, *J* 17.5 and 3.5, $CH_2CONHCO$), 2.09 (2H, t, *J* 7.5, $C_7H_{15}CH_2CONH$), 1.95−1.82 (2H, m, $NCHCH_2CH_2CONH$), 1.48 (2H, br qn, *J* 7.0, CH_2), 1.30−1.17 (10H, m, CH_2), 0.84 (3H, br t, *J* 7.0, CH_3). ^{13}C NMR: δ_C 173.4, 172.7 (×2) (C═O), 49.4 (NCH), 35.7, 31.7, 31.3, 29.2, 29.0 (×2), 25.6, 24.8, 22.5 (CH_2), 14.4 (CH_3). HRMS (+ESI) $C_{14}H_{24}N_2O_3Na$: calcd 291.1685; found 291.1674.

(*S*)-3-(Heptanoylamino)glutarimide 14. Method D was used (101 mg, 42%): mp 119−120 °C; $[\alpha]^{20}_D$ (*c* 0.63, MeOH) −37.1; ν_{max}/cm^{-1} 1716 (C═O, imide), 1646 (C═O, amide), 1548 (N−H, amide). Anal. ($C_{12}H_{20}N_2O_3$) C, H, N: calcd C 60.0, H 8.4, N 11.7; found C 59.9, H 8.5, N 11.6. ^{1}H NMR: δ_H 10.73 (1H, br s, CONHCO), 8.09 (1H, d, *J* 8.5, $C_6H_{13}CONH$), 4.50 (1H, q, *J* 8.5, NCH), 2.69 (1H, ddd, *J* 17.5, 7.5, and 3.5, CH_2-CONHCO), 2.46 (1H, dt, *J* 17.5 and 3.5, $CH_2CONHCO$), 2.10 (2H, t, *J* 7.5, $C_5H_{11}CH_2CONH$), 1.95−1.83 (2H, m, $NCHCH_2$-CH_2CONH), 1.48 (2H, br qn, *J* 7.0, CH_2), 1.31−1.18 (6H, m, CH_2), 0.84 (3H, t, *J* 7.0, CH_3). ^{13}C NMR: δ_C 173.1, 172.3 (×2) (C═O), 49.0 (NCH), 35.4, 31.0 (×2), 28.3, 25.3, 24.5, 22.1 (CH_2), 14.0 (CH_3). HRMS (+ESI) $C_{12}H_{20}N_2O_3Na$: calcd 263.1372; found 263.1368.

(*S*)-3-(Pentanoylamino)glutarimide 15. Method D was used (62 mg, 29%): mp 144−145 °C; $[\alpha]^{20}_D$ (*c* 0.35, MeOH) −40.3; ν_{max}/cm^{-1} 1699 (C═O, imide), 1618 (C═O, amide), 1550 (N−H, amide). Anal. ($C_{10}H_{16}N_2O_3$) C, H, N: calcd C 56.6, H 7.6, N 13.2; found C 56.2, H 7.5, N 12.9. ^{1}H NMR: δ_H 10.74 (1H, br s, CONHCO), 8.10 (1H, d, *J* 8.5, C_4H_9CONH), 4.50 (1H, q, *J* 8.5, NCH), 2.74−2.63 (1H, m, $CH_2CONHCO$), 2.46 (1H, dt, *J* 17.5 and 3.5, $CH_2CONHCO$), 2.10 (2H, t, *J* 7.5, $C_3H_7CH_2$-CONH), 1.96−1.83 (2H, m, $NCHCH_2CH_2CONH$), 1.47 (2H, br qn, *J* 7.5, CH_2), 1.27 (2H, sext, *J* 7.5, CH_2CH_3), 0.85 (3H, t, *J* 7.5, CH_3). ^{13}C NMR: δ_C 173.1, 172.3 (×2) (C═O), 49.0 (NCH), 35.1, 31.0, 27.5, 25.3, 24.5 (CH_2), 13.8 (CH_3). HRMS (+ESI) $C_{10}H_{16}N_2O_3Na$: calcd 235.1059; found 235.1047.

(*S*)-3-(2-Propylpentanoylamino)glutarimide 16. Method D was used (201 mg, 79%): mp 146−147 °C; $[\alpha]^{20}_D$ (*c* 0.50, MeOH) −47.2; ν_{max}/cm^{-1} 1703 (C═O, imide), 1651 (C═O, amide), 1517 (N−H, amide). Anal. ($C_{13}H_{22}N_2O_3$) C, H, N: calcd C 61.4, H 8.7, N 11.0; found C 61.3, H 8.8, N 11.0. ^{1}H NMR: δ_H 10.71 (1H, br s, CONHCO), 8.14 (1H, d, *J* 8.0, Pr_2-CHCONH), 4.50 (1H, ddd, *J* 13.5, 8.5, and 5.5, NCH), 2.69 (1H, ddd, *J* 17.5, 13.0 and 6.0, $CH_2CONHCO$), 2.46 (1H, dt, *J* 17.0 and 4.0, $CH_2CONHCO$), 2.17 (1H, m, $Pr_2CHCONH$), 1.99−1.80 (2H, m, $NCHCH_2CH_2CONH$), 1.50−1.37 (2H, m, CH_2), 1.34−1.14 (6H, m, CH_2), 0.83 (3H, t, *J* 7.0, CH_3), 0.82 (3H, t, *J* 7.0, CH_3). ^{13}C NMR: δ_C 176.8, 174.9, 174.0 (C═O), 49.1 (NCH), 45.6 (CHCO), 35.3, 35.2, 31.2, 24.7, 20.4 (×2) (CH_2), 14.4 (×2) (CH_3). HRMS (+ESI) $C_{13}H_{22}N_2O_3Na$: calcd 277.1528; found 277.1531.

(*S*)-3-(Undecanoylamino)glutarimide 17. (*S*)-3-(Undec-10-enoylamino)glutarimide (294 mg, 1 mmol) was dissolved in EtOAc (20 mL), and 10% Pd on carbon (50 mg) was added. The reaction mixture was stirred under hydrogen (1 atm) at ambient temperature for 15 h and then was filtered. The solution was reduced in vacuo, and the residue was recrystallized from EtOAc to give (*S*)-3-(undecanoylamino)glutarimide as a white solid (278 mg, 94%): mp 131−132 °C; $[\alpha]^{20}_D$ (*c* 0.6, MeOH) −18.3; ν_{max}/cm^{-1} 1716 (C═O, imide), 1650 (C═O, amide), 1547 (N−H, amide). Anal. ($C_{16}H_{28}N_2O_3$) C, H, N: calcd C 64.8, H 9.5, N 9.5; found C 64.8, H 9.6, N 9.5. ^{1}H NMR: δ_H 10.76 (1H, br s, CONHCO), 8.11 (1H, d, *J* 8.5, $C_{10}H_{21}CONH$), 4.49 (1H, dt, *J* 10.0 and 8.0, NCH), 2.69 (1H, ddd, *J* 18.0, 11.0, and 7.0, $CH_2CONHCO$), 2.45 (1H, dt, *J* 17.5 and 4.0, CH_2-CONHCO), 2.08 (2H, t, *J* 7.5, $C_9H_{19}CH_2CONH$), 1.95−1.80 (2H, m, $NCHCH_2CH_2CONH$), 1.47 (2H, br qn, *J* 6.5, CH_2), 1.25−1.18 (14H, m, CH_2), 0.82 (3H, br t, *J* 7.0, CH_3). ^{13}C NMR: δ_C 173.7, 173.0 (×2) (C═O), 49.7 (NCH), 36.0, 32.1, 31.7, 29.7 (×2), 29.6, 29.5, 29.3, 26.0, 25.1, 22.9 (CH_2), 14.7 (CH_3). HRMS (+ESI) $C_{16}H_{28}N_2O_3Na$: calcd 319.1998; found 319.1983.

(*S*)-3-(9-Carboxynonanoylamino)glutarimide 18. (*S*)-3-(undec-10-enoylamino)glutarimide (294 mg, 1 mmol) was dissolved in CCl_4 (2 mL) and CH_3CN (2 mL), and a solution of $NaIO_4$ (875 mg, 4.1 mmol) was added. Ruthenium trichloride hydrate (13 mg, 0.06 mmol) was added, and the reaction mixture was stirred for 4 h. The reaction was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried (Na_2SO_4) and reduced in vacuo, and the residue was recrystallized from EtOAc to give (*S*)-3-(9-carboxynonanoylamino)-

glutarimide as a white solid (268 mg, 86%): mp 126−127 °C; $[\alpha]^{20}_D$ (*c* 0.50, MeOH) −30.2; ν_{max}/cm^{-1} 1719 (C═O, imide), 1651 (C═O, amide), 1543 (N−H, amide). Anal. ($C_{14}H_{24}N_2O_3$) C, H, N: calcd C 57.7, H 7.8, N 9.0; found C 57.5, H 7.9, N 8.6. ^{1}H NMR: δ_H 11.98 (1H, br s, OH), 10.77 (1H, s, CONHCO), 8.12 (1H, d, *J* 8.5, $HO_2CC_8H_{16}CONH$), 4.50 (1H, br q, *J* 8.5, NCH), 2.69 (1H, ddd, *J* 18.0, 11.0, and 7.0, $CH_2CONHCO$), 2.45 (1H, dt, *J* 17.5 and 3.5, $CH_2CONHCO$), 2.15 (2H, t, *J* 7.5, $C_7H_{14}CH_2CO$), 2.08 (2H, t, *J* 7.5, $C_7H_{14}CH_2CO$), 1.94−1.80 (2H, m, $NCHCH_2CH_2CONH$), 1.52−1.40 (4H, m, CH_2), 1.28−1.17 (8H, m, CH_2). ^{13}C NMR: δ_C 173.4, 171.9, 171.2, 171.1 (C═O), 49.2 (NCH), 35.6, 34.0, 31.2, 29.0 (×2), 28.9, 28.8, 25.5, 24.8, 24.7 (CH_2). HRMS (+ESI) $C_{15}H_{24}N_2O_5Na$: calcd 335.1583; found 335.1571.

(*S*)-1-Methyl-3-(undec-10-enoylamino)glutarimide 19. (*S*)-3-(Undec-10-enoylamino)glutarimide (294 mg, 1 mmol) was dissolved in DMF (2 mL), and $CsOH·H_2O$ (168 mg, 1 mmol) was added. Methyl iodide (0.124 mL, 2 mmol) was then added, and the reaction mixture was stirred at ambient temperature for 16 h. The reaction mixture was partitioned between water (20 mL) and EtOAc (3 × 20 mL), and the combined organic layers were washed with 0.1 M HCl (2 × 50 mL), dried (Na_2SO_4), and reduced in vacuo. The residue was subjected to silica column chromatography (diethyl ether) and then recrystallized from EtOAc to give (*S*)-1-methyl-3-(undec-10-enoylamino)-glutarimide as a white solid (160 mg, 52%): mp 82−83 °C; $[\alpha]^{20}_D$ (*c* 1.01, MeOH) −16.0; ν_{max}/cm^{-1} 1675 (C═O, imide), 1640 (C═O, amide), 1532 (N−H, amide). Anal. ($C_{17}H_{28}N_2O_3$) C, H, N: calcd C 66.2, H 9.2, N 9.1; found C 66.0, H 9.2, N, 8.9. ^{1}H NMR: δ_H 8.15 (1H, d, *J* 8.5, CONH), 5.78 (1H, ddt, *J* 17.0, 10.0, and 6.5, $CH═CH_2$), 4.96 (1H, dq, *J* 17.0 and 2.0, $CH═CH_2$), 4.90 (1H, ddt, *J* 10.0, 2.0, and 1.0, $CH═CH_2$), 4.49 (1H, ddd, *J* 12.5, 8.5, and 6.5 NCH), 2.95 (3H, s, NCH_3) 2.77 (1H, dt, *J* 17.0, 12.0, and 6.0, $CH_2CONHCO$), 2.63 (1H, dt, *J* 17.0 and 3.5, $CH_2CONHCO$), 2.09 (2H, t, *J* 7.0, CH_2CONH), 1.98 (2H, br q, *J* 7.5, $CH_2CH═CH_2$), 1.95−1.80 (2H, m, $NCHCH_2CH_2CONCH_3$), 1.48 (2H, br qn, *J* 7.0, CH_2), 1.37−1.18 (10H, m, CH_2 × 5). ^{13}C NMR: δ_C 172.5, 172.4, 172.2 (C═O), 139.2 ($CH═CH_2$), 114.9 ($CH═CH_2$), 49.8 (NCH), 35.6, 33.5, 31.4, 29.0 (×2), 28.9, 28.8, 28.6, 26.8, 25.5, 23.8 (CH_2 and CH_3). HRMS (+ESI) $C_{17}H_{28}N_2O_3Na$: calcd 331.1998; found 331.1982.

(*S*)-3-(Undec-10-enoylamino)tetrahydropyridine-2-one 20. L-Ornithine hydrochloride (1.69 g, 10 mmol) was suspended in dry methanol (50 mL), and HCl gas was bubbled through the mixture for 15 min. The reaction mixture was heated at reflux for 2 h and was then reduced in vacuo to give a thick oil. The oil was redissolved in MeOH (50 mL) under argon, MeONa (1.08 g, 20 mmol) was added, and the reaction mixture was stirred for 2 h at ambient temperature. The reaction mixture was filtered under argon, and the organic phase was reduced in vacuo to give a colorless oil. This oil was then dissolved in $CHCl_3$ (50 mL), and to the solution was added triethylamine (1.40 cm^3, 10 mmol) and undec-10-enoyl chloride (2.03 g, 10 mmol). The reaction was stirred for 15 h at ambient temperature, the solvent was removed under reduced pressure, and the residue was partitioned between EtOAc (3 × 50 mL) and water (50 mL). The combined organic layers were washed with 0.1 M HCl (2 × 50 mL), dried (Na_2SO_4), and reduced in vacuo. The residue was subjected to silica column chromatography (EtOAc/MeOH) and recrystallized from EtOAc to give (*S*)-3-(undec-10-enoylamino)tetrahydropyridine-2-one as a white solid (1.79 g, 64%): mp 90−91 °C; $[\alpha]^{20}_D$ (*c* 0.50, MeOH) −4.0; ν_{max}/cm^{-1} 1667 (C═O, lactam), 1634 (C═O, amide), 1535 (N−H, amide). Anal. ($C_{16}H_{28}N_2O_2$) C, H, N: calcd C 68.5, H 10.1, N 10.0; found C 68.5, H 10.1, N 9.9. ^{1}H NMR: δ_H 7.90 (1H, d, *J* 8.0, $C_{10}H_{19}CONH$), 7.51 (1H, br s, NCHCONH), 5.77 (1H, ddt, *J* 17.0, 10.0, and 6.5, $CH═CH_2$), 4.97 (1H, br d, *J* 17.0, $CH═CH_2$), 4.91 (1H, br d, *J* 10.0, $CH═CH_2$), 4.10 (1H, ddd, *J* 10.0, 8.0, and 6.5, NCH), 3.13−3.04 (2H, m, CH_2NH), 2.05 (2H, t, *J* 7.5, CH_2CO), 1.98 (2H, br q, *J* 7.0, $CH_2CH═CH_2$), 1.94−1.87 (1H, m, CH_2CH_2NH), 1.78−1.62 (2H, m, $CH_2CH_2CH_2N$), 1.54 (1H, qd, *J* 10.5 and 4.5, $CH_2CH_2CH_2N$), 1.50−1.42 (2H, m, CH_2), 1.33 (2H, br qn, *J* 6.5, CH_2), 1.27−1.18 (8H, m, CH_2 × 4). ^{13}C NMR: δ_C 173.3, 171.4 (C═O), 140.3 ($CH═CH_2$), 116.1 ($CH═CH_2$), 50.2 (NCH), 42.5 (NCH_2), 36.7, 34.7, 30.2 (×2), 30.0, 29.9, 29.7, 29.3, 26.7, 22.4 (CH_2). HRMS (+ESI) $C_{16}H_{28}N_2O_2Na$: calcd 303.2048; found 303.2055.

Biological Assays. Compounds were tested for their ability to inhibit chemokine-induced migration using the microtiter format trans-well migration assay, exactly as described previously.[14] As noted in the text, assays were performed either using the human myelomonocytic cell line THP-1 (which responds to a range of chemoattractants including MCP-1, MIP-1α, RANTES, SDF-1α, and fMLP but not C5a or IL-8) or using freshly prepared human peripheral blood neutrophils (which respond to C5a or IL-8). All migration assays were performed in Gey's balanced salt solution containing 1 mg/mL endotoxin-free BSA (in the absence of fetal calf serum), allowing migration to occur for 2 h at 37 °C. All compounds were solubilized in DMSO and added to both the top and bottom compartments of the trans-well migration plate at a constant final DMSO concentration of 1%. The number of cells undergoing migration was measured using the vital dye MTT, as previously described,[14] and is expressed as a percentage of the number of cells undergoing migration in the presence of 1% DMSO vehicle only. Values are given as the mean and standard error from triplicate determinations.

The effect of NR58,4 **10** on TNF-α upregulation in vivo was determined as follows. Adult male CD-1 mice ($n = 6$ per group) were pretreated with various agents (vehicle, NR58,4, thalidomide or NR58-3.14.3) by subcutaneous injection 45 min prior to an acute inflammatory challenge with 750 μg of bacterial lipopolysachharide (from *E. coli* 0111:B4; Sigma L-4130) via the intraperitoneal route. After a further 3 h, the animals were sacrificed and blood was drawn by cardiac puncture. The level of TNF-α in serum was determined using the Quantikine ELISA for murine TNF-α (R&D Systems) and is reported as the mean ± standard error for each group.

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