Development of Potent Non-Carbohydrate Imidazole-Based Small Molecule Selectin Inhibitors with Antiinflammatory Activity

Deborah H. Slee,* Suzanne J. Romano, Jinghua Yu, Truc N. Nguyen, Judy K. John, Neil K. Raheja, Frank U. Axe, Todd K. Jones, and William C. Ripka

Ontogen Corporation, 2325 Camino Vida Roble, Carlsbad, California

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A novel series of non-carbohydrate imidazole-based selectin inhibitors has been discovered via high-throughput screening using a P-selectin ELISA-based assay system. The initial lead **1** had an IC_{50} of 17 μM in the P-selectin ELISA; this potency was significantly improved via an extensive SAR exploration. One of the current lead compounds (**29**) has an IC_{50} of 300 nM in a P-selectin ELISA; it also has good activity in P- and E-selectin cell adhesion assays and shows efficacy in vivo. These compounds represent a novel series of sLe^X mimetics with antiinflammatory activity. Their unique profile supports our interest in their further evaluation as drug candidates for the treatment of inflammation. Herein we describe the syntheses, optimization, and SAR of this series of novel potent selectin antagonists.

Introduction

The inflammatory response is mediated by a series of chemoattractants, cytokines, and cell adhesion molecules (CAMs) which work in a programmed, sequential manner to direct immune cells to the inflamed tissue. This has been termed "the leukocyte-endothelial cascade".[1,2] Three known families of CAMs participate in this cascade: the selectins, the integrins, and the immunoglobulin superfamily. The first step, rolling of leukocytes and lymphocytes along the blood vessel wall, is mediated by the selectins.[1−5] A number of natural ligands for the selectins have been identified. The specific carbohydrate moieties necessary for selectin binding, or the common motifs recognized by all three selectins, are the sialylated and fucosylated tetrasaccharide sialyl Lewis^x (sLe^x) and a related structure, sialyl Lewis^a (sLe^a).[6−8] The selectins have been implicated in a number of inflammatory disease states, such as asthma,[9−11] atopic dermatitis, contact dermatitis, (i.e., cutaneous inflammation),[12−15] and rheumatoid arthritis[16−18] among others. The aforementioned studies have clearly demonstrated that selectin-mediated rolling of immune cells along vascular endothelium is a key early event in the pathogenesis of acute inflammation, making it a good target for therapeutic intervention. A selectin inhibitor would be expected to attenuate inflammatory reactions and alleviate the symptoms of inappropriate immune responses by reducing cellular infiltration.

Many reports describing sugar-based selectin inhibitors,[14,19−25] in addition to several reports of peptidic inhibitors of the selectin/ligand interaction, have been published.[26,27] Typically these classes of inhibitors suffer from a lack of potency, poor pharmacokinetic properties, and are often difficult to synthesize. More importantly, there have been few reports[28,29] of non-carbohydrate, non-peptidyl antagonists of the selectin/ligand interaction; and in these cases only ELISA data have been reported. It has been our approach to develop a program for discovering potent small molecule inhibitors of selectin−ligand binding, utilizing cell-based assays, with the aim of developing a novel class of antiinflammatory therapeutics.

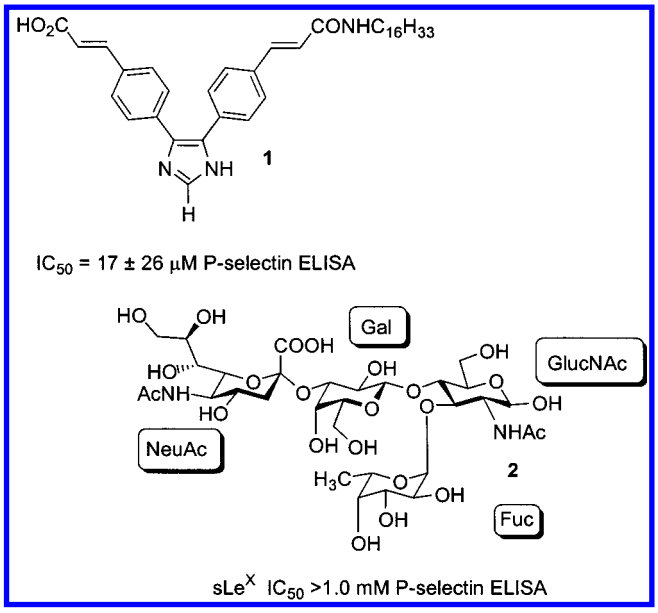

Figure 1.

The original lead imidazole-based compound **1** (IC_{50} = 17 ± 26 μM, Figure 1, Table 3) was discovered via high-throughput screening of our compound library against P-selectin using an ELISA-based assay system. This ELISA assay measures the inhibition of binding of a recombinant selectin protein to a synthetic sLe^x analogue.[30] Recent in vivo studies with knockout mice and selectin antibodies suggest that it may be necessary to block both P- and E-selectin in order to obtain a potent antiinflammatory response.[31,32]

Herein we report the syntheses and optimization of a series of novel potent imidazole-based P- and E-

* To whom correspondence should be addressed. Tel: 760-930-0100. Fax: 760-930-0955. E-mail: debbie.slee@ontogen.com.

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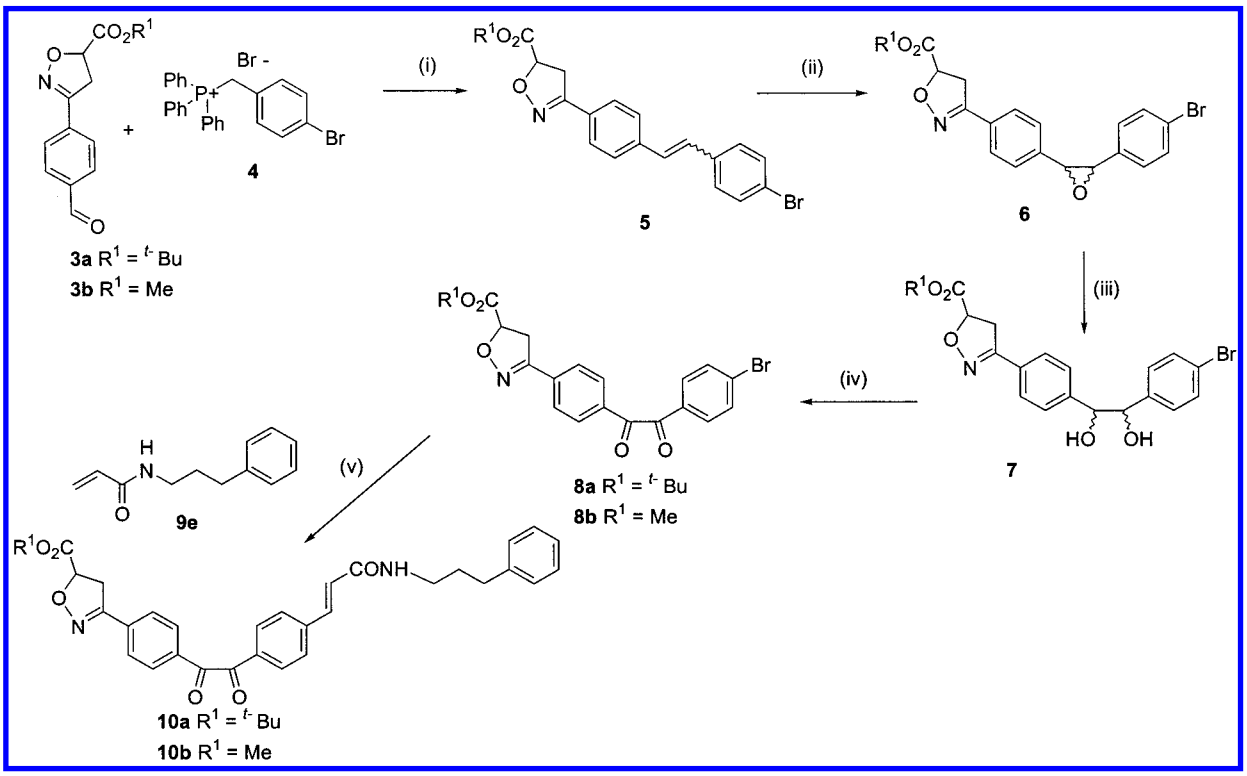
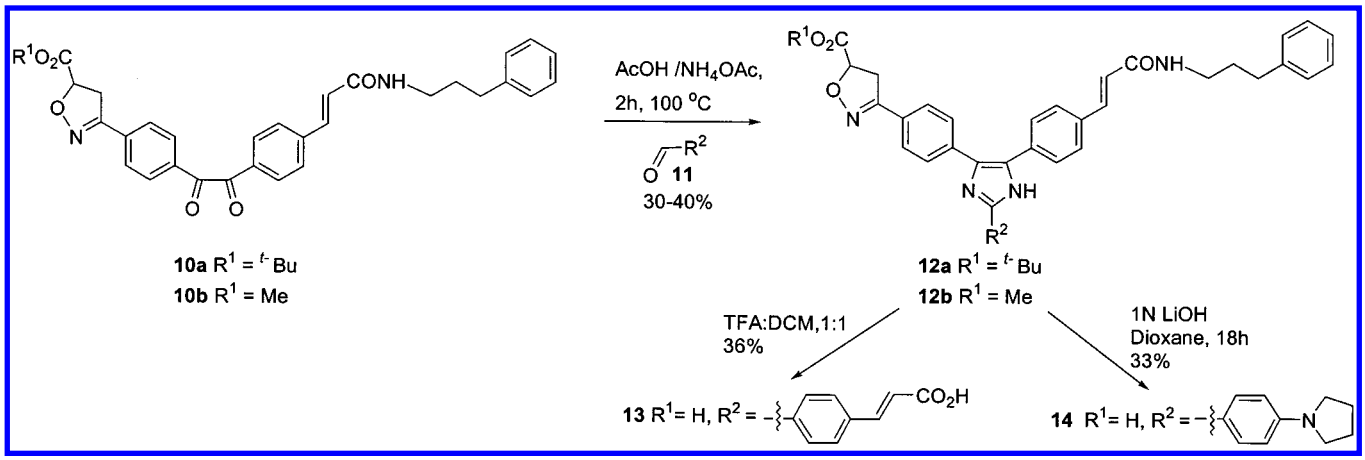
Scheme 1[a]



[a] Reagents and conditions: (i) K^{t}BuO, DMSO/THF, 2 h, 53%; (ii) mCPBA, DCM, 40 °C, 91%; (iii) formic acid, 0 °C, THF, 5 h, 64%; (iv) TEMPO, DCM, 0.7 M NaBr, buffered bleach, quantitative; (v) $Pd(OAc)_2$, TEA, P(*o*-tolyl)$_3$, 90% crude.

Scheme 2



selectin antagonists, derived from lead compound **1**, which have been readily prepared from commercially available starting materials.

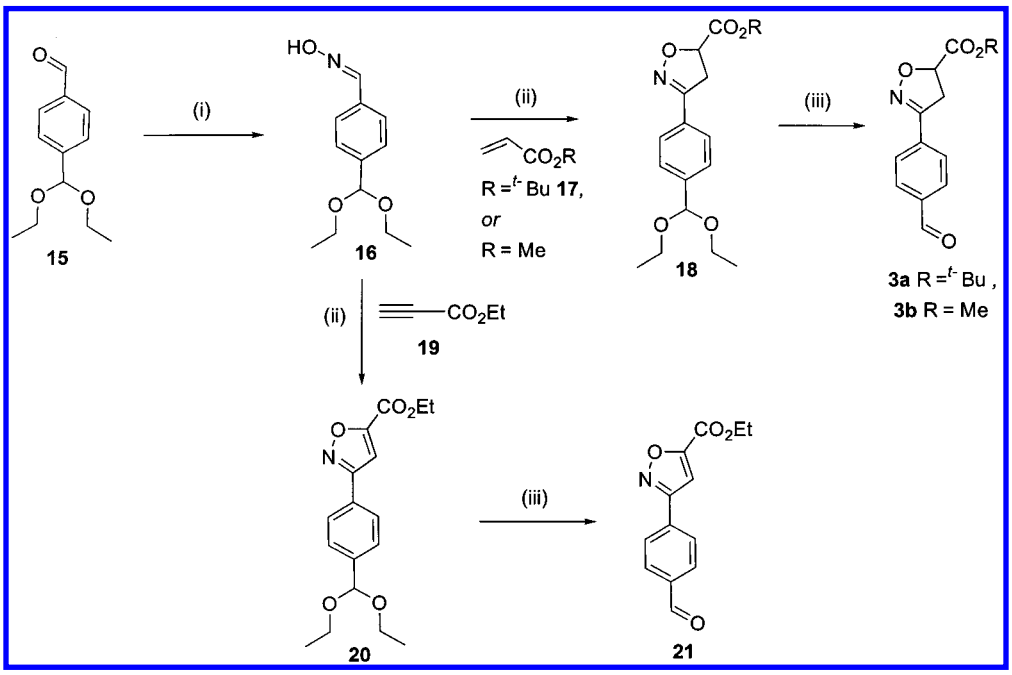
Chemistry

The imidazole-based selectin inhibitors were prepared using the general procedures shown in Schemes 1, 2, and 4. These schemes are illustrative of the approach used for the synthesis of imidazoles **1, 13**, **14**, and **27**–**31** (Table 3). The routes differ in the methodology used for the preparation of the required intermediate diones. The imidazole ring synthesis were carried out using similar methodology.[33] In the first example, shown in Schemes 1 and 2, the synthesis of imidazole **12** is outlined (**12a** → **13**; **12b** → **14**).

Scheme 1 illustrates the synthesis of the desired intermediate dione **10**. A Wittig olefination with **4** and either the *tert*-butyl or methyl ester of aldehyde **3** provided alkene **5** as a 1:1 mixture of geometrical isomers. Epoxide formation employing mCPBA was followed by ring opening with formic acid and subsequent hydrolysis of the resulting formic acid ester in situ to provide diol **7**. TEMPO oxidation of **7** furnished the dione intermediate **8**. This dione was further functionalized via a Heck reaction with acrylamide **9e**, synthesized via reaction of 3-phenyl-1-propylamine with acryloyl chloride (Scheme 5), to give the fully derivatized dione **10**. The dione **10** was condensed with NH_4OAc and the appropriate aldehyde **11** in acetic acid to give imidazole **12** (Scheme 2). Final deprotection gave the final desired imidazoles **13** and **14**.

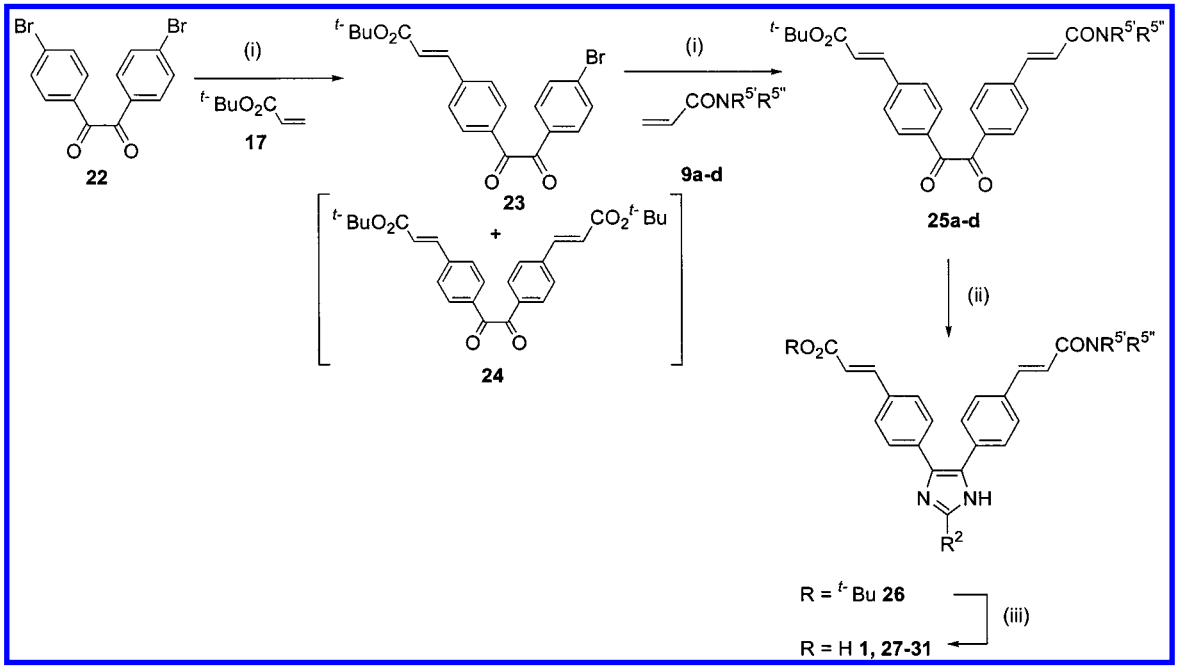
Preparation of the aldehydes **3** and **21**, shown in Scheme 3, began with the commercially available monodiethyl acetal of terephthalaldehyde **15**, which, upon

Scheme 3[a]



[a] Reagents and conditions: (i) dioxane, hydroxylamine·HCl, TEA, 72 h, 95%; (ii) bleach, THF, ~18 h; (iii) Amberlite IR120, THF/H_2O, 5 h, 92%.

Scheme 4[a]



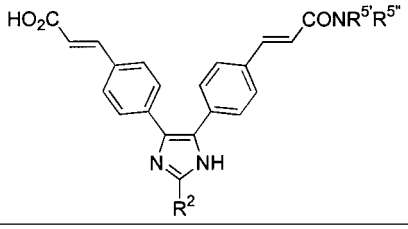
[a] Reagents and conditions: (i) $Pd(OAc)_2$, TEA, P(*o*-tolyl)$_3$, 40%; (ii) NH_4OAc, AcOH, R^2CHO = **3**, **21**, or paraformaldehyde, 100 °C, 36−92%; (iii) TFA:DCM 1:1 (for yields, see Table 1).

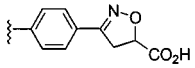
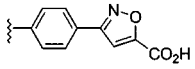
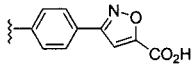
condensation with hydroxylamine, furnished oxime **16**. 1,3-Dipolar cyclization of **16** with either *tert*-butyl acrylate **17** or ethyl propiolate **19** in the presence of bleach, followed by saponification, provided the requisite aldehydes **3** and **21** in excellent overall yield.[34]

Scheme 4 shows the general synthetic route employed for the synthesis of diones **25a**−**d** from 4,4′-dibromobenzil, via sequential Heck reactions with *tert*-butyl protected acrylic acid **17**, and the appropriate acrylamide **9**. The di-*tert*-butyl ester side product **24** is carried through the reaction sequence and is easily removed from diones **25a**−**d** by column chromatography. The diones can then be reacted with NH_4OAc and the appropriate aldehyde to give the desired imidazole of general formula **26**. Deprotection of the *tert*-butyl protecting group of the intermediate of general formula **26** gives the desired imidazoles **1** and **27**−**31**. The yields for the last two steps in Scheme 4 are shown in Table 1.

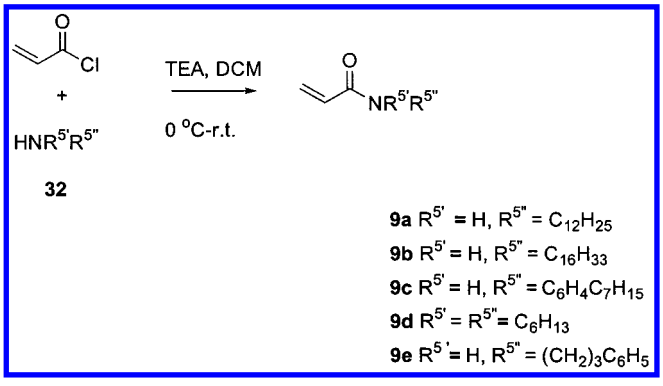
Scheme 5 illustrates the general approach used for the synthesis of the acrylamides **9a**−**e** used in Schemes 1 and 4. Acryloyl chloride was reacted with the ap-

Table 1. Final Compound Yields for Scheme 4



No.	$R^{5'}R^{5''}$	R^2	% Yield R = ^{t}Bu	% Yield R=H after recrystallization
1	$R^{5'} = H$, $R^{5''} = C_{16}H_{33}$	H	92	60
27	$R^{5'} = H$, $R^{5''} = C_6H_4C_7H_{15}$	H	68	18
28	$R^{5'} = R^{5''} = C_6H_{13}$	H	36	28
29	$R^{5'} = H$, $R^{5''} = C_{16}H_{33}$		72	69
30	$R^{5'} = H$, $R^{5''} = C_{16}H_{33}$		48	75
31	$R^{5'} = H$, $R^{5''} = C_{12}H_{25}$		48	55

Scheme 5



propriate amine **32** to give the acrylamide **9a**−**e** quantitatively. Acrylamides **9a**−**e** were then used directly without purification.

Modeling

The crystal structure of E-selectin is available from the Protein Data Bank (PDB). A number of published studies have established the key structural features of sLe^x necessary for binding to the selectin proteins. These include (1) the X-ray crystal structure of E-selectin,[35] (2) affinity data that have been obtained via chemical modification of the sLe^x ligand[36−46] or genetic modification of the E-[35,47,48] or P-selectin[49−52] proteins, (3) the force field derived model of sLe^x,[53−56] and (4) a number of studies utilizing NMR.[57−60]

In addition, the structure of complexes of sugar ligands with carbohydrate recognition domains of two closely related members of the C-type animal lectin family, rat serum and liver mannose-binding proteins (MBPs), have been resolved by X-ray crystallography.[61,62] These studies revealed that terminal mannose, *N*-acetylglucosamine, or fucose residues interact directly with the protein and indirectly through a bound calcium. Additional mutagenesis studies by the same group analyzed the mechanism of oligosaccharide binding to the selectin cell adhesion molecules by transferring regions of the carbohydrate recognition domains of E- and P-selectin to the corresponding sites of the homologous rat serum mannose binding protein.[63] One of the main findings of these studies was that affinity for the sLe^x ligand seemed to be dependent on the presence of a number of lysine residues and that the sialic acid portion of the ligand interacts near the sequence Lys-Lys-Lys which corresponds to residues 111−113 of

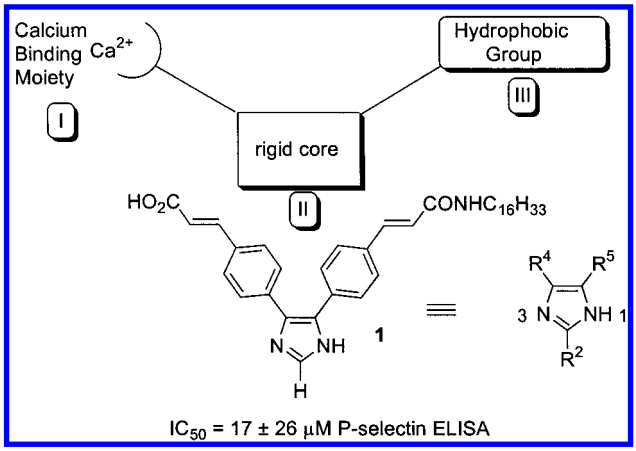


Figure 2. Criteria believed to be essential for a potent selectin inhibitor.

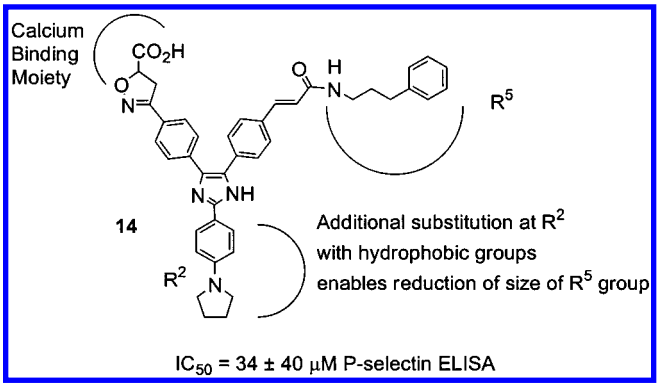
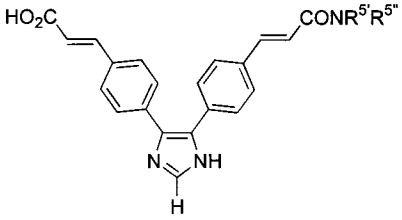


Figure 3. Additional substitution at R^2 allows reduction in size of the R^5 substituent.

Table 2. Alternative Hydrophobic Groups at R^5 as Replacements for a Straight Chain Alkyl Moiety Based on Calculated Π Values (Substituent log *P* Values)



No.	$R^{5'}R^{5"}$	Calculated Π values	P-Selectin Cell IC_{50} μM
1	$R^{5'}$ = H, $R^{5"}$ = $C_{16}H_{33}$	6.07	40±36 (n=5)
Reference	$R^{5'}$ = H, $R^{5"}$ = $C_{12}H_{25}$	4.31	–
27	$R^{5'}$ = H, $R^{5"}$ = $C_6H_4C_7H_{15}$	4.01	44±21 (n=3)
28	$R^{5'}$ = $R^{5"}$ = C_6H_{13}	4.13	21±17 (n=3)

E-selectin. Several independent studies also suggest the importance of Lys 111 and Lys 113 for ligand binding.[35,47,49−51]

The human P-selectin lectin domain shares a high sequence homology of >80% (with no insertions or deletions) with the human E-selectin lectin domain which is available as a high-resolution structure from the PDB as mentioned previously.[35] Our model of P-selectin was obtained from the X-ray structure of E-selectin by simple substitution of the appropriate amino acids. Recently, Camphausen has reported the structure of both E- and P-selectin with a bound sialyl Lewis^x ligand. In this study, the structure of P-selectin was indeed found to adopt a conformation similar to that of E-selectin, and the sLe^x binding site was highly conserved as predicted in our model.

Complexes were generated manually by docking inhibitors to the binding epitope of P-selectin, such that the proposed calcium binding carboxylate group bound the calcium ion, and key amino acid residues involved in sLe^x binding were blocked. These initial structures were energy minimized, and the lowest energy structure was selected. The final structure was subjected to a Monte Carlo docking protocol as described in ref 26, to determine whether there were any other lower energy docked complexes.[65] The docked complexes of the inhibitors with P-selectin were energy minimized with the Discover program[66] using the consistent valence force field (CVFF) of Hagler.[67]

Biology

High-throughput screening utilized an ELISA-based assay system developed at Nippon Organon K. K. (formerly Kanebo Ltd., New Drug Discovery Laboratories) in Osaka, Japan.[30] For lead optimization, we have developed a cell−selectin protein and a cell−cell assay system. Inhibitors were screened against both E- and P-selectin (see Table 3).

The animal models used to evaluate these selectin inhibitors in vivo include a mouse thioglycollate induced peritonitis model of inflammation[68] and a model of selectin-mediated rolling in vivo using an intravital microscopy system.[69] In this model, selectin-mediated rolling in cytokine-stimulated dorsal skin of anesthetized mice is measured.[70]

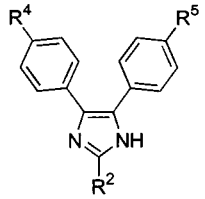
Results and Discussion

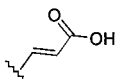
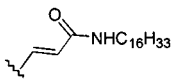
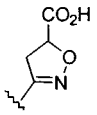
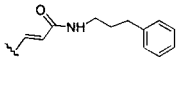
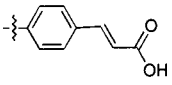
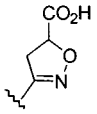
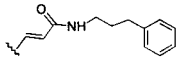
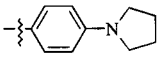
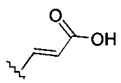
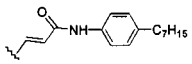
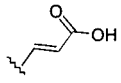
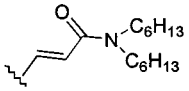
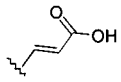
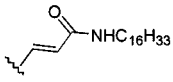
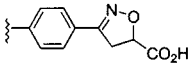
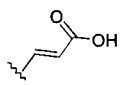
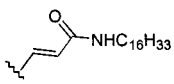
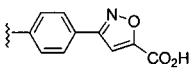
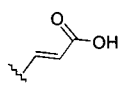
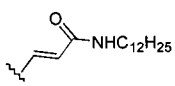
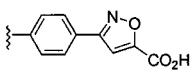
The original imidazole-based lead compound **1** (IC_{50} = 17 ± 26 μM (n = 40), P-selectin ELISA, 98 μM P-selectin cell−cell assay), was optimized via a combination of solid support[71] and solution phase chemistry. The synthesis of focused libraries on solid support was carried out using our OntoBLOCK system.[72]

Overall, three minimum criteria appeared to be essential for activity: (I) a calcium binding moiety, (II) a rigid template/core, and (III) a hydrophobic moiety (Figure 2).

Several different groups were tested as linkers for the carboxylic acid at R^4. We found that 4,5-dihydro-isoxazole-5-carboxylic acid, phenoxy acetic acid (not shown), and cinnamic acid, which are thought to act as calcium-binding moieties at R^4, gave equivalent activities. However, incorporation of the 4,5-dihydro-isoxazole-5-carboxylic acid moiety increased the aqueous solubility of this series. The *tert*-butyl ester precursors

Table 3. Biological Assay Data[a]



No.	R^4	R^5	R^2	P-Selectin ELISA IC_{50} μM	P-Selectin Cell IC_{50} μM	P-Selectin Cell/Cell IC_{50} μM	E-Selectin Cell/Cell IC_{50} μM (approx.)
1			H	17±26 (n=40)	40±36 (n=5)	98±125 (n=2)	>100
13				100±100 (n=3)	21±24 (n=18)	123±138 (n=3)	NT
14				34±40 (n=5)	50±25 (n=3)	NA	NT
27			H	16±16 (n=3)	44±21 (n=3)	NT	NT
28			H	NT	21±17 (n=3)	NA	NA
29				0.29±0.33 (n=35)	9±16 (n=49)	14±19 (n=24)	30 (n=2)
30				0.13±0.05 (n=2)	2.7±1.7 (n=4)	8±6 (n=10)	20 (n=2)
31				3±2 (n=9)	290±461 (n=11)	500±700 (n=2)	NA

[a] NT = not tested. NA = not active.

that we tested (e.g., **26**) were found to be inactive, providing evidence that the carboxylic acid moiety is essential for activity.

We were seeking to replace the long fatty side chain of **1**; however, preliminary SAR information indicated that the general hydrophobic character of a hexadecyl chain was required for potency. From our combinatorial libraries we found that removal of the hydrophobic moiety at R^5 resulted in loss of activity. If a substituted phenyl group was introduced at R^2, good activity was maintained in vitro in the ELISA and P-selectin cell assays when the alkyl chain (R^5) was replaced with the smaller propyl phenyl group as illustrated by imidazole **14** (Figure 3). Activity was lost, however, when the R^5 side chain was reduced to a benzyl group. Many different amines were used to couple to the R^5 side chain through the acrylamide moiety. Coupling of amines that were more hydrophilic in character, such as tryptamine or *N*-(3-aminopropyl)morpholine for example, resulted in loss of potency. It was found that overall, the most potent derivatives in the cell-based assay systems contain the C-16 alkyl chain at R^5. Interestingly, reduction of the double bonds in either of the R^4 and/or R^5 side chains when R^5 was dodecylalkyl or less, resulted

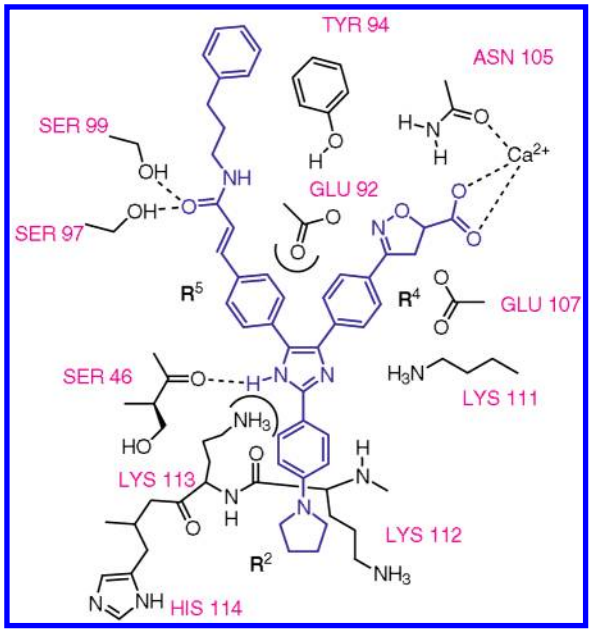


Figure 4. Schematic representation of **14** showing the proposed interactions with the P-selectin binding site.

in complete loss of potency in the cell-based assays, presumably due to the loss of structural rigidity. Thus implying that the direction of the hydrophobic group is important.

To find an alternative side chain with similar hydrophobic character, groups with similar calculated Π values (substituent log *P* values) were evaluated. Theoretical Π values for R^5 were calculated for the corresponding amine using the MOE software package.[73] The computed values for $NC_{12}H_{25}$ (4.31) and $NC_{16}H_{33}$ (6.07) were used as reference ($NC_{12}H_{25}$ was the smallest substituent that gave acceptable potency). Suitable side chains with similar log *P* values included a phenylheptyl moiety (compound **27**, Π value of 4.01) and a bishexyl moiety (compound **28**, Π value of 4.13), as shown in Table 2. Compound **27** has an IC_{50} of 44 μM which is comparable to compound **28** (IC_{50} of 21 μM) and compound **1** (IC_{50} of 40 μM) in the P-selectin cell assay (Table 3).

Our proposed model of how the imidazole-based inhibitors bind to P-selectin is illustrated in Figure 4. The inhibitors were modeled on the assumption that the acid functionality at R^4 binds to calcium, filling out the coordination sphere in a manner similar to the way the fucose moiety is believed to bind in the natural ligand sLe^x. This places the imidazole ring in close proximity to LYS 111 and LYS 113 where it may interact via hydrogen bonding, again analogous to the natural ligand sLe^X **2**. The amide group at R^5 can interact with amino acid side chains of SER 97 and SER 99 as well as TYR 94. The hydrophobic chains at R^5 may possibly provide a hydrophobic driving force to aid in keeping the inhibitor bound. The rigid aromatic rings at R^2 are hydrophobic in nature and tend to interact with the hydrophobic side chains of residues such as LYS 112.

Many commercially available aldehydes were employed to synthesize a series of imidazoles substituted at the R^2 position. Most of these substitutions were well tolerated when the R^4 and R^5 substituents met the basic requirements of a calcium binding moiety and a suitable hydrophobic group, respectively. Close examination of the proposed mode of binding suggested that a suitably positioned carboxylic acid moiety might pick up a key ionic interaction with LYS 112. Indeed the most potent analogues were found when a phenyl ring, para-substituted with a carboxylic acid-containing moiety, was added at the R^2 position. One of the most potent analogues is the diacid **29**, with an IC_{50} of 0.29 μM in our P-selectin ELISA assay (14 μM P-selectin cell−cell assay) (Table 3). We postulate that this imidazole derivative **29** binds in a similar way as compound **14** with the additional acid moiety picking up an ionic interaction with the terminal amino moiety of LYS-112 in P- or E-selectin as illustrated in Figure 5 (cross-eyed stereoview).

Overall, when we compare the proposed binding mode of our inhibitors to that of the known natural ligand sLe^X **2** (Figure 1), we postulate that the two carboxylic acid moieties act as replacements for the critical fucose and sialic acid groups of sLe^X, with a triaryl imidazole unit replacing the lactosamine core. This proposed binding mode is similar to that discussed by Kogan et al.[11] for the mannose-containing inhibitor TBC-1269. If the mode of binding is indeed similar, our series of compounds suggest that the proposed calcium binding-mannose functionality of TBC-1269 can be replaced by a simple carboxylic acid moiety resulting in a completely novel sLe^x mimetic that contains no sugar moieties.

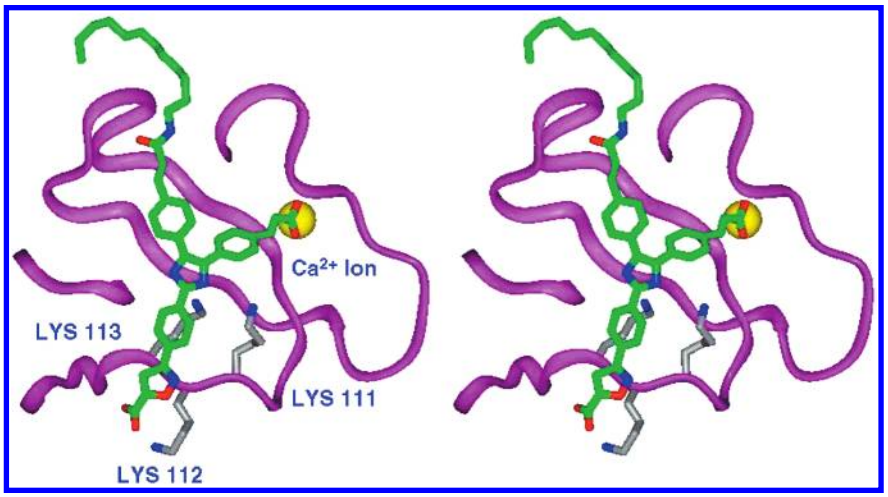


Figure 5. Stereoview of compound **29** bound to P-selectin illustrating the proposed binding mode.

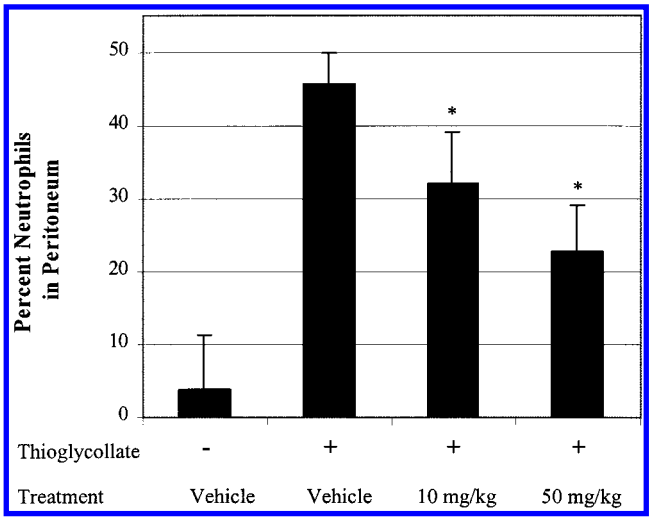


Figure 6. Treatment with compound **29** at 10 and 50 mg/kg reduces neutrophil influx in an in vivo model of inflammation. Balb/c mice were dosed with compound or vehicle (buffered saline) by intravenous injection immediately before intraperitoneal challenge with thioglycollate. Total cells and neutrophils in the peritoneum were quantified 4 h later ($n = 4-5$ mice per group). *$p < 0.01$ by unpaired, two-tailed t-test.

It was found that the chiral center of the 4,5-dihydroisoxazole-5-carboxylic acid moiety at R^2 of compound **29** could be removed to give the isoxazole **30** which has an IC_{50} of 0.13 μM in our P-selectin ELISA assay and 8 μM in our P-selectin cell−cell assay; this is approximately 2-fold more potent than the equivalent isoxazoline derivative **29**.

When the alkyl chain at R^5 is shortened to a dodecyl chain (compound **31**, Table 3), activity is maintained in the P-selectin ELISA assay system, or when changed to a propyl phenyl moiety (compound **13**, Table 3), activity is maintained in the P-selectin/cell assay system; however, in both cases, potency is lost in the cell−cell assay.

A number of templates were explored as alternatives to the imidazole core. It was found that either one of the nitrogens in the imidazole core could be replaced with sulfur to give a thiazole moiety, suggesting that the imidazole was functioning primarily as a rigid scaffold. This also suggests that any interaction between the selectin protein and the imidazole core is not important for binding. A simple appropriately substituted phenyl ring also gave active compounds. However, both the thiazole and phenyl derivatives were distinctly less aqueous soluble than the imidazole derivatives.

Compounds **29** and **30** have excellent activity in our in vitro P- and E-selectin cell-based assays (Table 3). Compound **29**, with potent cell−cell activity against both P- and E-selectin, was used for in vivo testing. The aqueous solubility of **29** is good as the bis-sodium salt (15 mg/mL), allowing us to use this form of the compound for our in vivo studies. Compound **29** was tested in the mouse thioglycollate-induced peritonitis model[68] and found to reduce the inflammatory response in a dose-dependent manner, causing a 30−50% reduction in cell infiltration at doses of 10−50 mg/kg iv (statistically significant results, Figure 6).[70] This compound also showed efficacy in a mouse model of IgE-dependent delayed-type hypersensitivity.[74]

To confirm that our inhibitors were effective under conditions of shear flow (i.e., under physiological conditions) and that the antiinflammatory effect we had observed in the thioglycollate model was via inhibition of selectin-mediated rolling, we tested compound **29** for its ability to inhibit selectin-mediated rolling in vivo using an intravital microscopy system.[69] This compound was found to inhibit cytokine-stimulated rolling in the dorsal skin of anesthetized mice at concentrations of 30−50 mg/kg iv.[70] A combination of P- and E-selectin monoclonal antibodies also blocked rolling in this system, demonstrating that the rolling was indeed mediated by selectins (data not shown).

Summary

A novel series of potent small molecule inhibitors that not only have excellent in vitro profiles but also have activity in vivo has been developed. A combination of parallel synthesis and structure-based design allowed rapid optimization, resulting in compounds with in vivo activity. We found what we believe to be the three minimal criteria that are essential for activity: (1) a calcium binding moiety, (2) a rigid template/core, and (3) a hydrophobic moiety (Figure 2). Of those that were tested, the carboxylic acid moiety was found to be optimal as the calcium binding moiety. If a substituted phenyl group was introduced at R^2, the alkyl chain (R^5) could be replaced with a smaller propyl phenyl moiety. However, the optimal group at the R^5 position was found to be a C-16 alkyl chain. A number of templates could be used as the rigid template, but the imidazole core was found to be optimal in terms of aqueous solubility. We found that many different substituted phenyl groups were tolerated at the R^2 position, but increased potency was observed when this ring was para-substituted with a carboxylic acid-containing functionality such as in compound **29**.

In general, the cell−selectin protein and cell−cell assay systems proved to be essential for lead optimization and seemed to give more predictive results in terms of identifying compounds with in vivo activity. These findings are similar to that of other groups.[75,76] Several members of this series were found to be active against both P- and E-selectin.

One of the more potent imidazole derivatives, compound **29**, identified as having good activity in all of the in vitro assays, has been shown to reduce inflammation in vivo and has also been demonstrated to inhibit selectin-mediated rolling in vivo. We believe that this is the first report of such a series of non-carbohydrate inhibitors with in vivo efficacy.

Experimental Section

P-Selectin ELISA Assay. An ELISA-type assay was used to screen for inhibitors of selectin−ligand interactions. A P-selectin-IgG chimera, constructed as described by Foxall,[77] and sialyl $Lewis^x$ pentaceramide were obtained from Kanebo, Ltd. (Osaka).[78]

Assays were performed essentially as described by Ohmoto et al.[30] Polystyrene microtiter plates (Falcon Pro-Bind) were coated with the sialyl $Lewis^X$ analogue at 40−100 pmol/well. Coated wells were blocked with 5% bovine serum albumin (BSA) in 50 mM imidazole buffer, pH 7.2, for 1 h at room temperature.

Compounds were diluted from DMSO stock solutions in assay buffer (50 mM imidazole buffer, pH 7.2, containing 1% BSA and 1 mM $CaCl_2$). Compounds were always run in duplicate or triplicate. A complex consisting of P-selectin IgG chimera, biotinylated goat F(ab′)2 anti-human IgG, and streptavidin-alkaline phosphatase conjugate was made in assay buffer. Selectin chimera was omitted from the complex for negative control ("background") wells. The complex and the test compounds (or vehicle controls) were combined in wells of a polypropylene microtiter plate and incubated for 30 min at room temperature. The complex−compound mixture was then added to the blocked, sialyl Lewis^X-ceramide coated plate and allowed to incubate for 45 min at 37 °C. After washing three to four times with 50 mM imidazole, the bound complex was detected using the colorimetric phosphatase substrate, *p*-nitrophenyl phosphate, at 1 mg/mL in 1 M diethanolamine containing 0.01% $MgCl_2$. After developing for 1−2 h at room temperature, the absorbance at 405 nm was measured in a Molecular Devices microplate reader. Percent inhibition was calculated by comparing the test compound result with the vehicle control after subtracting the background from each. IC_{50} values were calculated by in-house data analysis software (OntoASSAY; Ontogen, Corp.) using standard algorithms.

Cell−Selectin Adhesion Assays. The ability of compounds to inhibit the adhesion of HL60 cells to purified selectin proteins was measured using a "cell−selectin" assay. Recombinant soluble P- and E-selectin proteins purchased from R&D Systems (Minneapolis, MN) were diluted to 2.5 μg/mL in Dulbecco's PBS containing calcium and magnesium (PBS^+). Falcon Pro-Bind microtiter plate wells were incubated with 50 μL of the P- or E-selectin protein solution for 1 h at 37 °C or overnight at 4 °C. The selectin protein was omitted from negative control ("background") wells. Coated wells were then washed three times with PBS^+ and then blocked with 1% BSA in PBS^+ for 1 h at room temperature. After blocking, the plates were washed three times with PBS^+. Compounds were diluted to 2× final test concentration in PBS^+ and added to the blocked, selectin-coated wells in a volume of 50 μL. Samples were always run in duplicate or triplicate. Compounds and vehicle controls were preincubated in the wells for ∼20 min at room temperature.

HL60 cells obtained from the ATCC (Manassas, VA) were cultivated in RPMI medium containing 10% heat-inactivated fetal bovine serum (FBS). For the assay, cells were harvested by centrifugation, washed once with PBS^+, and resuspended in PBS^+ at a concentration of 2×10^6 cells/mL. Cells were added directly to the compound-containing wells in a volume of 50 μL per well, bringing the compound to its final test concentration in a total volume of 100 μL. Cells and compound were incubated on the selectin-coated wells for 45 min at 37 °C. Unbound cells were removed using a vacuum manifold and a single wash with 200 μL of PBS^+ (added slowly using a manual multichannel pipettor). Retained cells were labeled directly on the plate by adding 5 μg/mL of the membrane-permeable fluorescent dye, calcein-AM, and incubating for 30 min at 37 °C. The signal was quantified in a Wallac Victor fluorescent microplate reader using 485 nm excitation and 535 nm emission. Percent inhibition and IC_{50} values were calculated as described above for the ELISA assay.

E-Selectin Cell−Cell Adhesion Assays. Cell−cell adhesion assays were used to determine if compounds could inhibit native selectin−native ligand interactions. Human umbilical vein endothelial cells (HUVEC; Clonetics, Inc., San Diego, CA) were used as a source of native E-selectin. Frozen stocks of HUVECs were expanded and propagated in EGM medium (Clonetics) for a maximum of four to five passages. The day before the assay was to be run, HUVEC cells were trypsinized and replated in Nunc 96-well tissue culture plates which were precoated with 2% gelatin. On the day of the assay, the HUVECs were activated with 10 ng/mL of IL-1β (R&D Systems, Minneapolis, MN). It was found that this treatment significantly upregulated expression of the E-selectin protein (data not shown). After a 4 h activation at 37 °C, the stimulation medium was removed, and the compounds diluted in cold RPMI to 2× the final test concentration were added. Triplicate test samples were incubated with the activated HUVECs for 20 min at 4 °C. It was found that 4 °C was the optimal temperature for detecting primarily selectin-mediated adhesion (unpublished observations).

HL60 cells were labeled by incubating with the membrane-permeable fluorescent dye, BCECF-AM, at 5 μg/mL for 30 min at 37 °C. Cells were then washed and resuspended in RPMI medium at 2×10^6 cells/mL and added to the compound-containing wells in a volume of 50 μL. After incubating for 30 min at 4 °C, unbound cells were removed by aspiration and washing two times with PBS^+. Bound cells were quantified by measuring fluorescence as described for the cell−selectin assay. Percent inhibition was calculated as described above. IC_{50} values were calculated by Probit analysis using Microsoft Excel.

P-Selectin Cell−Cell Adhesion Assay. The procedure for the P-selectin cell−cell adhesion assay was similar to that of the E-selectin cell−cell assay described above, except that Chinese hamster ovary cells transfected with human P-selectin (CHO-Psel) served as the source of native P-selectin. These cells, which were kindly provided by Dr. G. Kansas (Northwestern Medical School, Chicago, IL), constitutively express P-selectin on their surface. The CHO-Psel cells were propagated in Hamm's F12 medium containing 10% FBS. The day before the assay, the cells were trypsinized and plated in 96-well tissue culture dishes. As described above for the E-selectin cell−cell assay, BCECF-labeled HL60 cells were allowed to adhere to the monolayers at 4 °C in the presence of test compounds, and bound cells were detected using a fluorescence plate reader. Calculations were done as described above.

Chemistry. Nuclear magnetic resonance spectra (^{1}H NMR) were measured on either a Varian 300 MHz or a Varian 400 MHz. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet), integration, peak assignment, and coupling constant (hertz). Elemental analyses (C, H, N) were performed by Quantitative Technology Inc., Whitehouse, NJ, and results were within 0.4% of calculated values except where noted. High-resolution MALDI-FTMS (HRMS) were recorded by the Scripps Research Institute Mass Spectrometry Facility, San Diego, CA. Mass spectra were measured using Atmospheric Pressure Chemical Formation (APcI) looking at positive and negative modes on a Micromass LCZ (3 keV with a probe temperature of 400 °C and a source block at 120 °C). LC spectra for LC/MS were measured using an eluant of CH_3CN (0.1% CF_3CO_2H)/H_2O (0.1% CF_3CO_2H) (V:V) on a Hewlett-Packard HP1100 HPLC, in the range 200−300 nm with a diode array detector (DAD); 5 μL per injection (Gilson 215 Autosampler) at an average concentration of 1 mg/mL; gradient: 10−100% CH_3CN in 5 min, 100% CH_3CN for 1 min, 100−10% CH_3CN in 0.2 min, 10% CH_3CN for 1.4 min; LC element split 1:4 directly into ion source (500 μL/min). Analytical HPLC was performed on two different reverse phase systems with a flow rate of 1.5 mL/min: HPLC system 1 − Hewlett-Packard HP1100 HPLC with Zorbax 150 × 4.6 mm C-8 column (5 μm particle sizes) and 200−300 nm DAD; 10−100% CH_3CN (solvent A) and 0.1% TFA in water (solvent B) were used as mobile phase with a run time of 15 min. HPLC system 2 − Thermo Separation Products HPLC with Kromsil 150 × 4.6 mm C-18 column (5 μm particle size) and 220 and 254 nm variable UV detector; 40−100% CH_3CN (solvent A) and 0.1% TFA in water (solvent B) were used as the mobile phase with a run time of 15 min. The purity of the final compounds is above 95% except where noted. The chromatography columns used for LC in LC/MS and HPLC were 50 × 4.6 mm C-8 with 5 μm particle sizes and Zorbax 150 × 4.6 mm C-8 with 5 μm particle sizes, respectively. The same gradient was used in HPLC as in LC for LC/MS. Reactions in solution phase were monitored by thin-layer chromatography (TLC) using Merck silica gel 60F-254-coated plates (0.25 mm thickness). Flash

chromatography was performed using E. Merck silica gel 60 (230−400 mesh ASTM).

General Methods. General Method 1: Synthesis of Aldehydes 3a, 3b, and 21 (Scheme 3). General procedure for synthesis of oxime **16**: The aldehyde **15** (10 g, 48 mmol) was dissolved in dioxane (40 mL). Triethylamine (20 mL) was added, followed by hydroxylamine hydrochloride (4.0 g, 58 mmol). The reaction mixture was sonicated for 3 h and then stirred at room temperature for 3 days. The progress of the reaction was monitored by ^{1}H NMR. The reaction was worked up by concentration in vacuo to about 50% of the original volume. Water (60 mL) was added, and the reaction mixture was extracted with diethyl ether (3 × 40 mL). The combined organic extracts were then dried ($MgSO_4$) and concentrated in vacuo. The oxime **16** was obtained, and the crude material was used in the next reaction (10 g, crude yield, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.0 (s, 1H), 7.4 (d, 2H, J = 8), 7.3 (d, 2H, J = 8), 5.4 (s, 1H), 3.5 (m, 4H), 1.1 (m, 6H).

The oxime **16** (25 g, 112 mmol) was dissolved in THF (200 mL). *tert*-Butyl acrylate **17** (29 g, 223 mmol) was added and the reaction mixture cooled to 0 °C. Bleach (5.25% sodium hypochlorite aqueous, 400 mL) was added, and the reaction mixture was allowed to warm to room temperature. When all of the starting material had been consumed, the reaction was worked up via addition of ethyl acetate (200 mL), followed by washing with 10% $Na_2S_2O_3$ (2 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo. The *tert*−butyl acrylate was removed by coevaporation with toluene (monitored by NMR) to give compound **18**. The acetal protecting group of **18** was removed by dissolving the isoxazoline acetal **18** in THF/water (300 mL/50 mL) followed by addition of acidic Amberlite IR-120 ion-exchange resin (2.0 g). The reaction mixture was stirred at room temperature for 5 h. The resin was then removed via filtration and the product extracted with DCM. The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The aldehyde **3a** was obtained as a pale yellow crystalline solid, which was recrystallized from DCM/hexane (22 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$): 10.01 (s, 1H), 7.95 (d, 2H, J = 8.1), 7.85 (d, 2H, J = 8.1), 5.1 (t, 1H, J = 9.6), 3.61 (d, 2H, J = 9.6), 1.5 (s, 9H).

The aldehyde input **3b** was synthesized according to general method 1, using methyl acrylate in place of *tert*-butyl acrylate to give aldehyde **3b** (R = Me) (92%). ^{1}H NMR (300 MHz, $CDCl_3$): 10.06 (s, 1H), 7.94 (d, 2H, J = 8.1), 7.86 (d, 2H, J = 8.4), 5.30−5.24 (m, 1H), 3.85 (s, 3H), 3.72−3.67 (m, 2H).

The aldehyde input **21** was synthesized according to general method 1, using ethyl propiolate **19** in place of *tert*-butyl acrylate to give aldehyde **21** as a white solid (1.8 g, 38%). TLC R_f = 0.5 (1:1 hexane/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): 10.10 (s, 1H), 8.03 (s, 4H), 7.33 (s, 1H), 4.49 (q, 2H, J = 7.2), 1.46 (t, 3H, J = 7.2).

General Method 2: Synthesis of Wittig Reagent 4 (Scheme 1). 4-Bromobenzyl bromide (10 g, 40 mmol) was added to triphenyl phosphine (11 g, 42 mmol), in *o*-xylene (50 mL). The mixture was heated to 150 °C overnight. The Wittig reagent **4** crystallizes out of solution and is collected by filtration as a white crystalline solid, which is washed with hexane and dried. The yield is quantitative. ^{1}H NMR (300 MHz, $CDCl_3$): 7.82−7.71 (m, 9H), 7.65−7.56 (m, 6H), 7.18 (d, 2H, J = 8.7), 7.06 (d, 2H, J = 8.6), 5.57 (d, 2H, J = 15.0).

General Method 3: Synthesis of Dione 8 via a Wittig reaction (Scheme 1). Wittig reaction to give alkene **5**: To the Wittig reagent **4** (22 g, 43 mmol) in dry DMSO (65 mL) was added potassium *tert*-butoxide (5.1 g, 43 mmol), and the mixture was stirred at room temperature. After 30 min, the aldehyde **3a** (11.4 g, 41 mmol) was added in dry THF (150 mL). The reaction was stirred for 1 h at room temperature and then quenched by pouring into ice water (100 mL). This mixture was then extracted with DCM (3 × 100 mL). The combined DCM extracts were washed with water (1 × 50 mL), saturated sodium bicarbonate (1 × 50 mL), and brine (1 × 50 mL). The mixture was dried over anhydrous sodium sulfate and concentrated to dryness. The crude product was purified by silica gel chromatography (eluting with hexane:ethyl acetate, 3:1) to give the desired cis and trans alkenes **5** as a pale yellow oil (9.4 g, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) cis isomer: 7.64 (d, 2H, J = 7.7), 7.55 (d, 2H, J = 8.2), 7.48 (d, 2H, J = 7.7), 7.40 (d, 2H, J = 8.5), 7.10 (s, 2H), 5.08 (t, 1H, J = 9.6), 3.6 (d, 2H, J = 9.6), 1.5 (s, 9H); trans isomer: 7.55 (d, 2H, J = 8.2), 7.35 (d, 2H, J = 8.5), 7.28 (d, 2H, J = 8.0), 7.10 (d, 2H, J = 8.2), 6.63 (d, 1H, J = 12.0), 6.57 (d, 1H, J = 12.0), 5.08 (t, 1H, J = 9.6), 3.60 (d, 2H, J = 9.9), 1.50 (s, 9H).

Preparation of epoxide **6**: The alkene **5** (9.4 g, 22 mmol) was dissolved in DCM (100 mL), and then mCPBA (5.0 g, 22 mmol, purity 57−86%) in DCM (100 mL) was added. The reaction was stirred at 40 °C for 10 h and then treated with 10% sodium sulfite until testing with starch paper was negative. The reaction mixture was then extracted with DCM. The combined organic extracts were washed with saturated sodium bicarbonate and brine and dried over anhydrous sodium sulfate. The product was concentrated to dryness. The product was purified via flash chromatography eluting with hexane:ethyl acetate (8:1 then 6:1). The desired epoxide **6** was obtained as a pale yellow foam (8.9 g, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of isomers: 7.47 (m, 2H), 7.27 (m, 2H), 7.17 (m, 2H), 7.01 (m, 2H), 4.97 (m, 1H), 4.35 (m, 2H), 3.49 (m, 2H), 1.48 (s, 9H).

Opening of epoxide **6** to give diol **7**: The epoxide **6** (11 g) was dissolved in THF (15 mL). The solution was cooled in an ice bath, and formic acid (30 mL) was added slowly followed by water (0.5 mL). The reaction was stirred at 0 °C for 5 h. Upon completion, the reaction was concentrated in vacuo. The residue was dissolved in THF (40 mL) and treated with 1 N NaOH (aqueous) until a color change was observed (yellow to brown). The reaction was monitored carefully by TLC. Upon completion, the product was extracted into ethyl acetate (2 × 200 mL), dried ($MgSO_4$), and concentrated in vacuo. The product was purified by column chromatography, eluting with 30% EtOAc in hexane, to give the desired diol **7** (7.2 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) 1:1 mixture of isomers: 7.56 (d, 2H, J = 8.0), 7.52 (d, 2H, J = 8.0), 7.38 (d, 2H, J = 8.0), 7.35 (d, 2H, J = 8.0), 7.19 (d, 2H, J = 8.0), 7.12 (d, 2H, J = 8.0), 7.03 (d, 2H, J = 8.0), 6.96 (d, 2H, J = 8.0), 5.1−4.95 (m, 1H), 4.90−4.80 (m, 3H), 4.70−4.55 (m, 2H), 3.60−3.49 (m, 4H), 1.50 (s, 18H).

Oxidation of diol **7** to give dione **8a**: The diol **7** (1.0 g, 2.2 mmol) was dissolved in dichloromethane (12 mL). To this mixture were added 0.7 M NaBr (1.5 mL, 1.0 mmol) and TEMPO (4.0 mg, 0.025 mmol), and the reaction mixture cooled to 0 °C. A freshly prepared buffered bleach solution (270 mg, $NaHCO_3$ dissolved in 16 mL of bleach (5.25% aqueous sodium hypochlorite) was added dropwise to the reaction mixture. The reaction mixture was then stirred for a further 15 min before work up. The reaction was quenched with 10% $Na_2S_2O_3$ aqueous (30 mL) and extracted with ethyl acetate (3 × 60 mL). The combined organic layers were then washed with water (1 × 30 mL) and brine (1 × 40 mL), dried ($MgSO_4$), and concentrated in vacuo, to afford the dione **8a** (0.8 g, quantitative) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): 8.01 (d, 2H, J = 8.5), 7.86 (d, 2H, J = 8.2), 7.83 (d,1H, J = 8.0), 7.69 (d, 2H, J = 8.2), 5.12 (t, 1H, J = 9.3), 3.62 (d, 2H, J = 9.4), 1.51 (s, 9H).

Aldehyde **3b** was used to synthesize the desired dione **8b** as the methyl ester using the methodology outlined for the synthesis of dione **8a** above, and crude was used in the following reaction.

Heck Reaction on Dione 8 To Give Dione 10 (Scheme 1). The dione **8a** or **8b** (1 equiv) was dissolved in DMF (to make 0.14 M solution), followed by addition of $Pd(OAc)_2$ (0.02 equiv), TEA (3 equiv), P(*o*-tolyl)$_3$ (0.09 equiv), and acrylamide (1.20 equiv). The reaction mixture was heated to 100 °C for 2 h. The reaction was then quenched via addition of water and extracted with methylene chloride. The combined organic layers were washed with 1 N HCl (aqueous) and water, dried (Na_2SO_4), and concentrated in vacuo, to give the desired derivatized diones **10a** and **10b**, respectively. The resulting dione was used for subsequent reactions without further purification.

Data for **10a** (quantitative yield, $R^1 = {}^t$Bu): ^{1}H NMR (300 MHz, $CDCl_3$): 8.02 (d, 2H, $J = 8.7$), 7.97 (d, 2H, $J = 8.1$), 7.82 (d, 2H, $J = 9$), 7.64−7.59 (m, 3H), 7.32−7.19 (m, 5H), 6.46 (d, 1H, $J = 15.6$), 5.77 (t, 1H, $J = 6$), 5.15−5.09 (m, 1H), 3.62−3.58 (m, 2H), 3.48−3.41 (m, 2H), 2.71 (t, 2H, $J = 7.8$), 1.98−1.88 (m, 2H), 1.51 (s, 9H).

Data for **10b** (quantitative yield, R^1 = Me): ^{1}H NMR (300 MHz, $CDCl_3$): 8.01 (d, 2H, $J = 6.6$), 7.96 (d, 2H, $J = 8.1$), 7.81 (d, 2H, $J = 9.0$), 7.65−7.52 (m, 3H), 7.35−7.14 (m, 5H), 6.44 (d, 1H, $J = 15.6$), 5.79 (t, 1H, $J = 5.0$), 5.26 (dd, 1H, $J = 10.5$, 7.2), 3.83 (s, 3H), 3.72−3.60 (m, 2H), 3.43 (q, 2H, $J = 6.3$), 2.70 (t, 2H, $J = 7.5$), 2.00−1.85 (m, 2H).

General Method 4: Synthesis of Acrylamide 9 (Scheme 5). Acrylamides **9a**−**e** were prepared by adding acryloyl chloride (1 equiv) to a cooled solution (0 °C) of the desired amine **32** (1.0 equiv) in dichloromethane (0.5 M) with triethylamine (1.0 equiv) as base. These acrylamides were used directly, without purification in the following Heck reaction.

General Method 5: Synthesis of Dione 25 (Scheme 4). To 4,4′-dibromobenzil **22** (5.0 g, 14 mmol, 1.0 equiv) in DMF (28 mL, 0.5 M) were added $Pd(OAc)_2$ (94 mg, 0.4 mmol, 0.03 equiv), P(*o*-tolyl)$_3$ (511 mg, 1.7 mmol, 0.12 equiv), TEA (3.9 mL, 28 mmol, 2.0 equiv), and *tert*-butyl acrylate (2.9 mL, 20 mmol, 1.45 equiv). The reaction was stirred at 100 °C for 1 h. Afterward, the acrylamide **9a**−**d** (7.5 mmol, 0.55 equiv) was added and the mixture stirred an additional hour. Upon completion, the mixture was diluted with ethyl acetate. The mixture was extracted with ethyl acetate (2 × 200 mL) and washed with water (2 × 100 mL). The aqueous layer was back extracted with an additional aliquot of ethyl acetate (250 mL). The combined organic phase was washed with water (1 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo to obtain a brown oil. The oil was purified by flash column chromatography, eluting with a hexane/ethyl acetate/dichloromethane mixture, to afford the desired product **25a**−**d** as a light brown solid (∼39%).

Data for **25a** ($R^{5'}$ = H, $R^{5''} = C_{12}H_{25}$): ^{1}H NMR (400 MHz, $CDCl_3$): 7.98 (d, 2H, $J = 6.3$), 7.97 (d, 2H, $J = 6.0$), 7.61−7.58 (m, 6H), 6.52 (d, 1H, $J = 11.7$), 6.49 (d, 1H, $J = 12.0$), 5.77 (t, 1H, $J = 4.2$), 3.39 (q, 2H, $J = 5.4$), 1.70 (brs, 2H), 1.59−1.54 (m, 9H), 1.33−1.30 (m, 18H), 0.88 (t, 3H, $J = 5.1$).

Data for **25b** ($R^{5'}$ = H, $R^{5''} = C_{16}H_{33}$): ^{1}H NMR (300 MHz, $CDCl_3$): 7.98 (d, 4H, $J = 7.2$), 7.69−57 (m, 6H), 6.51 (d, 1H, $J = 15.9$), 6.49 (d, 1H, $J = 15.9$), 5.77 (brs, 1H), 3.39 (q, 2H, $J = 5.4$), 1.82 (brs, 2H), 1.52 (s, 9H), 1.29 (s, 26H), 0.90 (t, 3H, $J = 5.1$).

Data for **25c** ($R^{5'}$ = H, $R^{5''} = PhC_7H_{15}$): ^{1}H NMR (300 MHz, $CDCl_3$): 8.44 (s, 1H), 7.97 (d, 2H, $J = 8.7$), 7.90 (d, 2H, $J = 8.7$), 7.71 (d, 1H, $J = 15.9$), 7.66−7.48 (m, 7H), 7.12 (d, 2H, $J = 8.7$), 6.77 (d, 1H, $J = 15.9$), 6.50 (d, 1H, $J = 15.9$), 2.50 (m, 2H), 1.50 (br s, 11H), 1.30 (br s,4H), 0.88 (t, 3H, $J = 6.9$).

Data for **25d** ($R^{5'} = R^{5''} = C_6H_{13}$): ^{1}H NMR (300 MHz, $CDCl_3$): 7.98 (d, 4H, $J = 8.1$), 7.70 (d, 1H, $J = 15.3$), 7.63 (d, 4H, $J = 8.4$), 7.59 (d, 1H, $J = 16.0$), 6.95 (d, 1H, $J = 15.3$), 6.48 (d, 1H, $J = 16.2$), 3.40 (br q, 4H, $J = 8.1$), 1.68−1.50 (m, 4H), 1.4−1.24 (m, 12H), 0.96−0.82 (m, 6H).

General Method 6: Synthesis of Imidazole (Schemes 2 and 4). Acetic acid (20 mL) was added to a mixture of the dione **10** or **25** (1.0 equiv), aldehyde (1.5 equiv), and NH_4OAc (30 equiv) and heated to 100 °C for ∼2 h. The reaction has to be monitored carefully if *tert*-butyl groups are present, as these will be removed with prolonged heating. The reaction mixture was extracted with ethyl acetate, washed with water, and then back extracted with ethyl acetate. The organic layers were combined, dried ($MgSO_4$), and concentrated in vacuo. The imidazole was purified by flash column chromatography, eluting with hexane/ethyl acetate (3:1). The compound fluoresces as a yellow spot on TLC under a long-wave UV lamp. The desired imidazole is obtained as a yellow or white solid.

General Method 7: Hydrolyses of a Methyl or Ethyl Ester (Also Removes *tert*-Butyl Esters). A mixture of appropriate ester (1.0 equiv), 1 N LiOH (15 equiv), and 1,4-dioxane (0.3 M of ethyl ester) was stirred at room temperature overnight. The reaction mixture was acidified with 1 N HCl and extracted with ethyl acetate. The ethyl acetate solution was washed with water and brine, dried over $MgSO_4$, and concentrated to dryness. The final acid was recrystallized using isopropyl alcohol and ethyl acetate.

General Method 8: Hydrolyses of a *tert*-Butyl Ester. The *tert*-butyl ester was dissolved in 50% TFA dichloromethane solution with ice bath. The reaction mixture was stirred at 0 °C for ∼1 h. The reaction mixture was then concentrated in vacuo. The product was precipitated with a mixture of acetonitrile (few drops) and ether, collected via filtration, and can be recrystallized from methanol/ethyl acetate.

3-[4-(2-[4-((*E*)-2-Carboxy-vinyl)-phenyl]-5-{4-[(*E*)-2-(3-phenyl-propylcarbamoyl)-vinyl]-phenyl}-1*H*-imidazol-4-yl)-phenyl]-4,5-dihydro-isoxazole-5-carboxylic Acid (13). Compound **13** was synthesized according to general method 7 from dione **10a** (0.73 g, 1.29 mmol) in acetic acid (6 mL), 4-formylcinnamic acid *tert*-butyl ester (0.36 g, 1.55 mmol), and NH_4OAc (3.0 g, 38.7 mmol). The resulting imidazole was purified by flash column chromatography eluting with DCM/MeOH (95:5) to give the protected imidazole 3-[4-(2-[4-((*E*)-2-*tert*-butoxycarbonyl-vinyl)-phenyl]-5-{4-[(*E*)-2-(3-phenyl-propylcarbamoyl)-vinyl]-phenyl}-1*H*-imidazol-4-yl)-phenyl]-4,5-dihydro-isoxazole-5-carboxylic acid *tert*-butyl ester **12a** as a yellow solid (0.42 g, 42%). ^{1}H NMR (300 MHz, DMSO-d_6): 8.15 (br s, 2H), 7.65−7.40 (br m, 12H), 7.30−7.10 (br m, 5H), 6.40−6.20 (br m, 3H), 5.04 (t, 1H, $J = 7.5$), 3.55 (d, 2H, $J = 7.5$), 3.34 (br s, 2H), 2.58 (br s, 2H), 1.84 (br s, 2H), 1.58 (s, 9H), 1.53 (s, 9H).

The bis-*tert*-butyl ester of **12a** was hydrolyzed according to general method 8 to give, after recrystallization, the desired imidazole 3-[4-(2-[4-((*E*)-2-carboxy-vinyl)-phenyl]-5-{4-[(*E*)-2-(3-phenyl-propylcarbamoyl)-vinyl]-phenyl}-1*H*-imidazol-4-yl)-phenyl]-4,5-dihydro-isoxazole-5-carboxylic acid **13** as a yellow solid (0.13 g, 36%). TLC: $R_f = 0.38$ (chloroform/isopropyl alcohol/formic acid, 80:20:1). ^{1}H NMR (300 MHz, DMSO-d_6): 8.20−8.14 (m, 3H), 7.86 (d, 2H, $J = 7.8$), 7.88 (d, 2H, $J = 8.1$), 7.69−7.57 (m, 7H), 7.45 (d, 1H, $J = 15.6$), 7.32−7.18 (m, 5H), 6.67 (d, 1H, $J = 15.9$), 6.63 (d, 1H, $J = 15.9$), 5.22−5.16 (m, 1H), 3.81−3.51 (m, 2H), 3.23−3.17 (m, 2H), 2.65−2.60 (m, 2H), 1.82−1.72 (m, 2H). HPLC-1: $t_R = 4.79$ min. HPLC-2: $t_R = 3.80$ min. HRMS: *m*/*z* 667.2540 $[M + H]^+$, expected 667.2551 $[M + H]^+$.

3-{4-[5-{4-[(*E*)-2-(3-Phenyl-propylcarbamoyl)-vinyl]-phenyl}-2-(4-pyrrolidin-1-yl-phenyl)-1*H*-imidazol-4-yl]-phenyl}-4,5-dihydro-isoxazole-5-carboxylic Acid (14). Compound **14** was synthesized according to general method 6 from dione **10b** (0.2 g, 0.35 mmol) in acetic acid (2.0 mL), with 4-pyrrolidin-1-yl-benzaldehyde (0.1 g, 0.38 mmol) and NH_4OAc (0.8 g, 11 mmol), to give 3-{4-[5-{4-[(*E*)-2-(3-phenyl-propylcarbamoyl)-vinyl]-phenyl}-2-(4-pyrrolidin-1-yl-phenyl)-1*H*-imidazol-4-yl]-phenyl}-4,5-dihydro-isoxazole-5-carboxylic acid methyl ester. The methyl ester **12b** was hydrolyzed according to general method 7 to give, after recrystallization, the desired imidazole 3-{4-[5-{4-[(*E*)-2-(3-phenyl-propylcarbamoyl)-vinyl]-phenyl}-2-(4-pyrrolidin-1-yl-phenyl)-1*H*-imidazol-4-yl]-phenyl}-4,5-dihydro-isoxazole-5-carboxylic acid **14** as a yellow solid (0.08 g, 33%). TLC: $R_f = 0.25$ (chloroform/isopropyl alcohol/formic acid, 80:20:1). ^{1}H NMR (300 MHz, DMSO): 8.16 (t, 1H, $J = 5.4$), 7.90 (d, 2H, $J = 9.0$), 7.70−7.56 (m, 8H), 7.43 (d, 1H, $J = 15.9$), 7.32−7.18 (m, 5H), 6.67−6.61 (m, 3H), 5.17 (dd, 1H, $J = 11.4$, $J = 6.9$), 3.79−3.55 (m, 2H), 3.35 (br s, 4H), 3.23−3.16 (m, 2H), 2.62 (t, 2H, $J = 7.8$), 1.98 (br s, 4H), 1.81−1.72 (m, 2H). MS (ESI): *m*/*z* 666.7 (100, $[M + H]$), calcd $C_{41}H_{40}N_5O_4$ ($[M + H]$) 666.3. HPLC-1: $t_R = 5.64$ min. HPLC-2: $t_R = 5.7$ min. HRMS: *m*/*z* 666.3095 $[M + H]^+$, expected 666.3075 $[M + H]^+$.

(*E*)-3-(4-{5-[4-((*E*)-2-Hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-4-yl}-phenyl)-acrylic Acid (1). Imidazole **1** was synthesized from dione **25b** (1.5 g, 2.38 mmol, 1.0 equiv) in acetic acid (14 mL) and DMSO (4 mL), with hexamethylenetetramine (1.67 g, 11.9 mmol, 5 equiv) and NH_4OAc (5.50 g, 71.4 mmol, 30 equiv). The resulting protected imidazole was purified by flash column chromatography eluting with a

gradient of 2−8% methanol in DCM to give (*E*)-3-(4-{5-[4-((*E*)-2-hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-4-yl}-phenyl)-acrylic acid *tert*-butyl ester **26** as a yellow solid (1.4 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.59 (s, 1H), 7.50−7.22 (m, 10H), 6.64 (br, s, 1H), 6.38 (d, 1H, J = 15.2), 6.28 (d, 1H, J = 16.0), 3.36−3.29 (m, 2H), 1.52−1.46 (m, 2H), 1.51 (s, 9H), 1.23 (br, s, 26H), 0.86 (t, 3H, J = 6.6).

The *tert*-butyl esters were removed according to general method 8 to give, after recrystallization from methanol/ethyl acetate, the desired imidazole (*E*)-3-(4-{5-[4-((*E*)-2-hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-4-yl}-phenyl)-acrylic acid **1** as a pale yellow solid (0.77 g, 60%). TLC: R_f = 0.13 (chloroform/isopropyl alcohol/formic acid, 80:20:1). ^{1}H NMR (300 MHz, DMSO-d_6): 8.49 (s, 1H), 8.11 (t, 1H, J = 5.6), 7.73 (d, 2H, J = 8.1), 7.60 (d, 2H, J = 8.4), 7.59 (d, 1H, J = 15.9), 7.52 (d, 2H, J = 8.4), 7.51 (d, 2H, J = 8.1), 7.42 (d, 1H, J = 15.6), 6.62 (d, 1H, J = 15.9), 6.56 (d, 1H, J = 16.2), 3.20−3.13 (m, 2H), 1.50−1.40 (m, 2H), 1.22 (br, s, 26H), 0.84 (t, 3H, J = 6.3). HPLC-1: t_R = 7.54 min. HPLC-2: t_R = 13.2 min. MS (ESI): m/z 584.8 (100, $[M + H]^+$), calcd $C_{37}H_{49}N_3O_3$ ($[M + H]^+$) 584.8. Anal. ($C_{37}H_{49}N_3O_3$) C, H, N.

3-(4-{4-[4-((*E*)-2-Carboxy-vinyl)-phenyl]-5-[4-(2-hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-4,5-dihydro-isoxazole-5-carboxylic Acid (29). Compound **29** was synthesized according to general method 6 from dione **25b** (0.5 g, 0.79 mmol) in acetic acid (5.5 mL), with 4-formyl-phenyl-4,5-dihydro-isoxazole-5-carboxylic acid *tert*-butyl ester **3a** (0.26 g, 0.95 mmol) and NH_4OAc (1.8 g, 23.8 mmol). The resulting imidazole was purified by flash column chromatography, eluting with hexane/ethyl acetate (3:1), to give 3-(4-{4-[4-((*E*)-2-*tert*-butoxycarbonyl-vinyl)-phenyl]-5-[4-(2-hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-4,5-dihydro-isoxazole-5-carboxylic acid *tert*-butyl ester **26** as a yellow solid (0.5 g, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): 8.01 (br, m, 2H), 7.70−7.20 (br, m, 10H), 6.40−6.10 (br, m, 3H), 5.10 (t, 1H, J = 9.3), 3.60 (d, 2H, J = 9.3), 3.30 (br, s, 2H), 1.58 (s, 9H), 1.56 (s, 9H), 1.57 (br, s, 2H), 1.30 (br, s, 26H), 0.85 (t, 3H, J = 7.5).

The *tert*-butyl esters were removed according to general method 8 to give, after recrystallization from methanol/ethyl acetate, the desired imidazole 3-(4-{4-[4-((*E*)-2-carboxy-vinyl)-phenyl]-5-[4-(2-hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-4,5-dihydro-isoxazole-5-carboxylic acid **29** as a pale yellow solid (0.3 g, 69%). TLC: R_f = 0.19 (chloroform/isopropyl alcohol/formic acid, 80:20:1). ^{1}H NMR (300 MHz, DMSO-d_6): 8.19 (d, 2H, J = 8.7), 8.10 (t, 1H, J = 5.4), 7.86 (d, 2H, J = 8.1), 7.75 (d, 2H, J = 8.4), 7.64−7.58 (m, 7H), 7.43 (d, 1H, J = 15.6), 6.65 (d, 1H, J = 15.9), 6.57 (d, 1H, J = 15.9), 5.25−5.19 (m, 1H), 3.84−3.61 (m, 2H), 3.19−3.13 (m, 2H), 1.50−1.40 (br, m, 2H), 1.22 (br, s, 25H), 0.84 (t, 3H, J = 6.60). MS (ESI): m/z 773.8 (30, $[M + H]^+$), calcd for $C_{47}H_{56}N_4O_6$ $[M + H]^+$ 773.4. Anal. ($C_{47}H_{56}N_4O_6 \cdot H_2O$) C, H, N.

3-(4-{4-[4-((*E*)-2-Carboxy-vinyl)-phenyl]-5-[4-(2-hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-isoxazole-5-carboxylic Acid (30). Imidazole **30** was synthesized according to general method 6 from dione **25b** (500 mg, 0.79 mmol) in acetic acid (4 mL), with 3-(4-formyl-phenyl)-isoxazole-5-carboxylic acid ethyl ester **21** (synthesized according to general method 1 using the appropriate alkyne) (290 mg, 1.2 mmol) and NH_4OAc (1.8 g, 23.7 mmol), which gave, after purification via column chromatography eluting with hexane/ethyl acetate/dichloromethane (2:1:1), 3-(4-{4-[4-((*E*)-2-*tert*-butoxycarbonyl-vinyl)-phenyl]-5-[4-(2-hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-isoxazole-5-carboxylic acid ethyl ester **26** (377 mg, 56%). TLC: R_f = 0.3 (1:1 H/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): 8.44 (d, 2H, J = 8.1), 8.11 (d, 2H, J = 8.4), 7.80−7.70 (m, 5H), 7.63 (d, 2H, J = 8.4), 7.53 (d, 3H, J = 7.8), 7.48 (s, 1H), 6.50 (d, 1H, J = 15.9), 6.48 (d, 1H, J = 15.9), 6.02 (brs, 1H), 4.67 (q, 2H, J = 7.2), 3.52 (q, 2H, J = 6.6), 1.86 (brs, 2H), 1.73 (s, 9H), 1.64 (t, 3H, J = 7.2), 1.44−1.41 (m, 26H), 1.07 (t, 3H, J = 6.6).

The *tert*-butyl and ethyl esters were removed according to general method 7 to give, after recrystallization from methanol/ethyl acetate, the desired imidazole 3-(4-{4-[4-((*E*)-2-carboxy-vinyl)-phenyl]-5-[4-(2-hexadecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-isoxazole-5-carboxylic acid **30** (217 mg, 75%) as a yellow solid. TLC: R_f = 0.3 (20% IPA/DCM, 1% formic acid). ^{1}H NMR (300 MHz, DMSO-d_6): 8.31 (d, 2H, J = 8.1), 8.19 (d, 2H, J = 8.4), 8.12 (t, 1H, J = 5.1), 7.92 (s, 1H), 7.78 (d, 2H, J = 8.1), 7.66−7.59 (m, 7H), 7.44 (d, 1H, J = 15.9), 6.67 (d, 1H, J = 15.6), 6.59 (d, 1H, J = 15.9), 3.16 (q, 2H, J = 6.0), 1.45 (t, 2H, J = 6.0), 1.23 (s, 26H), 0.84 (t, 3H, J = 5.7). LC/MS: LC retention time = 4.18 min. MS (APcI): m/z 771.0 (100, $[M + H]^+$), calcd $C_{47}H_{55}N_4O_6$ ($[M + H]^+$) 771.4. Anal. ($C_{47}H_{54}N_4O_6 \cdot H_2O$) C, H, N.

3-(4-{4-[4-((*E*)-2-Carboxy-vinyl)-phenyl]-5-[4-(2-dodecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-isoxazole-5-carboxylic Acid (31). Imidazole **31** was synthesized according to general method 6 from dione **25a** (300 mg, 0.52 mmol) in acetic acid (2 mL), with 3-(4-formyl-phenyl)-isoxazole-5-carboxylic acid ethyl ester **21** (synthesized according to general method 1 using the appropriate alkyne) (192 mg, 0.78 mmol) and NH_4OAc (1.2 g, 15.6 mmol), which gave, after purification via column chromatography eluting with DCM:methanol (95:5), 3-(4-{4-[4-((*E*)-2-*tert*-butoxycarbonyl-vinyl)-phenyl]-5-[4-(2-dodecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-isoxazole-5-carboxylic acid ethyl ester **26** (200 mg, 48%).

The ethyl and *tert*-butyl esters were hydrolyzed according to general method 7 to give, after recrystallization from methanol/ethyl acetate, the desired imidazole 3-(4-{4-[4-((*E*)-2-carboxy-vinyl)-phenyl]-5-[4-(2-dodecylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-2-yl}-phenyl)-isoxazole-5-carboxylic acid **31** (35 mg, 55%) as a yellow solid. TLC: R_f = 0.3 (20% IPA/DCM, 1% formic acid). ^{1}H NMR (300 MHz, DMSO-d_6): 8.24 (d, 2H, J = 8.4), 8.14 (t, 1H, J = 4.5), 8.08 (d, 2H, J = 8.4), 7.83 (s, 1H), 7.72 (d, 2H, J = 8.1), 7.63−7.59 (m, 7H), 7.42 (d, 1H, J = 15.6), 6.64 (d, 1H, J = 15.6), 6.54 (d, 1H, J = 15.9), 3.15 (t, 2H, J = 4.5), 1.44 (t, 2H, J = 5.7), 1.23 (s, 18H), 0.83 (t, 3H, J = 6.3). LC/MS: LC retention time = 3.72 min. MS (APcI): 715.1 (100, $[M + H]^+$), calcd $C_{43}H_{47}N_4O_6$ ($[M + H]^+$) 715.9. Anal. ($C_{43}H_{46}N_4O_6 \cdot 2H_2O$) C, H, N.

(*E*)-3-[4-(5-{4-[(*E*)-2-(4-Heptyl-phenylcarbamoyl)-vinyl]-phenyl}-1*H*-imidazol-4-yl)-phenyl]-acrylic Acid (27). Imidazole **27** was synthesized according to general method 6 from dione **25c** (300 mg, 0.52 mmol) in acetic acid (6 mL), with hexamethylene tetramine (360 mg, 2.58 mmol) and NH_4OAc (1.19 g, 15.5 mmol), to give, after purification via column chromatography eluting with DCM:methanol (95:5), (*E*)-3-[4-(5-{4-[(*E*)-2-(4-heptyl-phenylcarbamoyl)-vinyl]-phenyl}-1*H*-imidazol-4-yl)-phenyl]-acrylic acid *tert*-butyl ester **26** (110 mg, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): 8.96 (br, s, 1H), 7.77 (br, s, 1H), 7.61 (d, 2H, J = 7.8), 7.61−7.20 (m, 10H), 7.09 (d, 2H, J = 8.0), 6.67 (d, 1H, J = 15.3), 6.27 (d, 1H, J = 15.9), 2.54 (br, t, 2H, J = 7.2), 1.60−1.48 (m, 2H), 1.51 (s, 9H), 1.34−1.20 (m, 8H), 0.87 (t, 3H, J = 6.6).

The *tert*-butyl ester was removed according to general method 8 to give, after recrystallization from methanol/ethyl acetate, the desired imidazole (*E*)-3-[4-(5-{4-[(*E*)-2-(4-heptyl-phenylcarbamoyl)-vinyl]-phenyl}-1*H*-imidazol-4-yl)-phenyl]-acrylic acid **27** (31 mg, 28%) as a yellow solid. TLC: R_f = 0.21 (chloroform/isopropyl alcohol/formic acid, 80:20:1). ^{1}H NMR (300 MHz, DMSO-d_6): 7.86 (s, 1H), 7.68 (br, d, 4H, J = 6.3), 7.61−7.44 (m, 6H), 7.11 (d, 1H, J = 15.0), 6.52 (d, 1H, J = 15.9), 3.45 (t, 4H, J = 7.2), 1.51 (br, m, 4H), 1.27 (br, s, 12H), 0.86 (t, 6H, J = 7.5). HPLC-1: t_R = 5.92 min. HPLC-2: t_R = 7.15 min. HRMS: m/z 528.3209 ($[M + H]^+$), expected 528.3221.

(*E*)-3-(4-{5-[4-((*E*)-2-Dihexylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-4-yl}-phenyl)-acrylic Acid (28). Imidazole **28** was synthesized according to general method 6 from dione **25d** (410 mg, 0.71 mmol) in acetic acid (5 mL), with hexamethylenetetramine (1.05 g, 21.4 mmol) and NH_4OAc (1.97 g, 26 mmol), to give, after purification via column chromatography eluting with DCM:methanol (95:5), (*E*)-3-(4-{5-[4-((*E*)-2-dihexylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-4-yl}-phenyl)-acrylic acid *tert*-butyl ester **26** (280 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.06 (s, 1H), 7.56−7.50 (m, 6H), 7.45 (d, 2H, J = 8.0), 7.42 (d, 2H, J = 8.0), 6.82 (d, 1H, J = 16.0), 6.34 (d,

1H, $J = 16$), 3.34−3.36 (m, 4H), 1.66−1.56 (m, 4H), 1.62 (s, 9H), 1.32 (br, s, 12H), 0.87 (t, 6H, $J = 6.8$).

The ^{t}−butyl ester was removed according to general method 8 to give, after recrystallization from methanol/ethyl acetate, the desired imidazole (*E*)-3-(4-{5-[4-((*E*)-2-dihexylcarbamoyl-vinyl)-phenyl]-1*H*-imidazol-4-yl}-phenyl)-acrylic acid **28**, (50 mg, 18%) as a yellow solid. TLC: $R_f = 0.15$ (chloroform/isopropyl alcohol/formic acid, 80:20:1). ^{1}H NMR (400 MHz, DMSO-d_6): 12.40 (br, s, 1H), 10.12 (s, 1H), 7.95 (s, 1H), 7.69 (d, 2H, $J = 8.0$), 7.63−7.51 (m, 10H), 7.14 (d, 2H, $J = 8.4$), 6.82 (d, 1H, $J = 16.0$), 6.53 (d, 1H, $J = 16.0$), 2.52 (t, 2H, $J = 8.0$), 1.58−1.50 (br, m, 2H), 1.30−1.22 (br, m, 8H), 0.85 (t, 3H, $J = 6.8$). HPLC-1: $t_R = 6.16$ min. HPLC-2: $t_R = 8.38$ min. HRMS *m*/*z* 534.274 ([M + H]$^+$), expected 534.2751.

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Supporting Information Available: ^{1}H NMR spectra of compounds **1**, **13**, **14**, and **27**−**31**. This material is available free of charge via the Internet at http://pubs.acs.org.

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