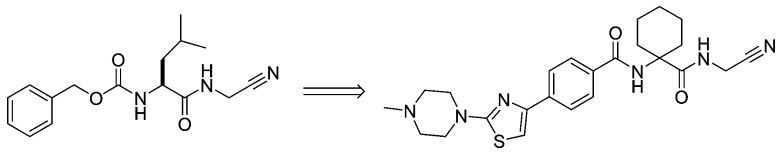
Journal of Medicinal Chemistry

Article

Design and Synthesis of Tri-Ring P Benzamide-Containing Aminonitriles as Potent, Selective, Orally Effective Inhibitors of Cathepsin K

James T. Palmer, Clifford Bryant, Dan-Xiong Wang, Dana E. Davis, Eduardo L. Setti, Robert M. Rydzewski, Shankar Venkatraman, Zong-Qiang Tian, Leland C. Burrill, Rohan V. Mendonca, Eric Springman, John McCarter, Tobee Chung, Harry Cheung, James W. Janc, Mary McGrath, John R. Somoza, Philip Enriquez, Z. Walter Yu, Robert M. Strickley, Liang Liu, Michael C. Venuti, M. David Percival, Jean-Pierre Falgueyret, Peppi Prasit, Renata Oballa, Denis Riendeau, Robert N. Young, Gregg Wesolowski, Sevgi B. Rodan, Colena Johnson, Donald B. Kimmel, and Gideon Rodan

J. Med. Chem., **2005**, 48 (24), 7520-7534• DOI: 10.1021/jm058198r • Publication Date (Web): 29 October 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 8 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Articles

Design and Synthesis of Tri-Ring P_3 Benzamide-Containing Aminonitriles as Potent, Selective, Orally Effective Inhibitors of Cathepsin K

James T. Palmer,*,† Clifford Bryant,† Dan-Xiong Wang,† Dana E. Davis,† Eduardo L. Setti,† Robert M. Rydzewski,† Shankar Venkatraman,† Zong-Qiang Tian,† Leland C. Burrill,† Rohan V. Mendonca,† Eric Springman,† John McCarter,† Tobee Chung,† Harry Cheung,† James W. Janc,† Mary McGrath,† John R. Somoza,† Philip Enriquez,† Z. Walter Yu,† Robert M. Strickley,† Liang Liu,† Michael C. Venuti,† M. David Percival,‡ Jean-Pierre Falgueyret,‡ Peppi Prasit,‡ Renata Oballa,‡ Denis Riendeau,‡ Robert N. Young,‡ Gregg Wesolowski,§ Sevgi B. Rodan,§ Colena Johnson,|| Donald B. Kimmel,§ and Gideon Rodan§

Celera Genomics, Inc., 180 Kimball Way, South San Francisco, California 94080, Merck Frosst Centre for Therapeutic Research, 16711 TransCanada Highway, Kirkland, Quebec, Canada H9H 3L1, Department of Bone Biology & Osteoporosis, Merck Research Laboratories, West Point, Pennsylvania, and Department of Laboratory Animal Resources, Merck Research Laboratories, West Point, Pennsylvania

Received February 28, 2005

We have prepared a series of achiral aminoacetonitriles, bearing tri-ring benzamide moieties and an aminocyclohexanecarboxylate residue at P_2. This combination of binding elements resulted in sub-250 pM, reversible, selective, and orally bioavailable cathepsin K inhibitors. Lead compounds displayed single digit nanomolar inhibition in vitro (of rabbit osteoclast-mediated degradation of bovine bone). The best compound in this series, **39n** (CRA-013783/L-006235), was orally bioavailable in rats, with a terminal half-life of over 3 h. **39n** was dosed orally in ovariectomized rhesus monkeys once per day for 7 days. Collagen breakdown products were reduced by up to 76% dose-dependently. Plasma concentrations of **39n** above the bone resorption IC_{50} after 24 h indicated a correlation between functional cellular and in vivo assays. Inhibition of collagen breakdown by cathepsin K inhibitors suggests this mechanism of action may be useful in osteoporosis and other indications involving bone resorption.

Introduction

Osteoporosis can be described as brittle bone disease, manifested by loss of bone mass and an increased tendency to fracture. The condition can be brought on by an imbalance of the functions of osteoblasts, responsible for bone formation, and osteoclasts, which are responsible for bone resorption.[1] The major structural components of extracellular bone are hydroxyapatite and protein, of which the latter is 90% type 1 collagen. During the bone resorption process, the multinucleated osteoclasts form resorption lacunae on the surface of the bone, into which protons and proteases are secreted. This acidic environment is conducive to the decalcification of the bone and is also suitable for the proteolytic degradation of the collagen component.

As a member of the papain family of cysteine proteases, including cathepsins B, L, S, F, V, W, O, H, C, and Z, cathepsin K's distribution renders it an attractive target for selective inhibition. Cathepsin K is the major cysteine protease expressed in osteoclasts.[2] Type 1 collagen is readily degraded by cathepsin K over a pH range of approximately 4−7. The abundance and selective location of this enzyme in the cells responsible for bone resorption suggest its potential as a target for osteoporosis therapeutics. Furthermore, a genetic deficiency in this enzyme in humans results in pcynodysostosis, a condition whose phenotype is characterized by short, stubby, and dense bones (osteopetrosis).[3] This syndrome indicates a clear connection between an imbalance in cathepsin K activity and the overall bone turnover process. A similar genetic mutation introduced experimentally in mice also results in an osteopetrotic phenotype.[4]

Inhibitor Design Motif. A number of recent reports on the inhibition of cathepsin K have focused on carbonyl-containing, transition state mimic-based inhibitors, including aldehydes,[5] aminomethyl ketones,[6] and hydroxymethyl ketones.[7] The challenges posed by inhibitors of such classes include, but are not limited to, high molecular weight, low oral bioavailability, and metabolic or chemical instability. Several reports have also issued describing the crystal structures of cathepsin K with vinyl sulfones,[8] substituted ketone-based inhibitors,[9] and ketobenzoxazoles.[10] The active site of cathepsin K in said cocrystal structures has revealed distinct opportunities for obtaining selectivity over other cysteine protease antitargets, such as cathepsins B, L, and S. The S_3 subsite of cathepsin K contains an aspartic acid residue (Asp^{61}) capable of forming ionic bonds/salt bridges with an appropriately designed P_3 moiety. Also, the wide opening of the S_2 pocket suggested accom-

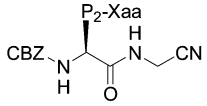
* To whom correspondence should be addressed. Tel.: (650) 866-6641. Fax: (650) 866-6654. e-mail: jim.palmer@celera.com.
† Celera Genomics, Inc.
‡ Merck Frosst Centre for Therapeutic Research.
§ Department of Bone Biology & Osteoporosis, Merck Research Laboratories.
|| Department of Laboratory Animal Resources, Merck Research Laboratories

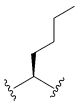
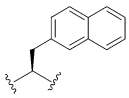
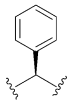
10.1021/jm058198r CCC: $30.25
Published on Web 10/29/2005

Table 1. Assay Conditions for Cathepsins K, B, L, and S. All Assays Were Performed with 8-Point Dose−Response Curves

	Cathepsin K	Cathepsin B	Cathepsin L	Cathepsin S
substrate	Z-Phe-Arg-AMC, 40 μM	Boc-Leu-Lys-Arg-AMC, 190 μM	Z-Phe-Arg-AMC, 10 μM	Z-Val-Val-Arg-AMC, 60 μM
buffer, pH	50 mM MES, 2.5 mM EDTA, 2.5 mM DTT, 10% DMSO, pH 5.5	50 mM BES, 2.5 mM EDTA, 0.001% Tween-20, 2.5 mM DTT, 10% DMSO, pH 6.0	50 mM MES, 2.5 mM EDTA, 2.5 mM DTT, 10% DMSO, pH 5.5	50 mM MES, 2.5 mM EDTA, 100 mM NaCl, 0.001% BSA, 10% DMSO, pH 6.5

Table 2. Inhibition Constants (K_i(app), μM) for CBZ-P_2-Xaa-GlyCN Derivatives

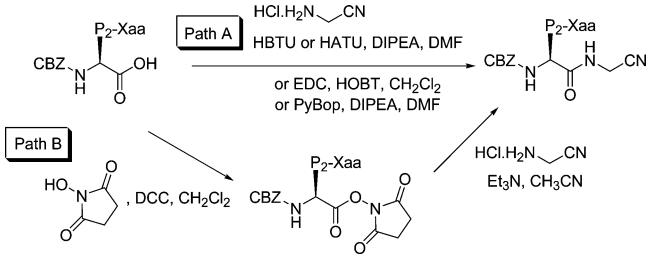


Entry	P_2-Xaa	Cat K	Cat B	Cat L	Cat S	Entry	P_2-Xaa	Cat K	Cat B	Cat L	Cat S
1		0.0347	123	2.79	0.143	**8**		1.8	>150	>150	4.2
2		0.49	>150	37	0.86	**9**		1.9	>150	>150	>150
3		30	3	0.054	0.18	**10**		0.23	63	23	0.15
4		11	>150	14	42	**11**		17	>150	88	3.5
5		1.5	70	25	14	**12**		>150	>150	>150	>150
6		13	>150	>150	13.6	**13**		1.5	110	>150	>150
7		0.35	>150	22	0.4	**14**		0.084	64	95	31

modation by a variety of P_2 binding elements capable of forming hydrophobic interactions. Last, the structures thus far solved all pointed to the crucial backbone interactions between the P_2-P_1 amide of the inhibitors and key residues of the enzyme (Gly[66], Asn[158]). With these facts in mind, we commenced our inhibitor design based on a modified dipeptide motif. We selected nitrile-based inhibitors as the framework for the studies described herein so as to lower molecular weight and provide a facile chemotype for rapid analogue generation.

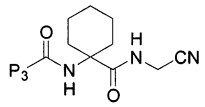
Dipeptide-based nitriles have been recently described in the preparation of cathepsin B[11] and cathepsin S-selective inhibitors,[12] as well as for cathepsin K-targeted inhibitors.[13] Starting with aminoacetonitrile as the reactive center, we prepared a series of carbobenzyloxy (CBZ)-protected dipeptide nitriles in order to probe the selectivity elements demanded by cathepsin K's active site. Using standard peptide-type coupling methods,[14] we converted CBZ-protected, commercially available amino acids to their aminoacetonitrile derivatives. Path A directly permitted us to couple the aminoacetonitrile moiety with the carboxylate, while path B involved the *N*-hydroxysuccinimidoyl active ester, which was readily displaced by aminoacetonitrile.

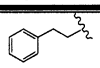
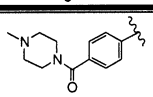
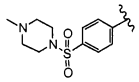
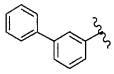
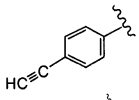
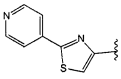
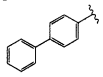
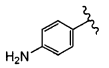
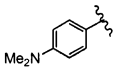
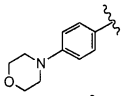
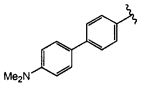
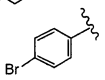
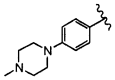
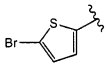
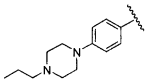
Scheme 1. Synthesis of CBZ-P_2-Xaa-GlyCN Derivatives



The inhibitors were then assayed against a panel of cysteine proteases using conditions tailored for the particular enzymes, cathepsins K, B, L, and S (Table 1). Table 2 shows the K_i(app) values (in μM units) obtained for this series following a 15 min preincubation of enzyme with inhibitor, prior to the addition of substrate. Values were obtained via 8-point dose−response curves. Apparent inhibition constants, K_i-(app)'s, were calculated from the velocity data generated at the various inhibitor concentrations using the software package, BatchKi (obtained from Dr. Petr Kuzmic, Biokin Ltd., Pullman, WA).[18] BatchKi provides a parametric method for the determination of inhibitor potency using a transformation of the tight binding inhibition model described by Morrison[19] and further refines the

Table 3. Inhibition Constants (K_i(app))of Human Cysteine Proteases by Amide-Ac6-aminoacetonitriles (all values in μM)



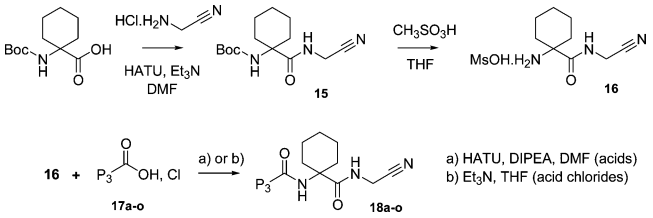
Entry	P_3	Cat K	Cat B	Cat L	Cat S	Entry	P_3	Cat K	Cat B	Cat L	Cat S
18a		0.41	99	>150	34	**18i**		0.014	13	>150	110
18b		0.23	7.9	7.9	6.2	**18j**		0.013	3.1	71	102
18c		0.21	8.3	22	1.9	**18k**		0.013	1.1	18	15
18d		0.15	9.3	>150	4	**18l**		0.0062	1.3	23	8.8
18e		0.032	10	45	6.5	**18m**		0.0033	5.2	48	35
18f		0.019	8.6	50	42	**18n**		0.002	0.68	3.2	5.7
18g		0.018	2.3	17	10	**18o**		0.0015	2.7	9.6	16
18h		0.016	3.1	5.9	1.4	**18p**		0.0014	2.9	18	28

K_i(app) values by nonlinear least-squares regression. The apparent inhibition constant is related to the true thermodynamic binding constant, K_i, by the following relationship: $K_i = K_i(\text{app})/(1 + [\text{substrate}]/K_m)$,[19] since substrates are typically supplied in the assays at their K_m, $K_i = K_i(\text{app})/2$.

From the examples prepared in this series, we observed that large P_2 amino acids (**3**, CBZ-Nal-GlyCN) favored cathepsins L and S, while simple P_2-leucine derivatives, such as **1**, **2**, **7**, and **10**, all afforded submicromolar K_i(app) values versus cathepsin K. Interestingly, the P_2-D-leucine derivative **6** retained modest potency against cathepsins K and S, and the racemic α-methylleucine derivative **8** also showed single digit micromolar potency against both enzymes. This suggested that cathepsin K's S_2 subsite might accommodate a bulky, α-substituted amino acid, and therefore we prepared the achiral cycloalkane-based derivatives **12**−**14**. We were gratified to discover that both cyclopentyl and cyclohexyl (Ac5, Ac6 respectively) afforded both potency and selectivity in this simple series. Armed with **14**, 84 nM versus cathepsin K, and several 100-fold selectivity over our antitargets, we embarked on a more elaborate series designed to maximize interactions between the S_3 subsite of the enzyme and the P_3 binding moiety of the inhibitor.

Initially, we explored the relevance of the linker group between the Ac6 amino group and the binding moiety. Since it was not clear at this point how important interactions were between the P_3−P_2 amide bond (or equivalent) and the active site, we prepared a series of

Scheme 2. Synthesis of P_3 Amides in the Ac6-GlyCN Series

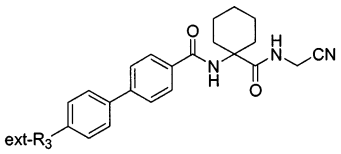


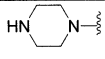
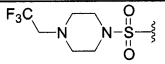
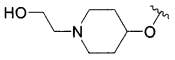
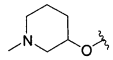
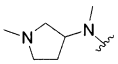
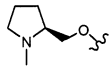
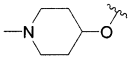
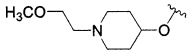
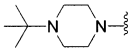
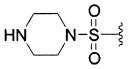
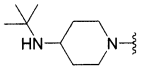
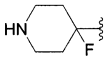
amides to probe this area of binding, using standard coupling procedures (Scheme 2).

Attempts to use deprotecting acids (TFA, HCl/dioxane, etc.) in removing the Boc group of **15** resulted in substantial formation of unwanted *tert*-butyl amide product. We proposed that this resulted from protonation of the nitrile by the acid; subsequently, the short-lived *tert*-butyl cation that results from Boc removal could be trapped by trace amounts of water to give *tert*-butyl alcohol, which could react with the highly electrophilic nitrilium ion, followed by tautomerization. This phenomenon was always observed with trace amounts of water in the system; we avoided this by ensuring that both the methanesulfonic acid and THF were scrupulously dry. Following isolation of **16**, we then coupled a series of acids (or acyl chlorides) via HATU methodology, or via simple acid chloride addition in the presence of base to afford **18a**−**o**. Table 3 shows inhibition data for this series.

We observed a clear requirement for sp^2 hybridization at the atom adjacent to the P_3 carbonyl (**18a** versus **14**, **18b**−**o**) and thus pursued regiochemical substitution

Table 4. Inhibition of Cathepsin K (K_i(app)) and in Vitro Potency (IC_{50}) in Bone Resorption Assay Functional Assay by Extended P_3 Biphenylamide Derivatives of the Ac6-GlyCN Motif (all values in μM)



Entry	ext-R_3	Cat K	Cat B	Cat L	Cat S	Bone res. IC_{50}	Entry	ext-R_3	Cat K	Cat B	Cat L	Cat S	Bone res. IC_{50}
19a		<.00025	0.42	7.9	4.9	0.077	**19h**		0.0061	56	0.54	5.5	0.32
19b		<.00025	0.64	9.1	16	0.059	**19i**		0.00067	0.87	2.3	13	0.010
19c		<.00025	0.34	2	8.7	0.033	**19j**		<.00025	0.59	4	10	0.024
19d		<.00025	0.53	5.6	12	0.020	**19k**		<.00025	0.54	1.2	6.2	0.021
19e		<.00025	0.51	11	7.9	0.013	**19l**		0.0054	1.8	0.42	2.5	0.037
19f		<.00025	0.38	0.43	9.1	0.020	**19m**		<.00025	76	1.6	3.9	0.040
19g		<.00025	0.28	0.9	7.9	0.035							

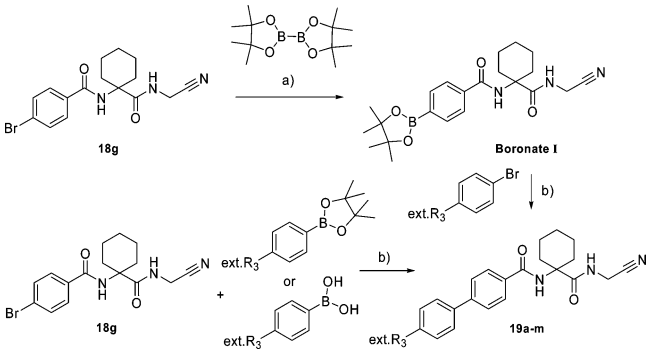
about the P_3 benzamide ring. On the basis of the 13-fold increase in cathepsin K potency comparing the 3-bromobenzamide **18b** versus the 4-bromobenzamide **18g**, and an even greater increase in potency between the *m*-biphenyl **18c** and the *p*-biphenyl **18l** (34-fold), we concluded that para-substituted benzamides would afford the most potent compounds. This conclusion was consistent with the 4-pyridylthiazolyl substituent in **18d** displaying intermediate cathepsin K potency of 150 nM, the geometry of the five-membered heterocycle placing the substituent closer to the 3-substituted benzamides than to the 4-substituted. By contrast, the 2-bromothiophene **18h**, considered to possess similar geometry to a bromophenyl group, was equipotent to **18g**.

The introduction of a "kink" in the 4-substituent (amide or sulfonamide, **18i**, **18j** respectively) appeared to offer no improvement in binding affinity. Substitution with additional rings, particularly those bearing somewhat basic moieties (**18m**−**o**), increased potency to single-digit nanomolar values, without significant loss in selectivity.

We realized that an additional aryl ring spacer between the P_3 benzamide and the basic moiety of the extended P_3 group might enhance activity, particularly by fixing the geometry of the lengthened group such that the Asp[61] residue might form a tighter interaction. To this end we prepared a series of 4-substituted biphenylamides, via Scheme 3 as the key step.

Transformation of **18g** to the corresponding pinacolboronate ester intermediate boronate **I** was effected by cross-coupling with pinacoldiborane using anhydrous conditions.[15] This material was isolated by precipitation as an off-white solid and was not purified further, but was subjected to Suzuki cross-coupling with appropriate aryl bromides. Alternatively, treatment of **18g** with

Scheme 3. Access to Extended P_3 Biphenylamides[a]



[a] (a) Pd(dppf)Cl_2·CH_2Cl_2, KOAc, DMF, 80 °C, 6 h; (b) Pd(dppf)Cl_2·CH_2Cl_2, DMF, 2 M Na_2CO_3, 80−85 °C, 3 h.

phenylboronates or phenylboronic acids substituted at the 4-position in the presence of palladium(II) catalyst and aqueous base in DMF under Suzuki-type conditions afforded the biphenylamides **19a**−**m**; elaborated examples required additional transformation following coupling.

In addition to assaying this series against cathepsins K, B, L, and S, we also assayed these compounds in an in vitro bone resorption assay that evaluates degradation of bovine bone by isolated rabbit osteoclasts (measuring the degradation of collagen using ELISA).[16] Table 4 shows the results comparing this series's selectivity as well as its potency against the isolated enzyme and in the functional cellular assay.

Clearly, the presence of a basic nitrogen distal from the point of attachment to the second aryl ring enhances activity against cathepsin K, resulting in sub-250 pM potency in most cases. This effect appeared to hold up even in the case of highly hindered amines (**19e**, **19m**).

Table 5. Intravenous PK Parameters for Extended P_3-Biphenylamide-Ac6 Nitriles

entry	Cl (mL/min/kg)	V_{ss} (L/kg)	MRT (min)	$\beta t_{1/2}$ (min)	C_{max} (μM)
19a	71	7.23	104	84	0.5
19b	231	25.3	110	123	0.22
19d	97	11.1	114	114	0.32
19e	67	11.2	172	144	0.24
19f	82	3.29	40	78	1.0
19g	37	1.76	48	47	0.66
19h	40	1.55	39	46	1.2
19i	104	8.46	81	90	0.25
19k	43	3.27	117	121	0.37
19m	84	10.7	130	116	0.36

entry	dose (mg/kg)	F (%)	$\beta t_{1/2}$ (min)	C_{max} (min)	T_{max} (min)
19a	10	21	113	0.14	120

[a] Abbreviations: CL = clearance; V_{ss} = volume of distribution; MRT = mean residence time; $\beta t_{1/2}$ = terminal half-life; C_{max} = peak concentration. T_{max} = time of peak concentration post-dose. F = oral bioavailability. Compounds were dosed intravenously at 0.5 mg/kg (iv) and 10 mg/kg for the oral study.

Having established sub-nM levels of potency versus cathepsin K in this series, along with sub-100 nM cellular potency as measured in the bone resorption functional assay, we turned our attention toward developing this series for in vivo efficacy studies. To this end, we explored derivatives in a cassette dosing mode in rats, whereby intravenous pharmacokinetic parameters (half-life, clearance, mean residence time (MRT), and volume of distribution) were obtained for up to four compounds per study. We used parameters obtained via this route to guide selection of candidates to be dosed in single compound, iv/po PK studies that followed. PK parameters for the cassette-dosed compounds are summarized in Table 5. Compounds were typically formulated in DMSO (<20%), PEG 400 (<20%), and water at 0.5 mg/mL/compound. A group of three rats were used in each study to obtain statistical data. Animals were dosed intravenously at 1 mL/kg to reach a final dose of 0.5 mg/kg/compound. Blood samples were taken at different time points, and plasma samples were prepared and analyzed by LC/MS/MS. Pharmacokinetic analysis was performed using WinNonlin Software.

Of this series, **19a**, bearing the 4-piperazinebiphenylamide moiety, was dosed in a single compound oral bioavailability experiment. The low peak concentration of 0.14 μM, when dosed at 10 mpk, was only twice that of the cellular potency as measured in the bone resorption assay and was not sustained over the course of several hours. This suggested that this series was not likely suitable for further development as a once-per-day preclinical candidate for osteoporosis treatment at this dose.

To improve oral bioavailability, we modified the tri-ring series such that the distal phenyl ring of the biphenyl array would be replaced with a thiazole moiety, attached to the para-position of the proximal phenyl through the 4-carbon of the thiazole. Syntheses of the heterocycles were achieved according to Scheme 4, wherein *p*-bromoacetylbenzoic acid **20** was coupled with appropriately constructed thioamides, thioureas, or thiocarbamates (Procedures A−E). The derivatized benzoic acids were then coupled to **16** according to the procedure earlier described (Scheme 2) giving inhibitors **39a**−**q**. Alternatively, treatment of 1,4-dibromothiazole with substituted piperazines (Procedure F) in the presence of base, followed by Suzuki coupling of the product(s) with boronate **I**, available in one step from **18g** via bis-pinacolatodiborane cross-coupling, afforded the desired products.

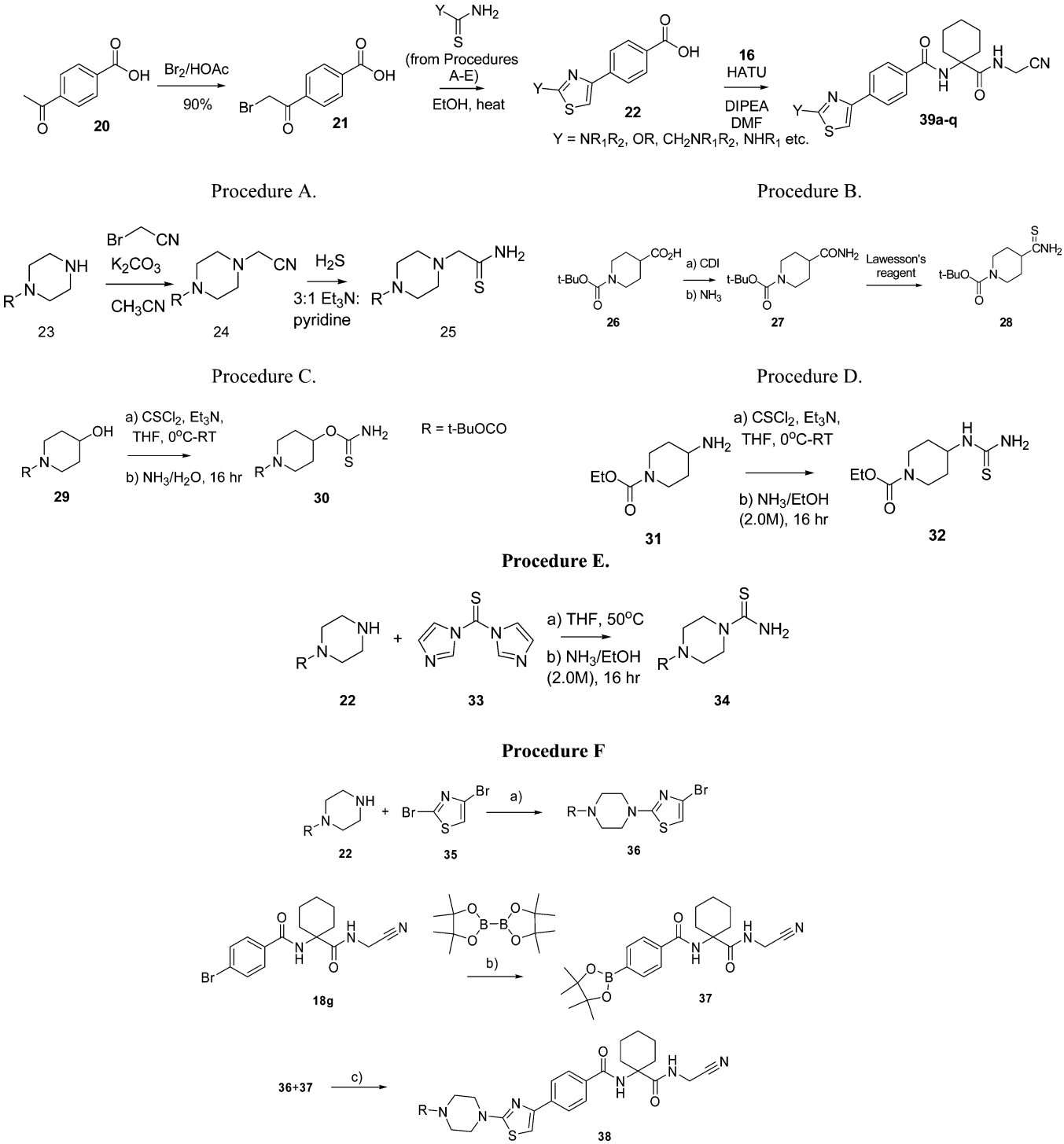
Inhibition data for cathepsins K, B, L, and S, osteoclast-based in vitro bone resorption data, and pharmacokinetic data for this series of thiazolebenzamides (**39a**−**m**) are described in Table 6. A clear requirement for sub-nM potency appeared in the shape of a charged moiety at the extended P_3 group; tertiary amines were favored over secondary and primary amines. In the bone resorption assay, substituted piperazines tended to be the most potent.

Compounds in this series bearing uncharged extended P_3 elements (**39a**,**h**) retained sub-10nM potency, but introduction of a charged moiety increased potency to sub-nM K_i levels. Tertiary amines at the extended position tended to be more potent than primary amines (**39e**). Potency was apparently independent of the distance or spacer atom (nitrogen, **39j**, oxygen, **39g**, or carbon, **39d**) between the piperazine/piperidine/morpholine ring and the thiazole, However, in the bone resorption assay, substituted piperazines tended to be the most potent (**39l**,**m)**. Compounds bearing secondary and primary amine moieties, as well as uncharged compounds, tended to show lower potency in the bone resorption assay, possibly due to cell permeability issues.

Within this series, several compounds (**39f**,**i**,**k)** displayed long half-lives/MRT's when dosed intravenously, suggesting possible suitability of this family for oral dosing and subsequent once per day administration in appropriate in vivo models. Having optimized the structural elements of the thiazole-benzamide series for potency (sub-nM versus Cat K), selectivity (≫100-fold versus cathepsins B, L, and S), and efficacy in in vitro systems (sub-10 nM IC50 in the bone resorption assay), we dosed the most potent compounds from this series in iv/po PK experiments to determine oral bioavailability. In an optimized series substituted at P_3 with piperidine and piperazine derivatives (Table 7) we were able to obtain oral bioavailability values (measured in rats) of 35−68%, with terminal half-lives ranging from 1.4 to over 5 h. All the compounds bore a tertiary amine at the distal position relative to the thiazole; this led us to conclude that cellular permeability, expected to be greater in examples with this type of functionality, was evident in both the cellular bone resorption assay and in oral bioavailability. Coupled with the ease of synthesis of members of this series, these parameters allowed us to conclude that an appropriate example for additional in vivo studies would be the methylpiperazine derivative **(39n)**, prepared according to Scheme 4, using procedure E to generate the intermediate thiourea for condensation with 4-bromoacetylbenzoic acid and subsequent HATU-mediated coupling with MsOH.Ac6-GlyCN (**16**).

X-ray Structural Analysis of 39n. At this point, a crystallographic analysis of **39n** revealed several distinct features. Figures 1 (distinct hydrogen bonding network) and 2 (surface plots) indicate that the extended P_3 moiety consisting of the methylpiperazine-thiazole-benzamide portion of the molecule is sufficiently close to the Asp^{61} of the S_3 pocket to be capable of forming

Scheme 4. Synthesis of P_3-Thiazolebenzoate Moieties General P_3-Thiazolebenzoate-Ac6-GlyCN Preparation[a]

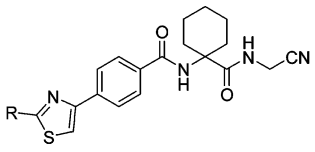


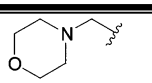
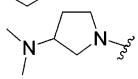
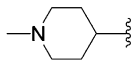
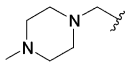
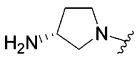
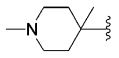
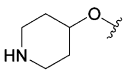
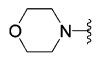
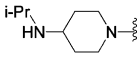
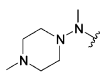
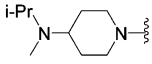
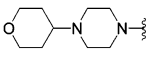
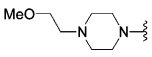
[a] (a) Et_3N, DMF; (b) $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, DMF, MeCOOK, 80−85 °C, 3 h; (c) $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, DMF, 2 M Na_2CO_3, 80−85 °C, 3 h.

an ionic interaction. The P_2 cyclohexyl ring is conformationally positioned with the P_3-P_2 amide nitrogen adopting an axial arrangement, with the carbonyl of the corresponding P_2-P_1 amide oriented equatorially. Key hydrogen bonds appear between the P_2-P_1 amide NH and the carbonyl of Asn^{158} (3.28 Å), and the P_2-P_1 amide carbonyl oxygen and the Gly^{66} NH (3.22 Å). An additional essential hydrogen bond between the P_3-P_2 amide NH and the carbonyl of Gly^{66} (2.91 Å) reveals the importance of the P_2 amino acid H-bond acceptors and donors within the enzyme's active site.

Kinetic Characterization of 39n. The reversibility of inhibition of human cathepsin K by **39n** was demonstrated in experiments in which a 1:1 enzyme−inhibitor mixture was preincubated for 15 min, followed by a 100-fold dilution into substrate-containing buffer. After dilution of the inhibitor to 0.05 nM, the cathepsin K enzyme activity was initially inhibited, but slowly recovered with a first-order rate constant of 0.023 s^{-1} ($t_{1/2}$ = 30 s). The final steady-state enzyme activity was 80−90% that of control, demonstrating the complete reversibility of the **39n**−cathepsin K complex. Consis-

Table 6. Inhibition (K_i(app)) Values, in Vitro Bone Resorption Inhibition Constants (IC_{50}), and Intravenous PK Parameters for Extended P_3-Thiazolobenzamide-P_3-Ac6-GlyCN Series

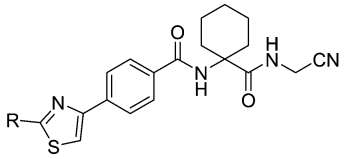


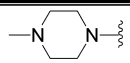
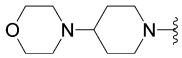
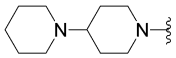
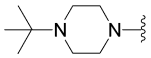
Entry/ synthesis procedure (A-F)	R	Cat K (μM)	Cat B (μM)	Cat L (μM)	Cat S (μM)	Bone res. IC_{50} (μM)	MRT (min)	$\beta t_{1/2}$ (min)	C_{max} (μM)
39a (A)		**0.0087**	3.8	2.2	0.25	0.21	15.6	ND	0.55
39b (E)		**0.00079**	1.7	12	26	0.11	41	38	0.3
39c (B)		**0.0016**	1.4	15	20	0.061	14	43	1.43
39d (A)		**0.00094**	1.5	19	42	0.06	75	77	0.43
39e (E)		**0.0014**	3	7.5	4.5	0.052	53.6	53	0.84
39f (B)		**0.00095**	1.1	7.6	30	0.046	140	137	0.44
39g (C)		**0.00057**	0.74	11	68	0.043	74	152	1.4
39h (E)		**0.0023**	3.1	74	13	0.035	28	29	1.4
39i (E)		**0.00059**	0.76	12	33	0.019	145	153	0.66
39j (D)		**0.00025**	0.89	8.9	21	0.011	62	92	0.47
39k (E)		**0.0016**	1.4	15	3.2	0.011	121	115	0.43
39l (E)		**0.00047**	0.84	>150	17	0.008	59	64	0.49
39m (E)		**0.00048**	1.3	6.4	20	0.004	41	118	1.78

tent with previous studies with biaryl nitrile cathepsin K inhibitors, **39n** was not a substrate for the nitrilase activity of Cat K.[13a]

Rhesus Monkey Bone Resorption Marker Study. A rhesus monkey bone resorption marker model, in which estrogen-deficiency bone loss was established by surgical oophorectomy (OVX), was used to evaluate the anti-bone resorptive activity of **39n**.[13a] Compound-treated monkeys (average age 15 y, all 3 y post OVX) were treated po, qd for 2 days with 1% carboxymethylcellulose (vehicle) and then treated with **39n** po, qd for 8−11 days at 15, 3, or 0.6 mg/kg. At the end of drug treatment, monkeys were treated for three additional days with vehicle. Vehicle monkeys received vehicle throughout the 13-day period. Twenty-four hour urine collections of both groups were made on the day before the start of drug dosing and during drug dosing. One additional urine collection was made 10−17 days after the last drug dose. The urine samples were frozen at −70 °C and N-telopeptides (NTx) and creatinine concentrations in each urine specimen were measured as described.[13a] The results are expressed as uNTx/Cre (nM/mM). As shown in Figure 3, the cathepsin K inhibitor **39n** dosed at 15 mg/kg po, qd decreased uNTx/Cre by an average of 76% relative to vehicle-treatment ($p < 0.001$). The suppression was detected after the second dose, the first sampling time. At 3 days after the last dose of **39n**, uNTx/Cre remained suppressed by 65% ($p = 0.007$). However, by 10 days after the last dose, uNTx/Cre in **39n**-treated monkeys had returned to control group levels. When **39n** was dosed at 3 mg/kg and 0.6 mg/kg po, qd, uNTx/Cre was decreased by an average of 68% ($p < 0.001$) and 31% ($p < 0.01$) relative to vehicle-treatment respectively (Figure 4). As shown in the single dose pharmacokinetic studies in intact rhesus monkeys (Figure 5), plasma levels of **39n** remained at or above the bone resorption IC_{50} for at least 24 h following the 15 or 3 mg/kg oral doses, but dropped below the bone resorption IC_{50} at the 0.6 mg/kg dose.

Table 7. Inhibition, in Vitro, and Pharmacokinetic Data for Selected Extended P_3-thiazolobenzamide-P_3-Ac6-GlyCN Compounds



Entry/ synthesis procedure (A-F)	R	Cat K (μM)	Cat B (μM)	Cat L (μM)	Cat S (μM)	Bone res. IC_{50} (μM)	Terminal $t_{1/2}$ (min)	C_{max} (μM) @ dose	F (%)
39n (E)		**.0002 (0.0005 vs. rabbit cat K)**	**1.0**	**6**	**47**	**0.005**	**204**	**1.4 @ 20 mpk, po**	**68**
39o (E)		**<.00025**	0.67	16	6.4	**0.003**	320	1.7 @ 10 mpk po	**48**
39p (E)		**0.00045**	0.69	8.8	42	**0.003**	201	0.36 @ 10 mpk, po	**35**
39q (F)		**0.00029**	0.91	8.8	19	**0.001**	100	0.8 @ 10 mpk, po	**44**

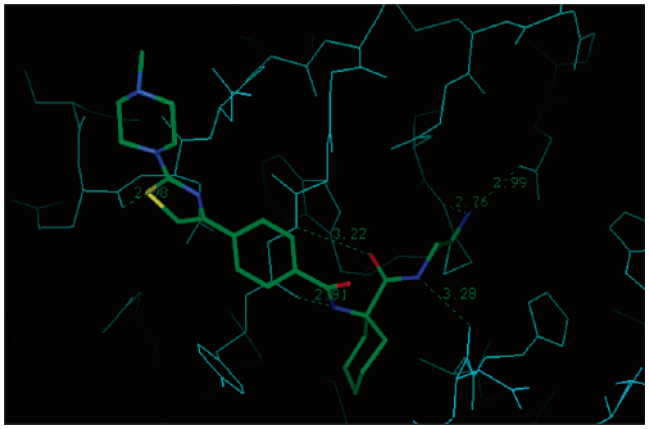


Figure 1. Hydrogen bonding network between Cat K active site residues and **39n**.

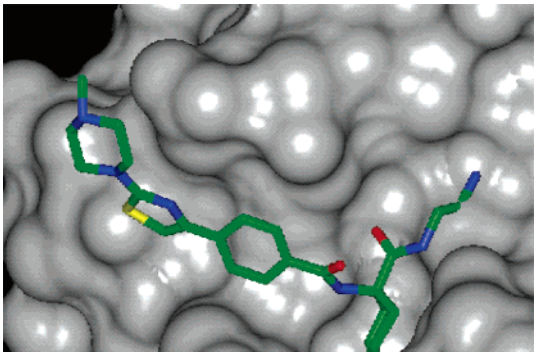


Figure 2. Surface plot depicting **39n** bound in active site of Cat K, covalently bound to catalytic cysteine.

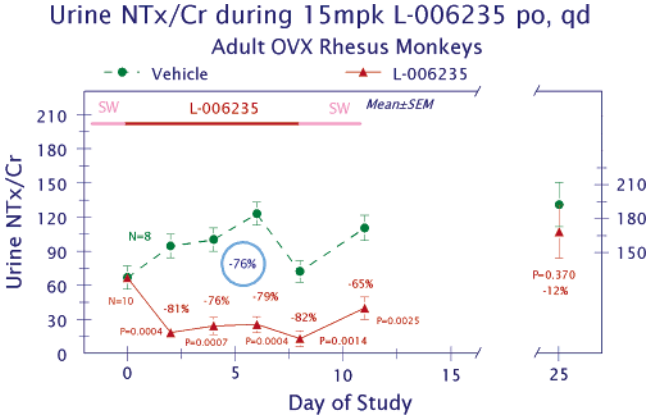


Figure 3. Ratio of N-telopeptides (NTx) to creatinine (Cr) during dosage of **39n** in adult OVX monkeys.

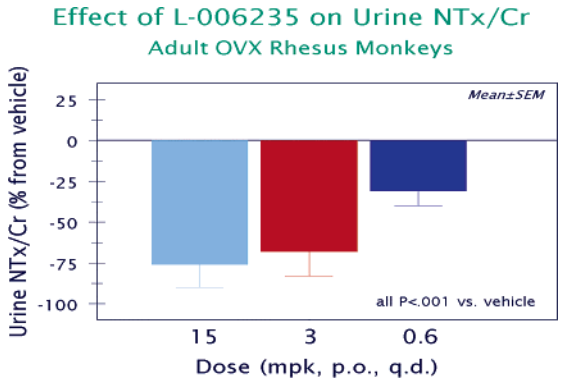


Figure 4. Dose response of **39n** in OVX monkey model.

Conclusions

Starting with a simple dipeptide nitrile scaffold (Z-Leu-GlyCN), a relatively potent (35 nM) but nonselective cathepsin K inhibitor, we were able to tune selectivity for cathepsin K versus the other antitargets of interest (cathepsins B, L, and S) by replacing the natural amino acid with an achiral, conformationally restricted aminocyclohexanecarboxylate (Ac6) moiety. By manipulating the P_3 binding elements, we discovered that para-substituted benzamides bearing basic groups afforded us single-digit nanomolar potency. By extending the benzamide further, through incorporation of an additional aromatic spacer, we were able to increase potency to sub-nanomolar levels against the enzyme. Furthermore, by retaining the basic amine group, again optimally substituted on the distal aromatic ring, we could translate enzyme potency to reasonable (10−100 nM) potency in the functional cellular assay of bone resorption. By exchanging the distal phenyl ring for a more stable thiazole heterocycle, we not only increased the cellular potency of this series but also the pharma-

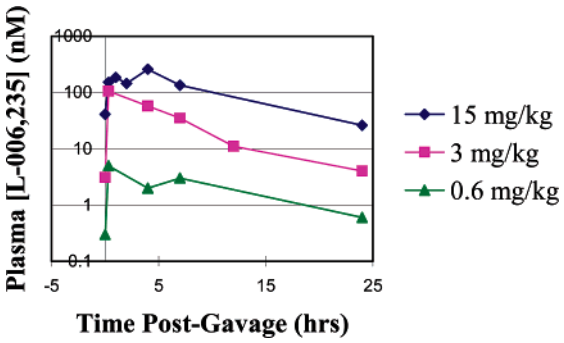


Figure 5. Day 8 PK from OVX monkey model.

cokinetic suitability of the chemotype. Several examples of sub-250 pM inhibitors bearing the charged alicyclic ring-thiazole-benzamide P_3 array proved to have high oral bioavailability in the rat, with half-lives of 3−5 h.

The compound bearing the overall most favorable attributes, **39n**, was dosed in a primate bone resorption marker model and indicated a clear dose−response in reducing collagen breakdown products as a ratio with creatinine, as measured in the urine. PK analysis at the end of the study indicated a correlation between plasma levels of the compound and the NTx/creatinine ratio; furthermore, it was clear that the in vitro bone resorption assay IC_{50} offered a highly predictive measure of the potential utility of this compound series in the in vivo model. This study suggests that inhibition of cathepsin K may be a viable therapeutic approach for the treatment of osteoporosis and other bone disorders displaying excessive levels of resorption.

Experimental Section

Unless otherwise noted, all nonaqueous reactions were carried out under a nitrogen atmosphere using commercial grade solvents and reagents. ^{1}H NMR spectra were recorded on a JEOL-270 or a General Electric QE−300 FT spectrophotometer. Chemical shifts are reported in ppm relative to tetramethylsilane, using the following abbreviations: s, singlet; d, doublet; t, triplet; dd, doublet of doublets, etc.; m, multiplet; b, broad. An asterisk (*) signifies an unresolved peak. Coupling constants (*J*) are reported in hertz (Hz) where relevant. Mass spectrometric analyses were obtained on an ABI−Sciex LC/MS, with electrospray ionization methodology. Flash chromatography was performed using EM silica gel 60 (230−400 mesh). Reverse phase preparative HPLC was performed using Varian (Rainin) Dynamax, or Gilson instruments using C_{18} columns. Elemental analyses were performed by Robinson Microlit Laboratories, Inc., Madison, NJ.

Synthesis of 1. A mixture composed of 2,5-dioxopyrrolidin-1-yl 2-benzyloxycarbonylamino-4-methylvalerate (Z-LeuOSu, 39.6 g, 0.109 mol), aminoacetonitrile hydrochloride (10.1 g, 0.109 mol), triethylamine (61 mL, 0.436 mol), DMF (40 mL), and acetonitrile (360 mL) was stirred at room temperature for 27 h. The mixture was filtered, concentrated to a volume of 100 mL, and poured into ice−water (1000 mL). The mixture was stirred until a precipitate had formed. The precipitate was collected, washed with water, and dried. The dry product was recrystallized from 55% ethanol/water (80 mL). The crystals were collected and recrystallized from 65% ethanol/water (70 mL). The crystals were collected and dried to provide benzyl (*S*)-1-cyanomethylcarbamoyl-3-methylbutylcarbamate (21.1 g, 0.067 mol, 61%) as white needles, mp 120−121 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 8.68 (t, J = 6 Hz, 1H), 7.54 (d, J = 8 Hz, 1H), 7.33 (m, 5H), 5.00 (Abq, 2H), 4.09 (d, J = 6 Hz, 2H), 4.03 (m, 1H), 1.24−1.64 (m, 3H), 0.84 (apparent t, J = 7 Hz, 6H) MS (electrospray): MH^+ 303.9 (100%). Analysis ($C_{16}H_{21}N_3O_3$): C, H, N.

Synthesis of 2. A mixture composed of benzyloxycarbonylnorleucine (0.425 g, 1.60 mmol), aminoacetonitrile hydrochloride (0.148 g, 1.60 mmol), PyBOP.RTM. (0.836 g, 1.60 mmol), *N*,*N*-diisopropylethylamine (0.84 mL, 4.83 mmol), and DMF (10 mL) was stirred at room temperature for 2.5 h. The mixture was concentrated, and the residue was taken up into dichloromethane. The dichloromethane mixture was washed with 1 N hydrochloric acid, water, and aqueous sodium bicarbonate, dried ($MgSO_4$), filtered, and concentrated. The crude material was purified from the residue by silica gel chromatography using 5% methanol in dichloromethane to provide the final product (435 mg, 1.52 mmol, 95%) as an oil. ^{1}H NMR (300 MHz, DMSO-d_6): 8.65 (bt, 1H), 7.55 (d, J = 7 Hz, 1H), 7.41 (bs, 5H), 5.00 (Abq, 2H), 4.08 (d, J = 6 Hz, 2H), 3.97 (m, 1H), 1.52 (m, 2H), 1.24 (m, 4H), 0.81 (bs, 3H). Analysis ($C_{16}H_{21}N_3O_3$): (C, H, N).

Synthesis of 3. This material was produced using the same procedure as for **2** starting with commercially available Z-Nal-OH. ^{1}H NMR (300 MHz, DMSO-d_6): 8.81 (bt, 1H), 7.84 (m, 4H), 7.48 (m, 3H), 7.21 (m, 5H), 4.95 (Abq, 2H), 4.40 (m, 1H), 4.12 (d, J = 7 Hz, 2H), 3.08 (m, 2H). Analysis ($C_{23}H_{21}N_3O_3$): (C, H, N).

Synthesis of 4. This material was produced using the same procedure as for **2** starting with commercially available Z-Thr-OH. ^{1}H NMR (300 MHz, DMSO-d_6): 8.55 (t, 1H), 7.42 (m, 5H), 7.08 (d, 1H), 5.03 (ABq, 2H), 4.18 (m, 2H), 3.95 (d, 2H), 1.04 (d, 3H). MS (APCI): MH^+ 292.

Synthesis of 5. This material was produced using the same procedure as for **1** starting with commercially available Z-IleOSu. ^{1}H NMR (300 MHz, DMSO-d_6): 8.72 (bt, 1H), 7.50 (d, J = 8 Hz, 1H), 7.35 (s, 5H), 5.02 (s, 2H), 4.13 (m, 2H), 3.85 (apparent t, 1H), 1.75 (m, 1H), 1.42 (m, 1H), 1.14 (m, 1H), 0.80 (m, 6H). Analysis ($C_{16}H_{21}N_3O_3$): (C, H, N).

Synthesis of 6. This material was produced using the same procedure as for **2** starting with commercially available Z-D-Leu-OH. mp 120−121°C.; ^{1}H NMR (300 Mz, DMSO-d_6): 8.68 (t, J = 6 Hz, 1H), 7.54 (d, J = 7 Hz, 1H), 7.33 (bs, 5H), 4.99 (ABq, 2H), 4.09 (d, J = 5 Hz, 2H), 4.00 (m, 1H), 1.28−1.65 (m, 3H), 0.83 (apparent t, J = 7 Hz, 6H); MS (electrospray): MH^+ 304 (100%);

Benzyl 1-cyanomethylcarbamoyl-4,4,4-trifluoro-3-methylbutylcarbamate (Z-F_3C(D,L-Leu)-GlyCN, 7): ^{1}H NMR (DMSO-d_6): 9.21 (s, 1 H); 8.01 (s, 1 H); 7.21 (m, 5 H); 5.12 (m, 1 H); 5.01 (s, 2 H); 4.21 (s, 2 H); 3.02 (m, 3 H); 1.01 (m, 3 H). MS (electrospray): MH^+ 358.2.

Benzyl 1-cyanomethylcarbamoyl-1,3-dimethylbutylcarbamate (Z-α-MeLeu-GlyCN, 8): ^{1}H NMR (DMSO-d_6): 9.21 (s, 1 H); 8.01 (s, 1 H); 7.21 (m, 5 H); 5.01 (s, 2 H); 4.01 (m, 3 H); 1.81 (m, 2 H); 0.86 (m, 6 H). MS (electrospray): MH^+ 318.2.

Benzyl (*S*)-1-cyanomethylcarbamoyl-2,2-dimethylpropylcarbamate (Z-t-Leu-GlyCN, 9): ^{1}H NMR (DMSO-d_6): 9.21 (s, 1 H); 8.01 (s, 1 H); 7.21 (m, 5 H); 5.01 (s, 2 H); 4.01 (s, 2 H); 3.88 (m, 1 H); 0.96 (s, 9 H). MS (electrospray): MH^+ 304.2.

Benzyl (*S*)-1-cyanomethylcarbamoyl-3,3-dimethylbutylcarbamate (Z-t-BuAla-GlyCN, 10): ^{1}H NMR (DMSO-d_6): 9.21 (s, 1 H); 8.01 (s, 1 H); 7.27 (m, 5 H); 5.01 (m, 2 H); 4.01 (m, 3 H); 1.55 (m, 2 H); 0.96 (s, 9 H). MS (electrospray): MH^+ 318.2.

Benzyl (*S*)-1-cyanomethylcarbamoyl-phenylmethylcarbamate (Z-Phg-GlyCN, 11): ^{1}H NMR (DMSO-d_6): 9.21 (s, 1 H); 8.01 (s, 1 H); 7.27 (m, 5 H); 5.01 (m, 2 H); 4.01 (m, 3 H); 1.55 (m, 2 H); 0.96 (s, 9 H). MS (electrospray): MH^+ 324.2. Analysis ($C_{18}H_{17}N_3O_3$): (C, H, N).

Benzyl [1-(cyanomethylcarbamoyl)-cyclopropyl]-carbamate (Z-Ac3-GlyCN, 12): Analysis ($C_{14}H_{15}N_3O_3$): (C, H, N).

Benzyl [1-(cyanomethylcarbamoyl)-cyclopentyl]-carbamate (Z-Ac5-GlyCN, 13): Analysis ($C_{16}H_{19}N_3O_3$): (C, H, N).

Benzyl [1-(cyanomethylcarbamoyl)-cyclohexyl]-carbamate (Z-Ac6-GlyCN, 14): Analysis ($C_{17}H_{21}N_3O_3$): (C, H, N).

Synthesis of 1-Amino-*N*-cyanomethylcyclohexanecarboxamide Methanesulfonate (MsOH·Ac6-GlyCN, 16). 1-*tert*-Butoxycarbonylaminocyclohexanecarboxylic acid (Boc-Ac6OH, 5.0 g, 21.0 mmol) was taken up in DMF (40 mL). The mixture was cooled in an ice bath and then sequentially treated with aminoacetonitrile hydrochloride (3.8 g, 42 mmol), HATU (8.25 g, 21 mmol), and triethylamine (8.0 mL, 63 mmol). The reaction was allowed to proceed for 4 h, and then the mixture was concentrated under vacuum. The residue was treated with saturated $NaHCO_3$ solution (40 mL) and ethyl acetate (150 mL). The organic layer was separated, sequentially washed with water, 1 M hydrochloric acid (20 mL), water, and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The product was purified from the residue using a plug of silica with ethyl acetate as eluent, giving **15** (Boc-Ac6-GlyCN). ^{1}H NMR (DMSO-d_6) δ 8.09 (s, 1H); 6.76 (s, 1H); 0.4.00 (d, 2H); 1.88−1.07 (m, 19H). MS: M + H$^+$ 282.0. This material was dissolved in anhydrous THF (20 mL) under N_2 and dry methanesulfonic acid (6.0 equiv) was added and the mixture stirred for approximately 12 h. The mixture was concentrated, and the residue was triturated with ether (100 mL) and dried under vacuum to provide **16** (5.5 g, >99% yield). MS (M + H)$^+$: 182.2.

Synthesis of P_3-Ac6-GlyCN Derivatives 18a−c, 18e−j, 18l−o (General Procedure). To a mixture of **16** (1 equiv), HATU (1 equiv), and P_3-CO_2H (1 equiv) in DMF (0.2 M concentration) at 0 °C was added diisopropylethylamine (3 equiv). The solution was allowed to warm to room temperature over 2 h. The reaction was quenched with an equal volume of saturated aqueous $NaHCO_3$, extracted with ethyl acetate, washed with water, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, evaporated to dryness, and purified by crystallization, flash chromatography, or reverse-phase HPLC as required. Yields ranged from 30 to 80%.

18a: ^{1}H NMR (DMSO-d_6): δ 8.04 (s, 1H); 0.7.65 (s, 1H); 7.20 (m, 5H); 4.00 (d, 2H); 4.02 (s, 2H); 2.77 (t, 2H); 2.45 (s,br, 2H);1.95−1.07 (m, 10H); MS: M + H$^+$ 314.0.

18b. ^{1}H NMR (DMSO-d_6): δ 8.27−8.14 (m, 2H); 8.08 (s, 1H); 7.82 (d, 1H); 7.71 (d, 1H); 7.40 (t, 1H); 4.02 (d, 2H); 2.15−1.10 (m, 10H). MS: M + H$^+$ 364.0 and 366.2.

Analysis ($C_{16}H_{18}BrN_3O_2$): (C, H, N).

18c. ^{1}H NMR (DMSO-d_6): δ 8.22 (s,br, 1H); 8.14 (d, 2H); 7.81 (t, 2H); 7.72 (d, 2H); 7.58−7.33 (m, 4H); 4.01 (d, 2H); 2.17−1.13 (m, 10H). MS: M + H$^+$ 362.0. Analysis ($C_{22}H_{23}N_3O_2$): (C, H, N).

Synthesis of 2-Pyridin-4-yl-thiazole-4-carboxylic Acid [1-(Cyanomethyl-carbamoyl)-cyclohexyl]-amide (18d). 2-(4-Pyridyl)-thiazole-4-carboxylic acid (209 mg, 1.01 mmol) and **16 (**279 mg, 1.01 mmol) were dissolved in 3.5 mL DMF. DIPEA (870 μL, 5.0 mmol) was added, and the mixture was stirred for 5 min. HATU (460 mg, 1.21 mmol) was added, and the solution was stirred at room-temperature overnight. The volatiles were removed under reduced pressure. The residue was dissolved in 50 mL EtOAc and stirred with 25 mL saturated aqueous $NaHCO_3$ for 30 min. The organic phase was washed with 3 × 15 mL water and 1 × 25 mL brine, dried over anhydrous $MgSO_4$, and filtered through a short plug of silica gel. The solvent was concentrated and dried under high vacuum to obtain 361 mg of the title compound as a tan solid in 97% crude yield. ^{1}H NMR (300 MHz, DMSO-d_6): 8.77 (d, J = 7 Hz, 2H), 8.50 (s, 1H), 8.46 (t, J = 6 Hz, 1H), 8.04 (d, J = 7 Hz, 2H), 7.91 (s, 1H), 4.07 (d, J = 6 Hz, 2H), 2.17 (apparent d, J = 14 Hz, 2H), 1.80 (apparent t, J = 13 Hz, 2H) 1.2−1.7 (m, 6H). MS (electrospray): M + H$^+$ 370.0.

18e. Analysis ($C_{16}H_{20}N_4O_2$): (C, H, N).

18f. ^{1}H NMR (DMSO-d_6): δ 8.11 (s, 1H); 7.76(d, 2H), 7.65 (s, 1H); 6.93 (d, 2H); 4.02 (d, 2H); 3.70 (s, br, 4H); 3.17 (s, br, 4H); 2.15−1.04 (m, 10H): MS: M + H$^+$ 370.6. Analysis ($C_{20}H_{26}N_4O_3$): (C, H, N).

18g. Analysis ($C_{14}H_{16}BrN_3O_2S$): (C, H, N).

18h. Analysis ($C_{14}H_{16}BrN_3O_2S$): (C, H, N).

Synthesis of *N*-[1-(Cyanomethylcarbamoyl)cyclohexyl]-4-ethynylbenzamide (18k). 4-Bromobenzoic acid (233 mg, 1.16 mmol), 1-aminocyclohexanecarboxyclic acid methyl ester hydrochloride (225 mg,1.16 mmol), and HBTU (493 mg, 1.3 mmol) were dissolved in 5 mL *N*,*N*′-dimethylformamide with excess triethylamine (1.0 mL, 7.17 mmol) and allowed to stir overnight. The reaction was quenched by the addition of excess 1.0 N HCl and transferred to a separatory funnel. The mixture was extracted twice with ethyl ether. The combined volume of ether was washed one time each with water, saturated aqueous sodium bicarbonate, and brine. The ether was dried over magnesium sulfate and then filtered through sintered glass. The filtrate was concentrated in vacuo to give methyl 1-(4-bromobenzoylamino)cyclohexanecarboxylate as a clear, colorless oil that solidified under continued vacuum. 363 mg, 92% yield. MS (electrospray): MH$^+$ 341.2 (100%). To a solution of this material (363 mg, 1.07 mmol) in 5 mL dry DMF in a sealed tube reaction vessel was added dichlorobis(triphenylphosphine)palladium(II) (25 mg, 0.036 mmol), CuI (10 mg, 0.053 mmol) triethylamine(0.3 mL, 2.15 mmol), and (trimethylsilyl)acetylene (0.158 mL, 1.12 mmol). The reaction mixture was placed under a blanket of nitrogen, sealed (to prevent escape of volatile (trimethylsilyl)acetylene), and stirred overnight at 75 °C. The reaction mixture was cooled to ambient temperature and transferred to a separatory funnel and extracted thrice with dichloromethane. The combined volume of dichloromethane was extracted thrice with water and once with brine. The dichloromethane solution was then dried over magnesium sulfate and filtered over sintered glass. The filtrate was then flushed through a short plug of Celite washing with more dichloromethane. The combined volume of the dichloromethane solution was evaporated to dryness. ^{1}H NMR showed presence of expected TMS protons. 290 mg yellow oil, 76% crude yield of methyl 1-[4-(trimethylsilanylethynyl)-benzoylamino]cyclohexanecarboxylate. This material was taken up with vigorous stirring in 10 mL tetrahydrofuran and 10 mL water. To the stirring solution was added excess lithium hyrdoxide. The opaque white reaction solution immediately turned dark brown. The reaction as allowed to stir 2 h. The reaction solution was quenched by the addition of excess 1.0 N HCl and transferred to a separatory funnel. The mixture was extracted thrice with ethyl acetate. The combined ethyl acetate volume was washed once each with 1.0 N HCl, water, and brine. The ethyl acetate solution was dried over magnesium sulfate and filtered over sintered glass. The filtrate was concentrated under reduced pressure. LCMS of residual oils showed the desired product (1-(4-ethynylbenzoylamino)-cyclohexanecarboxyclic acid) as the major species. MS (electrospray): MH$^+$ 271.7 (100%). The crude 1-(4-ethynylbenzoylamino)-cyclohexanecarboxyclic acid (110 mg, 0.406 mmol), HBTU (161 mg, 0.425 mmol), and aminoacetonitrile (50 mg, 0.540 mmol) was dissolved with vigorous stirring in 10 mL DMF and 2 mL triethylamine. The reaction was allowed to stir overnight. The reaction was quenched by the addition of 1.0 N HCl and transferred to a separatory funnel. The mixture was extracted thrice with ethyl ether. The combined ethereal volume was washed once each with 1.0 N HCl, water, saturated aqueous sodium bicarbonate, and brine. The ether solution was dried over magnesium sulfate and filtered through sintered glass. The filtrate was concentrated under reduced pressure. The residual oils were purified by reversed phase preparative HPLC to give the title compound. 36 mg, 29% yield. Proton NMR (400 MHz, DMSO-d_6): δ 8.2 (s, 1H) 8.0 (d, 1H) δ 7.8 (d, 2H) δ 7.6 (d, 2H) δ 4.2 (d, 2H) δ 2.1 (s, 1H) δ 1.8−0.8 (m, 10H). MS (electrospray): MH$^+$ 309.1 (100%).

18l: Analysis ($C_{22}H_{23}N_3O_2$): (C, H, N).

18m. ^{1}H NMR (DMSO-d_6): δ.8.13 (s, 1H); 7.73 (d, 2H) 7.57 (s, 1H) 6.68 (d, 2H) 4.23−4.05 (s,br, 6H) 4.02 (s, 2H) 2.93 (s, br, 6H) 2.17−1.03 (m, 10H). MS: M + H$^+$ 329.4.

18n: Analysis ($C_{24}H_{28}N_4O_2$): (C, H, N).

18o: Analysis ($C_{21}H_{29}N_5O_2$): (C, H, N).

Synthesis of Biphenyl Amide Derivatives 19a−m (illustrative procedure). To a solution of **18g** (3.9 g, 10.7 mmol) in degassed DMF (50 mL) were added 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi-1,3,2-dioxaborolane (Frontier Scientific, 3.0 g, 11.8 mmol), potassium acetate (3.15 g, 32.1 mmol), and $PdCl_2$(dppf).CH_2Cl_2 (Strem Chemical, 0.3 g, 0.37 mmol). The

mixture was heated at 80 °C for 6 h. The solution was cooled, diluted with a 4:1 solution of ethyl acetate:THF (500 mL), washed with 1:1 brine:water (500 mL) and brine (500 mL), and then rapidly filtered through a short plug containing layers (top:bottom) of Celite:activated charcoal:silica gel:$MgSO_4$). The solution was concentrated, and the residue was dissolved in CH_2Cl_2. Hexane was added, and the resulting precipitate was allowed to stir for 2 h. The intermediate *N*-[1-(cyanomethyl-carbamoyl)-cyclohexyl]-4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzamide (**37**) was collected by filtration as a slightly off-white solid, weight 3.1 g (70%). MS (M + H): 412. The material was used without further purification.

To a solution of **37** (0.21 g, 0.5 mmol), 4-piperazin-1-yl-phenyl bromide hydrochloride (0.28 g, 1.0 mmol), and 2 M aqueous Na_2CO_3 (1.5 mL) in degassed DMF (5 mL) was added $PdCl_2$ (dppf)·CH_2Cl_2 (25 mg, 0.03 mmol). The mixture was stirred at 80 °C in a sealed tube for 3 h, cooled to room temperature, diluted with 4:1 ethyl acetate/THF (100 mL), washed with 1:1 brine:water (50 mL) and brine (50 mL), filtered through a short plug containing layers (top:bottom) of Celite:activated charcoal:silica gel:$MgSO_4$), concentrated under reduced pressure, and purified by preparative reverse phase HPLC to yield the product (157 mg, 35%) as the trifluoroacetamide salt. A small portion was converted to the free base by stirring with K_2CO_3 in acetonitrile, filtering through Celite, from which white crystals were obtained as a monohydrate.

19a. ^{1}H NMR (DMSO-d_6) (400 MHz): δ 8.23 (1H, t, J = 4.5 Hz), 8.01 (1H, s), 7.96 (2H, d, J = 6.6 Hz), 7.26 (2H, d, J = 6.6 Hz), 7.31 (2H, d, J = 6.6 Hz), 7.04 (2H, d, J = 6.6 Hz), 4.08 (2H, d, J = 4 Hz), 3.14 (4H, 2xd, J = 3, 3 Hz), 2.87 (4H, 2xd, J = 3, 3 Hz), 2.15 (2H, m), 1.78 (2H, m), 1.56 (5H, m), 1.28 (1H, m). ^{13}C NMR (DMSO-d_6) (500 MHz): δ 174.8, 165.9, 151.23, 142.5, 132.04, 128.6, 128.2, 127.1, 124.8, 117.6, 115.1, 59.1, 48.7, 45.3, 31.5, 27.4, 24.9, 21.0. LC/MS: M + 1: 446. Analysis: Calcd for $C_{26}H_{31}N_5O_2$·H_2O, C, 67.36; H, 7.18; N, 15.11. Found: C, 67.54, H, 6.86, N, 15.14.

19b. ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.24 (1H, t, J = 5.5 Hz), 8.02 (1H, s), 7.95 (2H, d), 7.70 (3H, m), 7.14 (2H, bs), 5.34 (1H, br), 4.05 (2H, d, J = 8.4 Hz), 3.78 (2H, m), 3.20 (6H), 2.18 (6H, m), 1.77 (2H, m), 1.53 (5H, m), 1.25 (1H, m). MS: M + H^+: 505.6.

19d. ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.20 (1H, t, J = 8.4 Hz), 8.00(1H, s), 7.95 (2H, d, J = 12.4 Hz), 7.71 (2H, d, J = 12.4 Hz), 7.65 (2H, d, J = 12.8 Hz), 7.06 (2H, d, J = 12.8 Hz), 4.44 (1H, m), 4.05 (2H, d, J = 8.4 Hz), 2.65 (2H, m), 2.20 (5H, m), 1.95 (2H, m), 1.70 (6H, m), 1.53 (5H, m), 1.26 (1H, m). LC/MS: M + 1: 475.4.

Synthesis of 19i. To a solution of 1-methylpiperidin-3-ol (2.2 g, 0.0191 mol) and 4-bromophenol (3.3 g, 0.0191 mol) in dry THF (20 mL), at 0 °C, were added a solution of triphenylphosphine (5.0 g, 0.0191 mol) in THF (15 mL) and diethyl azodicarboxylate (3.0 mL, 0.0191 mL). After stirring for 18 h at room temperature, under nitrogen, the mixture was diluted with diethyl ether (50 mL) and treated with 1 N HCl (50 mL). The aqueous solution was further extracted with diethyl ether (2 × 20 mL), and the pH was adjusted to about 10 by adding 1 N NaOH. This mixture was extracted several times (3×) with DCM (50 mL), and the combined organic extracts were dried over magnesium sulfate. After evaporation of the solvent, the product was obtained as a thick oil (3.1 g, 60%), LC/MS: M + 1: 271. This material was coupled with **37** using the palladium-assisted cross-coupling procedure illustrated for **19a**. ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.21 (1H, t, J = 5.4 Hz), 8.00 (1H, s), 7.95 (2H, d, J = 8.4 Hz), 7.72 (2H, d, J = 8:4 Hz), 7.67 (2H, d, J = 8.8 Hz), 7.05 (2H, d, J = 8.8 Hz), 4.05 (2H, m), 3.88 (1H, m), 2.96 (1H, m), 2.60 (1H, m), 2.37 (3H, s), 2.20 (2H, m), 1.95 (2H, m), 1.70 (6H, m), 1.53 (5H, m), 1.26 (1H, m). Analysis: C, 70.86%; H, 7.22%; N, 11.81%. Found: C, 68.35%; H, 7.79%; N, 11.43%. LC/MS: M + 1: 475.4.

19j. ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.20 (1H, t, J = 5.4 Hz), 8.00 (1H, s), 7.95 (2H, d, J = 8.4 Hz), 7.71 (2H, d, J = 8.4 Hz), 7.65 (2H, m), 7.05 (2H, d, J = 8.9 Hz), 4.05 (2H, J = 5.4 Hz), 3.90 (2H, m), 2.95 (1H, m), 2.37 (3H, s), 2.10 (2H, m), 2.00 (2H, m), 1.70 (6H, m), 1.53 (5H, m), 1.26 (1H, m). MS: M + 1: 474.4. Analysis ($C_{28}H_{34}N_4O_3$): (C, H, N).

General Synthesis of 4-*N*-Alkylpiperazines, Illustrated for 4-*N*-*tert*-Butylpiperidine. To a mixture of *tert*-butyl-4-oxopiperidine-1-carboxylate (7.0 g, 35.13 mmol) and *tert*-butylamine (7.41 mL, 70.26 mmol) under nitrogen was added titanium isopropoxide (12.55 mL, 43.91 mmol) at room temperature and stirred. A white precipitate was observed after 15 min. After 2 h and 30 min, 50 mL ethanol was added followed by 700 mg PtO_2. This was then hydrogenated overnight using a Parr reactor at 50 psi hydrogen pressure. The reaction was then filtered and concentrated to give 5.81 g of *tert*-butyl 4-*tert*-butylaminopiperidine-1-carboxylate**.** ^{1}H NMR (DMSO-d_6) (270 MHz): δ 3.79 (4H, m), 2.78 (1H, t), 2.61 (1H, m), 1.61 (4H, m), 1.36 (9H, s), 1.03 (9H, s). MS: M + H^+: 256.6. **Note:** In the above procedure 2 equiv of the amine was used because the excess amine could be evaporated. In the case of higher boiling point amines 1.2 stoichiometric equivalents of the amine to 1 stoichiometric equivalent of the ketone is used.

4-*tert*-Butylamino-piperidine-1-carboxylic acid *tert*-butyl ester (5.81 g) was treated with 100 mL 4 M HCl in dioxane at room temperature and stirred for 1 h. LC/MS shows reaction complete. The reaction was concentrated under reduced pressure and the residue triturated with ether (3 × 100 mL). This was then dried under vacuum to yield 5.63 g of *tert*-butylaminopiperidin-4-yl-amine. ^{1}H NMR (DMSO-d_6) (270 MHz): δ 3.31 (2H, m), 2.92 (3H, m), 2.06 (4H, m), 1.39 (9H, s). MS: M + H^+: 256.6. This material was then coupled with **37** as described earlier to provide **19m**.

General P_3-Thiazolebenzoate-Ac6-GlyCN Procedure. Synthesis of Substituted Thiazolebenzoic Acids. 4-Acetylbenzoic acid (**20**, 20 g, 128.2 mmol) was taken up in 200 mL of acetic acid. The acid was dissolved by heating the solution to 80 °C. Bromine (7.0 mL, 128.2 mmol) was added (exothermic reaction) and stirred for 1 h while the solution cooled to room temperature. The product, **21**, was filtered and washed with ether and dried. Yield: 20 g (67%). ^{1}H NMR (DMSO-d_6, ppm): δ 10.1 (br. s. 1 H), 7.81 (m, 4 H), 5.51 (s, 2 H).

21 was condensed with thioamides, thioureas, thiocarbamates (**25**, **28**, **30, 32**, and **34** as described in procedures A−E below) to form the requisite thiazolebenzoic acids.

General Synthesis Procedure A, Illustrated for Preparation of 39d. Potassium carbonate (31.1 g, 225 mmol), 1-methylpiperazine (**23**, R= Me; 18.3 mL, 165 mmol), and bromoacetonitrile (10.4 mL, 150 mmol) were stirred in acetonitrile (450 mL) for 75 h. The solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography on silica gel using 9:1 CH_2Cl_2:MeOH eluent to obtain 11.41 g (55%) of **24** as a pale amber oil.

24 (13.04 g, 93.7 mmol) was stirred in 400 mL of 3:1 triethylamine:pyridine for 30 min. The stirred suspension was saturated with hydrogen sulfide via a gas dispersion frit. A white precipitate formed during introduction of the H_2S. The flask was sealed and stirred overnight, concentrated, and the residue was boiled in ethyl acetate (250 mL) for 15 min. The supernatant was decanted and cooled, yielding 4.20 g small brown crystals by filtration. A second crop of 0.94 g was obtained (37% yield of **25**.

25 (1.73 g, 10.0 mmol) was dissolved in anhydrous THF (40 mL). A solution of 4-(2-bromoacetyl)benzoic acid (2.43 g, 10.0 mmol in anhydrous THF (20 mL) was added. The mixture was stirred at 70 °C for 5 h, cooled, and filtered. The solids were washed with Et_2O and dried under high vacuum over P_2O_5 to obtain 3.40 g (85%) of 4-[2-(4-methyl-piperazin-1-ylmethyl)-thiazol-4-yl]-benzoic acid hydrobromide as an orange solid. This material was coupled with **16** in the presence of HATU and diisopropylethylamine as described previously, in 7% yield after reverse phase HPLC using a 20 → 65% MeCN/H_2O + 0.05% TFA gradient elution. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.58 (br s, 1H), 8.26* (s, 1H), 8.24 (t, J = 6 Hz, 1H), 8.05* (s, 1H), 8.03 (d, J = 9 Hz, 2H), 7.96 (d, J = 9 Hz, 2H), 4.06 (m, 2H), 3.38−3.48 (m, 2H), 3.03−3.17 (m, 4H), 2.82 (s, 3H), 2.52−

2.60 (m, 2H), 2.07−2.19 (m, 2H), 1.70−1.84 (m, 2H), 1.46−1.60 (m, 5H), 1.2−1.36 (m, 1H). MS (ES): MH^+ 480.6.

Synthesis of 39a. General synthesis procedure A (starting with morpholine and bromoacetonitrile) was employed. The final step (HATU-mediated condensation between **16** and 4-(2-morpholin-4-ylmethylthiazol-4-yl)-benzoic acid) proceeded in 11% isolated yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23* (t, J = 6 Hz, 1H), 8.22* (s, 1H), 8.05* (s, 1H), 8.03 (d, J = 9 Hz, 2H), 7.96 (d, J = 9 Hz, 2H), 4.06 (d, J = 6 Hz, 2H), 3.90 (s, 2H), 3.63 (t, J = 5 Hz, 4H), 2.56 (t, J = 5 Hz, 4H), 2.08−2.20 (m, 2H), 1.68−1.84 (m, 2H), 1.48−1.62 (m, 5H), 1.20−1.38 (m, 1H). Calcd exact mass: 467.1991. MS (ES) MH^+ 467.4.

General Synthesis Procedure B, Illustrated for Preparation of 39c. Boc-isonipecotic acid (**26**, 3.07 g, 13.4 mmol) and 1,1-carbonyldiimidazole (2.53 g, 15.6 mmol) were dissolved in 50 mL THF and stirred at room temperature for 1.5 h. The solution was cooled in an ice bath, and 15 mL (30 mmol) of a 2.0 M solution of NH_3 in MeOH was added. The solution was stirred 30 min at 0 °C and then overnight at room temperature. A white precipitate formed. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc (150 mL) and washed with 2 × 50 mL 10% aqueous citric acid, 1 × 50 mL 10% aqueous $NaHCO_3$, and 1 × 75 mL brine. The solution was dried over anhydrous $MgSO_4$ and concentrated under vacuum to give 2.50 g of BOC-isonipecotamide, **27**, as a white solid. This material was dissolved in THF (50 mL). Lawesson's reagent (4.47 g (11 mmol) was added. The mixture was heated at reflux under nitrogen for 3 h, cooled, and allowed to stand overnight, before being concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL) and washed with 1 × 75 mL 10% aqueous citric acid, 1 × 75 mL 10% aqueous $NaHCO_3$, 2 × 75 mL water, and 1 × 75 mL brine. The solution was dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by flash chromatography to obtain 1.26 g of *tert*-butyl 4-thiocarbamoylpiperidine-1-carboxylate, **28**, as a white solid in 38% yield.

28 (1.26 g, 5.16 mmol) and 1.25 g (5.14 mmol) 4-(2-bromoacetyl)benzoic acid (1.25 g, 5.14 mmol) were dissolved in anhydrous THF (100 mL) and heated at 70 °C for 3 h. The solvent was removed under reduced pressure to obtain 2.55 g of a white solid, which was dissolved in DMF (20 mL). **16** (1.40 g, 5.0 mmol) and DIPEA (4.5 mL, 26 mmol) were added and stirred for 5 min. HATU (2.08 g, 5.47 mmol) was added. The mixture was stirred at room temperature for 3 h. The solvents were removed under reduced pressure. The residue was dissolved in EtOAc (300 mL), washed with 1 × 75 mL 10% aqueous citric acid, 1 × 75 mL water, 1 × 75 mL 10% aqueous Na_2CO_3, 1 × 75 mL water, and 1 × 75 mL brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by radial chromatography using EtOAc eluent to obtain 1.11 g of 4-(4-{4-[1-(cyanomethyl-carbamoyl)-cyclohexylcarbamoyl]-phenyl}-thiazol-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester in 39% yield.

This material (208 mg, 0.376 mmol) was dried under high vacuum, dissolved in 1.0 mL anhydrous THF, and stirred until homogeneous. The solution was cooled in an ice bath, and anhydrous methanesulfonic acid (74 μ, 1.1 mmol) was added. The mixture was allowed to stand at room-temperature overnight. The solution was concentrated at 20 °C, and the residue was triturated three times with Et_2O. The product, **39c**, was purified by reverse phase preparative TLC using 7:3 MeCN:H_2O eluent giving 116 mg (56%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.3−8.6 (br s, 1H), 8.26 (t, J = 6 Hz, 1H), 8.21 (s, 1H), 8.07 (s, 1H), 8.04 (d, J = 9 Hz, 2H), 7.97 (d, J = 9 Hz, 2H), 4.06 (d, J = 6 Hz, 2H), 3.35−3.50 (m, 2H), 3.07 (t, J = 13, 2H), 1.85−2.30 (m, 6H), 1.68−1.84 (m, 2H), 1.48−1.60 (m, 5H), 1.2−1.36 (m, 1H). Calcd exact mass: 451.2042. MS (electrospray): MH^+ 451.2 (100%).

Synthesis of 39f. Isonipecotamide (10.0 g, 78.0 mmol) was placed in a 250 mL Parr hydrogenation bottle and dissolved in 70 mL MeOH. Aqueous formaldehyde (37%) (17.5 mL (235 mmol) was added. The solution was stirred for 3 h. Raney nickel (2.25 g of a 50% w/w slurry) in water was added. The mixture was hydrogenated on a Parr apparatus at 60 psi H_2 for 3 h, filtered, and concentrated. Trituration of the white solid residue with 3 × 15 mL acetone and drying under high vacuum gave 1.45 g (51%) of 1-methylpiperidine-4-carboxamide as a white solid.

To a rapidly stirred solution of this material (5.65 g, 39.7 mmol) in anhydrous $CHCl_3$ (100 mL) was added thionyl chloride (29.0 mL, 398 mmol) at a fast dropping rate. A lumpy precipitate formed during addition. The mixture was then heated at reflux for 20 h. Excess solvent and $SOCl_2$ were removed by distillation. The residue was dissolved in CH_2Cl_2 (50 mL), and then 5% aqueous NH_4OH (150 mL) was added and stirred for 15 min. The phases were separated. The aqueous phase was extracted further with CH_2Cl_2 (2 × 50 mL). The organics were combined, washed with water (2 × 50 mL) and brine (75 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was distilled (72−74 °C/1.3 kPa) to obtain 2.54 g (52%) of 1-methylpiperidine-4-carbonitrile as a pale yellow oil.

A solution of 2.5 g (20 mmol) of this material in anhydrous THF was added dropwise to 15 mL (30 mmol) of a clear orange 2.0 M solution of lithium diisopropylamide in THF/heptane/ethylbenzene at −10 °C, stirred at 0 °C for 1.5 h, cooled to −10 °C, and treated with CH_3I (1.5 mL, 24 mmol) dropwise. The solution was stirred at 0 °C for 1.5 h, quenched with ethanol (1.5 mL), diluted with water (25 mL), stirred for 15 min, and filtered through Celite, washing the Celite with ether (50 mL). The aqueous phase was extracted with ether (3 × 25 mL). The combined organic extracts were washed with brine (75 mL), dried over $MgSO_4$, filtered, and concentrated to obtain 2.2 g of a clear yellow liquid. This material was dissolved in Et_2O and converted to its hydrochloride salt with 4.0 M HCl/dioxane. The precipitate was filtered and dried under high vacuum to obtain 1.51 g (43%) of 1,4-dimethylpiperidine-4-carbonitrile hydrochloride. The synthesis of **39f** was then accomplished via the general synthesis procedure B outlined above. ^{1}H NMR (300 MHz, DMSO-d_6): 8.23 (t, J = 6 Hz, 1H), 8.17 (s, 1H), 8.04 (d, J = 9 Hz, 2H), 8.02* (s, 1H), 7.96 (d, J = 9 Hz, 2H), 4.06 (d, J = 6 Hz, 2H), 2.40−2.50 (m, 2H), 2.04−2.38 (m, 6H), 2.14* (s, 3H), 1.70−1.86 (m, 4H), 1.46−1.62 (m, 5H), 1.36* (s, 3H), 1.20−1.40 (m, 1H). Calcd exact mass: 479.2355. MS (ES): MH^+ 479.8 (100%).

General Synthesis Procedure C, Illustrated for Preparation of 39g. Di-*tert*-butyl dicarbonate (75.5 g, 346 mmol) was dissolved in 250 mL anhydrous CH_2Cl_2 under N_2. The solution was cooled in an ice bath, and 4-hydroxypiperidine (25.1 g, 248 mmol) dissolved in 200 mL anhydrous CH_2Cl_2 was added, followed by Et_3N (35 mL,250 mmol), keeping the reaction temperature below 10 °C. The mixture was then stirred at room temperature for 4 h, diluted with methanol (100 mL), and stirred for 60 min. The volatiles were removed on a rotary evaporator and the residue dried under reduced pressure. The residue was dissolved the residue in EtOAc (300 mL), washed with 1 × 100 mL 10% aqueous citric acid, 1 × 100 mL water, 1 × 100 mL 10% aqueous NaOH, 1 × 100 mL water, and 1 × 100 mL brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to give 38.0 g (76%) of Boc-4-hydroxypiperidine (**29**: R= Boc) as a white solid.

29 (18.47 g, 91.8 mmol) and Et_3N (14.1 mL, 101 mmol) were dissolved in THF (250 mL) and cooled in an ice bath. Thiophosgene (7.0 mL, 92 mmol) was added dropwise, maintaining a reaction temperature below 15 °C. The mixture was stirred at room temperature for 1 h, cooled again in an ice bath, and treated with 35 mL (252 mmol) of 28% aqueous NH_4OH. The solution was stirred at room temperature for 3 h, concentrated, dissolved in EtOAc (300 mL), washed with 2 × 100 mL 10% aqueous citric acid, 1 × 100 mL 10% saturated aqueous $NaHCO_3$, 1 × 100 mL water, and 1 × 100 mL brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. The product was purified by flash chromatography on silica gel using 3:1 CH_2Cl_2:EtOAc eluent to obtain 1.57 g of **30** R = Boc, 7% yield.

30 (1.57 g, 6.03 mmol) was dissolved in 15 mL anhydrous THF. A solution of **21** (1.47 g, 6.03 mmol) was added in anhydrous THF (15 mL). The reaction was heated at 70 °C for 6 h, cooled to room temperature, filtered, and concentrated.

The residue was purified by flash chromatography on silica gel using 9:1 CH_2Cl_2:MeOH eluent, giving 1.70 g (70%) of Boc-4-[4-(4-carboxyphenyl)-thiazol-2-yloxy]-piperidine as a cream colored solid. This material (982 mg, 2.43 mmol) and **16** (673 mg, 2.43 mmol) were dissolved in DMF (10 mL). DIPEA (1.7 mL, 9.8 mmol) was added. The solution was stirred for 5 min. HATU (1.02 g, 2.69 mmol) was added, and the mixture was stirred for 4 h. The solvents were removed under reduced pressure. The residue was dissolved in EtOAc (150 mL), washed with 1 × 50 mL 10% aqueous citric acid, 1 × 50 mL water, 1 × 50 mL 10% aqueous Na_2CO_3, 1 × 50 mL water, and 1 × 50 mL brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel using 1:1 CH_2Cl_2:EtOAc eluent, to obtain 1.08 g (78%) of Boc-4-(4-{4-[1-(cyanomethylcarbamoyl)-cyclohexylcarbamoyl]-phenyl}-thiazol-2-yloxy)-piperidine.

To a solution of 274 mg (0.482 mmol) of this material in THF (1.0 mL) at 0 °C was added MsOH (94 μL, 1.4 mmol). The mixture was allowed to stand at room-temperature overnight. The mother liquor was decanted. The precipitate was triturated three times with 2:1 Et_2O:THF. The residue was purified by reverse phase HPLC using a 20 → 65% MeCN/H_2O + 0.05% TFA gradient elution to obtain 42 mg (15%) of **39g** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.58 (br s, 1H), 8.24 (t, J = 6 Hz, 1H), 8.04 (s, 1H), 7.94 (s, 4H), 7.67 (s, 1H), 5.29 (m, 1H), 4.06 (d, J = 6 Hz, 2H), 3.08−3.35 (m, 4H), 1.92−2.33 (m, 6H), 1.68−1.84 (m, 2H), 1.45−1.60 (m, 5H), 1.2−1.36 (m, 1H). Calcd exact mass: 467.1991. MS (electrospray): MH^+ 468.2 (100%).

General Synthesis Procedure D, Illustrated for Preparation of 39j. (4-Methyl-piperazin-1-yl)-thiourea was synthesized following the procedure according to ref 17. A suspension of this compound (6.6 g, 0.038 mol) and 4-(2-bromo-acetyl)-benzoic acid (9.2 g, 0.038 mol) in ethanol (300 mL) was refluxed for 2 h. The reaction mixture was cooled at 4 °C for about 1 h, and the yellow solid that formed was filtered and washed with ethyl ether. The product, 4-[2-(4-methylpiperazin-1-ylamino)-thiazol-4-yl]-benzoic acid hydrobromide, was obtained as a yellow solid (12.8 g, 84%, LC/MS: M + 1: 319.0).

To a suspension of the hydrobromide salt (2.5 g, 0.0062 mol) in methanol (20 mL) was bubbled HCl gas for 15 min. The solution was left stirring overnight at room temperature. The solid that crashed out from the solution was filtered and washed with ethyl ether. Methyl 4-[2-(4-methylpiperazin-1-ylamino)thiazol-4-yl]-benzoate dihydrochloride was obtained as an yellow solid (2.5 g, 100%, LC/MS, M + 1: 332.6.

To a solution this material (3.0 g, 0.0074) in dry DMF (15 mL) at 0 °C, a 60% suspension of NaH in oil (900 mg, 0.023 mol) was added and the reaction mixture stirred for 15 min. Then, methyl iodide (0.500 mL, 0.00814 mol) was added and the mixture stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water and brine. After drying over sodium sulfate, the solution was concentrated to dryness to obtain a yellow solid (2.0 g, LC/MS, M + 1: 346.2). The solid was dissolved in MeOH (100 mL) and a solution of NaOH (924 mg, 0.023 mol) in water (20 mL) was added. The reaction mixture was stirred overnight at room temperature. The methanol was removed under vacuum, and the pH of the aqueous solutions was acidified to pH 5 by adding 1 N HCl. The mixture was extracted several times with ethyl acetate, and the combined organic layers were washed with brine and dried over sodium sulfate. After evaporation of the solvent, the title compound was obtained as a yellow solid (0.820 mg, 33% overall yield). ^{1}H NMR (DMSO-d_6): d 7.93 (4H, m), 7.44 (1H, s), 3.40 (4H, m), 3.27 (3H, s), 3.21 (4H, m), 3.16 (3H, s). This material was coupled with **16** as described previously to obtain the title compound. ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.18 (1H, t, J = 5.4 Hz), 7.95 (1H, s), 7.88 (5H, s), 4.03 (2H, d, J = 5.4 Hz), 3.10 (3H, s), 2.82 (4H, m), 2.19 (3H, s), 2.10 (4H, m), 1.74 (2H, m), 1.52 (5H, m), 1.25 (1H, m). Analysis: C, 60.58%; H, 6.71%, N, 1978%. Found: C, 59.11%; H, 7.02%; N, 19.08%. LC/MS: M + 1: 496.5.

General Synthesis Procedure E, Illustrated for Preparation of 39n. 4-Methylpiperazinyl thiourea (**34**, 15.0 g, 95 mmol) was added to THF (500 mL) containing **21** (22.0 g, 95.0 mmol). The mixture was refluxed for 3 h. The product, **22** (Y = *N*-methylpiperazinyl) was filtered, washed with acetone and ether, and dried. The yield was 22.0 g, 60%. The product was characterized by LC-MS (FAB LC-MS. 304.2 ($M + H^+$)).

To a stirred solution of thiocarbonyldiimidazole (23, 17.8 g, 100 mmol) in THF (300 mL) at room temperature was added *N*-methyl piperazine (**23,** R = Me, 10.0 g, 100 mmol). The reaction mixture was stirred at room temperature for 1 h and then at 55 °C for 1 h. The mixture was cooled to room temperature, and approximately 20 mL of THF was removed under vacuum. Ammonia in ethanol (200 mL of a 2.0 M solution) was added. The mixture was stirred overnight. The reaction was then heated at 55 C for 2 h and cooled to room temperature. The product, **22** Y = *N*-methyl piperazine, was filtered, washed with ether, and dried. Yield 15.0 g (90%). ^{1}H NMR (DMSO-d_6, ppm): δ 7.5 (br. s. 2 H), 3.68 (m, 4 H), 2.23 (m, 4 H), 2.13 (s, 3 H).

22, Y = *N*-methyl piperazine (20 g, 50.80 mmol), was taken up in DMF (350.0 mL), and **16** (15.0 g, 50.8 mmol) was added, followed by triethylamine (27.6 mL, 200 mmol) and HATU (20.0 g, 50.8 mmol). The solution was stirred overnight at room temperature. DMF was removed under vacuum. Saturated sodium bicarbonate (200 mL) was added, and the mixture was stirred for 30 min. Water (100 mL) and ether (100 mL) were added, and the mixture was stirred for an additional 15 min. The solution was filtered. The solid was washed with water twice and dried. The solid was then taken up in 10% methanol in dichloromethane and dried over anhydrous magnesium sulfate. (This is done to remove any traces of water, which interferes with the recrystallization procedure.) The solvent was removed in vacuo. This was then redissoved in hot 10% methanol in dichloromethane. The solution was allowed to cool to room temperature, and ether was added till the solution became turbid. This was then stirred for 30 min, and the product was filtered, washed with ether, and dried. The product was characterized by LC-MS and NMR. Yield (20 g, 80%). ^{1}H NMR (DMSO-d_6, ppm): δ 8.13 (m, 1 H), 8.03 (m, 4 H), 7.51 (s, 1 H), 4.89−4.13 (m, 5 H), 3.21−3.67 (m, 6 H), 2.81 (s, 3 H), 2.11−2.23 (m, 2 H), 1.55−1.72 (m, 6 H). ^{13}C NMR (DMSO-d_6, ppm): δ 21.27, 27.59, 31.67, 38.56, 45.71, 47.91,-53.69,59.27, 104.31, 117.77,125.07, 128.16, 133.34, 137.10, 149.78, 165.96, 170.49, 174.92. MS (ES): 466.4 ($M + H^+$). Analysis ($C_{28}H_{34}N_4O_3$): (C, H, N)

39b. Synthesized via general procedure E: ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.22 (1H, t, J = 5.4 Hz), 8.00 (1H, s), 7.96 (4H, s), 7.44 (1H, s), 5.15 (1H, m), 4.05 (2H, d, J = 5.7 Hz), 3.31 (6H, s), 3.04 (2H, s), 2.89 (2H, s), 2.30 (3H, s), 2.11 (4H, m), 1.76 (2H, m), 1.53 (5H, m), 1.26 (1H, m). LC/MS: M + 1: 481.6.

39e. Synthesized via general procedure E: ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.22 (1H, t, J = 5.5 Hz), 8.00 (1H, s), 7.95 (2H, d, J = 8.4 Hz), 7.90 (2H, d, J = 8.4 Hz), 7.39 (1H, s), 4.05 (2H, d), 3.75 (1H, m), 3.50 (4H, m), 2.30 (3H, s), 2.11 (4H, m), 1.77 (2H, m), 1.53 (5H, m), 1.27 (1H). MS (ES): M + H^+: 453.2.

39i. Synthesized via general procedure E: ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.22 (1H, t), 8.00 (1H, s), 7.91 (2H, s), 7.90 (2H, s), 7.46 (1H, s), 4.05 (2H, d), 3.83 (1H, m), 3.46 (5H, m), 3.15 (2H, t), 2.09 (4H, t), 1.75 (2H, m), 1.53 (6H, m), 1.23 (3H, s), 1.21 (3H, s). MS: M + H^+: 508.8.

39k. **Synthesized via general procedure E:** ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.22 (1H, t), 8.00 (1H, s), 7.91 (2H, s), 7.90 (2H, s), 7.43 (1H, s), 4.06 (2H, t,m), 2.74(1H, m), 2.65 (3H, m), 2.49 (1h, m), 2.09 (5H, m), 1.69 (5H, m), 1.53 (8H, m), 1.23 (3H, s), 1.21 (3H, s). MS: M + H^+: 523.6**39n**. MS: M + H^+: 503.4.

39l. Synthesized via general procedure E: ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.22 (1H, t), 8.00 (1H, s), 7.91 (2H, s), 7.90 (2H, s), 7.43 (1H, s), 4.04 (2H, d), 3.87 (2H, m), 3.45 (4H, m), 2.62 (5H, t), 2.49 (2H, m), 2.09 (2H, m), 1.69 (4H, m), 1.53 (8H, m). MS: M + H^+: 537.6.

39n. Synthesized via general procedure E. ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.19 (1H, t, J = 5.4 Hz), 7.97 (1H, s), 7.92 (2H, d, J = 8.8 Hz), 7.88 (2H, d, J = 8.8 Hz), 7.42 (1H, s), 4.04 (2H, d, J = 5.7 Hz), 3.46 (6H, m), 3.30 (3H, s), 2.55 (4H, m), 2.10 (2H, m), 1.74 (2H, m), 1.52 (5H, m), 1.25 (3H, m). LC/MS: M + 1: 511.6.

39p. **Synthesized via general procedure E:** ^{1}H NMR (DMSO-d_6) (270 MHz): δ 8.20 (1H, t), 8.00 (1H, s), 7.90 (4H, s), 7.39 (1H, s), 4.01 (4H, d, m), 3.02 (3H, t), 2.09 (2H, m), 1.74 (5H, m), 1.49 (17H, m). MS: M + H$^+$: 535.4.

General Procedure F, Illustrated for 39q. A 1:1 mixture of *tert*-butylpiperazine[13a] (**23**, R = t-Bu) and 2,4-dibromothiazole (**35**) in DMF (in the presence of 2 equiv of triethylamine) was heated for 3 h, cooled, and purified by flash chromatography (10% MeOH/CH_2Cl_2) to give **36** (R = t-Bu) in 50% yield. This material was coupled with **37** using the palladium-assisted cross-coupling procedure as described for the biphenylamide derivatives **19a**−**m**, above.

Enzyme Assays. All enzymes used in these studies, with the exception of human cathepsin B, were produced by Celera Genomics. Cathepsin B was from human liver and was purchased from Athens Research and Technology (Athens, GA). The substrates used in these studies were purchased from the following vendors: Z-Phe-Arg-AMC, Boc-Leu-Lys-Arg-AMC, and Z-Val-Val-Arg-AMC were from Bachem (Torrance, CA) and Z-Leu-Arg-AMC was from Calbiochem-Novabiochem (San Diego, CA). Bovine serum albumin was purchased from the Sigma Chemical Company (St. Louis, MO). Routine buffer components and all other chemicals used in these experiments were of the highest available quality.

Methods. Enzyme inhibition studies were performed under several sets of conditions, and each set was tailored to provide the optimal activity of the given cathepsin being assayed. Each cathepsin was incubated with the inhibitor, present at variable concentrations, under the conditions specified below. Enzyme and inhibitor were incubated together for 30 min at room temperature (21 to 24 °C) in 96-well U-bottom, microtiter plates (Falcon, from Becton Dickinson, Franklin Lakes, NJ). After the preincubation phase, reactions were initiated with the addition of the 7-amino-4-methylcoumarin (AMC) substrate specified below. The hydrolysis of these substrates yields 7-amino-4-methylcoumarin which was monitored fluorometrically (excitation at 355 nm and emission at 460 nm) using an FMAX 96-well plate reader from Molecular Devices (Sunnyvale, CA) interfaced with a Macintosh computer. Under these experimental conditions, 1 μM of product produces a fluorescence signal of approximately 125 units. The velocity of the enzyme-catalyzed reaction was obtained from the linear portion of the progress curves (usually the first 5 min after addition of substrate).

Specific assay conditions for each cathepsin tested are reported below.

Human Cathepsin K. The buffer used to assay this enzyme consisted of 50 mM MES (pH 5.5), 2.5 mM D,L-dithiothreitol (DTT), 2.5 mM ethylenediaminetetraacetic acid (EDTA), and 10% dimethyl sulfoxide (DMSO). Recombinant human cathepsin K was supplied at 1 nM. Substrate, Z-Phe-Arg-AMC, was supplied at 40 μM.

Human Cathepsin B. The buffer used to assay this enzyme consisted of 50 mM MES (pH 6.0), 2.5 mM DTT, 2.5 mM EDTA, 0.001 Tween-20, and 10% DMSO. Human liver cathepsin B was supplied at 1 nM. Substrate, Boc-Leu-Lys-Arg-AMC, was supplied at 190 μM.

Human Cathepsin L. The buffer used to assay this enzyme consisted of 50 mM MES (pH 5.5), 2.5 mM DTT, 2.5 mM EDTA, and 10% DMSO. Recombinant human cathepsin L was supplied at 500 pM. Substrate, Z-Phe-Arg-AMC, was supplied at 10 μM.

Human Cathepsin S. The buffer used to assay this enzyme consisted of 50 mM MES (pH 6.5), 2.5 mM β-mercaptoethanol (BME), 2.5 mM EDTA, 100 mM NaCl, 0.001% bovine serum albumin (BSA), and 10% DMSO. Recombinant human cathepsin S was supplied at 500 pM. Substrate, Z-Val-Val-Arg-AMC, was supplied at 60 μM.

Acknowledgment. The authors thank Joanne Fanucchi for help with preparation of this manuscript and Drs. Jochen Knolle and Ann Welton for helpful discussions.

Supporting Information Available: Elemental analysis data for compounds **1**-**3**, **5**, **11**−**14**, **18b**,**c**,**e**−**i**,**n**,**o**, **19j**, and **39n**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Peck, W. A.; Burckhardt, P.; Christiansen, C.; Fleisch, H. A.; Genant, H. K.; Gennari, C.; Martin, T. J.; Martini, L.; Morita, R.; Ogata, E.; Rapado, A.; Shulman, L. E.; Stern, P. H.; Young, R. T. T.; Barrett-Connor, E.; Brandi, M. L.; Chesnut, C. H.; Delmas, P. D.; Heaney, R. P.; Johnston, C. C.; Kanis, J. A.; Lindsay, R.; Meunier, P. J.; Papapoulos, S. E.; Reginster, J. Y.; Riggs, B. L.; Riis, B. J.; Seeman, E.; Wasnich, R. D. Consensus development conference: diagnosis prophylaxis and treatment of osteoporosis. *Am. J. Med*. **1993**, *94*, 646−650.

(2) (a) Bromme, D.; Okamoto, K.; Wang, B. B.; Biroc, S. Human Cathepsin O2, a matrix protein-degrading cysteine protease expressed in osteoclasts. *J. Biol. Chem.* **1996**, *271*, 2126−2132. (b) Bossard, M. J.; Tomaszek, T. A.; Thompson, S. K.; Amegadzie, B. Y.; Hanning, C. R.; Jones, C.; Kurdyla, J. T.; McNulty, D. E.; Drake, F. H.; Gowen, M.; Levy, M. A. Proteolytic activity of human osteoclast cathepsin K. Expression, purification, activation, and substrate identification. *J. Biol. Chem.* **1996**, *271*, 12517−12524. (c) Tezuka, K.; Tezuka, Y.; Maejima, A.; Sato, T.; Nemoto, K.; Kamioka, H.; Hakeda, Y.; Kumegawa, M. Molecular cloning of a possible cysteine proteinase predominantly expressed in osteoclasts. *J. Biol. Chem*. **1994**, *269*, 1106−1109.

(3) (a) Gelb, B. D.; Moissoglu, K.; Zhang, J.; Martignetti, J. A.; Bromme, D.; Desnick, R. J. Cathepsin K; isolation and characterization of the murine cDNA and genomic sequence, the homologue of the human pycnodysostosis Gene. *Biochem. Mol. Med*. **1996**, *59*, 200−206. (b) Johnson, M. R.; Polymeropoulos, M. H.; Vos, H. L.; Oritz de Luna, R. I.; Francomano, C. A. A nonsense mutation in the cathepsin K gene observed in a family with pycnodysostosis. *Genome Res*. **1996**, *6*, 1050−1055. (c) Gelb, B. D.; Shi, G.-P.; Chapman, H. A.; Desnick, R. J. Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science* **1996**, *273*, 1236−1238.

(4) (a) Saftig, P.; Hunziker, E.; Wehmeyer, O.; Jones, S.; Boyde, A.; Rommerskirch, W.; Detlev, J. D.; Schu, P.; von Figura, K. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin K deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13453−13458. (b) Gowen, M.; Lazner, F.; Dodds, R.; Kapadia, R.; Field, J.; Tavaria, M.; Bertoncello, I.; Drake, F.; Zavarselk, S.; Tellis, I.; Hertzog, P.; Debouck, C.; Kola, I. Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. *J. Bone Miner. Res*. **1999**, *14*, 1654−1663.

(5) Votta, B. J.; Levy, M. A.; Badger, A.; Bradbeer, J.; Dodds, R. A.; James, I. E.; Thompson, S.; Bossard, M. J.; Carr, T.; Conner, J. R.; Tomaszek, T. A.; Szewczuk, L.; Drake, F. H.; Veber, D. F.; Gowen, M. Peptide aldehyde inhibitors of cathepsin K inhibit bone resorption both in vitro and in vivo. *J. Bone Miner. Res.* **1997**, *12*, 1396−1406.

(6) (a) Marquis, R. W.; Ru, Y.; LoCastro, S. M.; Zeng, J.; Yamashita, D. S.; Oh, H.-J.; Erhard, K. F.; Davis, L. D.; Tomaszek, T. A.; Tew, D.; Salyers, K.; Proksch, J.; Ward, K.; Smith, B.; Levy, M.; Cummings, M. D.; Haltiwanger, R. C.; Trescher, G.; Wang, B.; Hemling, M. E.; Quinn, C. J.; Cheng, H.-Y.; Lin, F.; Smith, W. W.; Janson, C. A.; Zhao, B.; McQueney, M. S.; D'Alessio, K.; Lee, C.-P.; Marzulli, A.; Dodds, R. A.; Blake, S.; Hwang, S.-H.; James, I. E.; Gress, C. J.; Bradley, B. R.; Lark, M. W.; Gowen, M.; Veber, D. F. Azepanone-Based Inhibitors of Human and Rat Cathepsin K. *J. Med. Chem*. **2001**, *44* (9), 1380−1395. (b) Marquis, R. W.; Ru, Y.; Yamashita, D. S.; Oh, H.-J.; Yen, J.; Thompson, S. K.; Carr, T. J.; Levy, M. A.; Tomaszek, T. A.; Ijames, C. F.; Smith, W. W.; Zhao, B.; Janson, C. A.; Abdel-Meguid, S. S.; D'Alessio, K. J.; McQueney, M. S.; Veber, D. F. Potent dipeptidyl ketone inhibitors of the cysteine protease cathepsin K. *Bioorg. Med. Chem.* **1999**, *7*, 581−588. (c) Marquis, R. W.; Yamashita, D. S.; Ru, Y.; LoCastro, S. M.; Oh, H.-J.; Erhard, K. F.; DesJarlais, R. L.; Head, M. S.; Smith, W. W.; Zhao, B.; Janson, C. A.; Abdel-Meguid, S. S.; Tomaszek, T. A.; Levy, M. A.; Veber, D. F. Conformationally constrained 1,3-diamino ketones: A series of potent and selective inhibitors of the cysteine protease cathepsin K. *J. Med. Chem*. **1998**, *41*, 3563−3567.

(7) Mendonca, R. V.; Venkatraman, S.; Palmer, J. T. Novel route to the synthesis of peptides containing 2-amino-1′- hydroxymethyl ketones and their application as cathepsin K inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12* (20): 2887−2891.

(8) McGrath, M. E.; Klaus, J. L.; Barnes, M. G.; Bromme, D. Crystal structure of human cathepsin K complexed with a potent inhibitor. *Nat. Struct. Biol*. **1997**, *4*, 105−109.
(9) (a) DesJarlais, R. L.; Yamashita, D. S.; Oh, H.-J.; Uzinskas, I. N.; Erhard, K. F.; Allen, A. C.; Haltwanger, R. C.; Zhao, B.; Smith, W. W.; Abdel-Meguid, S. S.; D'Allesio, K.; Janson, C. A.; McQueney, M. S.; Tomaszek, T. A.; Levy, M. A.; Veber, D. F. Use of X-ray cocrystal structures and molecular modeling to design potent and selective non-peptide inhibitors of cathepsin K. *J. Am. Chem. Soc.* **1998**, *35*, 9114−9115. (b) Yamashita, D. S.; Smith, W. W.; Zhao, B.; Janson, C. A.; Tomaszek, T. A.; Bossard, M. J.; Levy, M. A.; Oh, H.-J.; Carr, T. J.; Thompson, S. K.; Ijames, C. F.; Carr, S. A.; McQueney, M.; D'Alessio, K. J.; Amegadzie, B. Y.; Hanning, C. R.; Abdel-Meguid, S.; DesJarlais, R. L.; Gleason, J. G.; Veber, D. F. Structure and Design of Potent and Selective Cathepsin K Inhibitors. *J. Am. Chem. Soc*. **1997**, *119* (46), 11351−11352.
(10) McGrath, M. E.; Sprengeler, P. A.; Hill, C. M.; Martichonok, V.; Cheung, H.; Somoza, J. R.; Palmer, J. T.; Janc, J. W., Peptide ketobenzoxazole inhibitors bound to cathepsin K. *Biochemistry* **2003**, *42* (51), 15018−15028.
(11) Greenspan, P. D.; Clark, K. L.; Tommasi, R. A.; Cowen, S. D.; McQuire, L. W.; Farley, D. L.; van Duzer, J. H.; Goldberg, R. L.; Zhou, H.; Du, Z.; Fitt, J. J.; Coppa, D. E.; Fang, Z.; Macchia, W.; Zhu, L.; Capparelli, M. P.; Goldstein, R.; Wigg, A. M.; Doughty, J. R.; Bohacek, R. S.; Knap, A. K. Identification of dipeptidyl nitriles as potent and selective inhibitors of cathepsin B through structure-based drug design. *J. Med. Chem.* **2001**, *44*, 4524−4534.
(12) Ward, Y. D.; Thomas, D. S.; Frye, L. L.; Cywin, C. L.; Morwick, T.; Emmanuel, M. J.; Zindell, R.; McNeil, D.; Bekkali, Y.; Giradot, M.; Hrapchak, M.; DeTuri, M.; Crane, K.; White, D.; Pav, S.; Wang, Y.; Hao, M.-H.; Grygon, C. A.; Labadia, M. E.; Freeman, D. M.; Davidson, W.; Hopkins, J. L.; Brown, M. L.; Spero, D. M. Design and synthesis of dipeptide nitriles as reversible and potent cathepsin S inhibitors. *J. Med. Chem.* **2002**, *45*, 5471−5482.
(13) (a) Robichaud, J.; Oballa, R.; Prasit, P.; Falgueyret, J.-P.; Percival, M. D.; Wesolowski, G.; Rodan, S. B.; Kimmel, D.; Johnson, C.; Bryant, C.; Venkatraman, S.; Setti, E.; Mendonca, R.; Palmer, J. T.; A novel class of nonpeptidic biaryl inhibitors of human cathepsin K. *J. Med. Chem*. **2003**, *46* (17), 3709−3727. (b) Bryant; C. M.; Palmer; J. T.; Rydzewski; R. M.; Setti; E. L.; Tian; Z-Q.; Venkatraman; S.; Wang; D-X. Compounds and compositions as protease inhibitors. U.S. Patent 6,593,327, July 15, 2003. (c) Gabriel, T.; Pech, M.; Rodriguez Sarmiento, R. M. β-Amino acid nitrile derivatives useful for the treatment of diseases which are associated with cysteine proteases. World Patent 2001/96285, *Chem. Abstr.* **2001**, *136*, 53544. (d) Missbach, M. Preparation of dipeptide cathepsin K inhibitors. World Patent 2001/58886, *Chem. Abstr.* **2001**, *135,* 180786. (e) Altmann, E.; Betschart, C.; Gohda, K.; Horiuchi, M.; Lattmann, R.; Missbach, M.; Sakaki, J.; Takai, M.; Teno, N.; Cowen, S. D.; Greenspan, P. D.; McQuire, L. W.; Tommasi, R. A.; Van Duzer, J. H. Dipeptide nitriles. World Patent 9924460, *Chem. Abstr.* **1999**, *130,* 352553.
(14) See, for example, Synthesis of Peptides and Peptidomimetics. In *Houben-Weyl Methods of Organic Chemistry*; Goodman, M., Felix, A., Moroder, L., and Toniolo, C., Eds.; Georg Thieme Verlag: Stuttgart, Germany, 2001; Volume E22A.
(15) Giroux, A.; Han, Y.; Prasit, P. One Pot Biaryl Synthesis via in situ Boronate Formation. *Tetrahedron Lett.* **1997**, *38*, 3841−3844.
(16) Falgueyret, J. P.; Oballa, R. M.; Okamoto, O.; Wesolowski, G.; Aubin, Y.; Rydzewski, R. M.; Prasit, P.; Riendeau, D.; Rodan, S. B.; Percival, M. D. Novel, nonpeptidic cyanamides as potent and reversible inhibitors of human cathepsins K and L. *J. Med. Chem.* **2001**, *44* (1), 94−104.
(17) Collins, J. L.; Blanchard, S. G.; Boswell, G. E.; Charifson, P. S.; Cobb, J. E.; Henke, B. R.; Hull-Ryde, E. A.; Kazmierski, W. M.; Lake, D. H.; Leesnitzer, L. M.; Lehmann, J.; Lenhard, J. M.; Orband-Miller, L. A.; Gray-Nunez, Y.; Parks, D. J.; Plunkett, K. D.; Tong, W.-Q. *N*-(2-Benzoylphenyl)-L-tyrosine PPAR Agonists. 2. Structure−Activity Relationship and Optimization of the Phenyl Alkyl Ether Moiety. *J. Med. Chem.* **1998**, *41* (25), 5037−5054.
(18) Kuzmic, P.; Sideris, S.; Cregar, L. M.; Elrod, K. C.; Rice, K. D.; Janc, J. W. High-throughput screening of enzyme inhibitors: Automatic determination of tight-binding inhibition constants. *Anal. Biochem.* **2000**, *281*, 62−67.
(19) Morrison, J. F. Kinetics of the reversible inhibition of enzyme-catalysed reactions by tight-binding inhibitors. *Biochim. Biophys. Acta* **1969**, *185*, 269−286.

JM058198R