A Novel Class of Nonpeptidic Biaryl Inhibitors of Human Cathepsin K

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A novel series of nonpeptidic biaryl compounds was identified as potent and reversible inhibitors of cathepsin K. The P2−P3 amide bond of a known amino acetonitrile dipeptide **1** was replaced with a phenyl ring, thereby giving rise to this biaryl series that retained potency vs cathepsin K and showed an improved selectivity profile against other cathepsins. Structural modification within this series resulted in the identification of compound (*R*)-**2**, a potent human cathepsin K inhibitor (IC_{50} = 3 nM) that is selective versus cathepsins B (IC_{50} = 3950 nM), L (IC_{50} = 3725 nM), and S (IC_{50} = 2010 nM). In an in vitro assay involving rabbit osteoclasts and bovine bone, compound (*R*)-**2** inhibited bone resorption with an IC_{50} of 95 nM. It was shown that, unlike some peptidic nitrile inhibitors of cysteine proteases, the nitrile moiety of (*R*)-**2** is not converted to the corresponding amide **3** by cathepsin K. This indicates that this class of nonpeptidic nitrile inhibitors is unlikely to be hydrolyzed by cysteine proteases. Furthermore, the inhibition of cathepsin K by compound (*R*)-**2** was shown to be fully reversible and not observably time-dependent. To demonstrate the efficacy of compound (*R*)-**2** in vivo, it was administered to ovariectomized (OVX) rhesus monkeys at 20 mg/kg, po once daily for 8 days, and a urinary marker of bone turnover, N-telopeptide of type I collagen (uNTx), was measured. During the eight-day dosing period, the mean reduction by compound (*R*)-**2** in uNTx was 80% ($p < 0.001$). This demonstrates that inhibition of cathepsin K leads to an inhibition of this bone resorption marker in OVX rhesus monkeys and strongly suggests that inhibition of cathepsin K is a viable therapeutic approach for the treatment of osteoporosis.

Introduction

Osteoporosis is characterized by low bone mass and accompanying microarchitectural deterioration that results in skeletal fragility and an increased risk of fracture. The bone loss associated with osteoporosis is brought about by an imbalance between bone resorption (performed by osteoclasts) and bone formation (performed by osteoblasts).[1] The extracellular matrix of bone is a composite of hydroxyapatite and protein, 90% of which is type I collagen. Bone resorption requires both decalcification of the hydroxyapatite by an acidic microenvironment and degradation of the protein matrix. These processes occur in the acidic lacunae beneath the osteoclast. Cathepsin K is a member of the papain superfamily of cysteine proteases that is abundantly and selectively expressed in osteoclasts[2−4] and has been shown to play a key role in osteoclast-mediated degradation of bone matrix. It is postulated that molecules that inhibit the activity of cathepsin K could serve as useful therapeutic agents against diseases such as osteoporosis and other bone disorders displaying excessive levels of resorption.

Cathepsin K is one of a growing number of cysteinyl cathepsins (B, H, L, S, C, K, O, F, V, X, and W),[5] all of which are involved in intracellular protein degradation. Some of these enzymes have been shown to have a broad tissue distribution (i.e., cathepsins L and B) whereas others possess a much more selective distribution (i.e., cathepsins K,[6] S,[7,8] and W[9]). The design of therapeutically useful cathepsin K inhibitors would therefore require a high degree of selectivity versus others members of this class of cysteine proteases.

Most of the current approaches to cathepsin inhibition involve molecules of a peptidic nature.[10,11] While these classes of compounds can achieve good potency and selectivity, they are often plagued by high molecular weights and poor pharmacokinetic profiles. Peptidic molecules bearing a nitrile warhead have been reported to be inhibitors of cathepsin K,[12−15] B,[16] and S.[17] Our present approach entailed the replacement of one peptidic bond of the known nitrile dipeptide **1**[18] with an aryl or heteroaryl substituent (see Figure 1, dipeptide **1** to biaryl **4**). This strategy provided us with several potent

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⊥ Celera.

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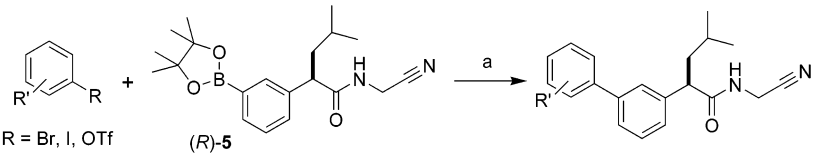
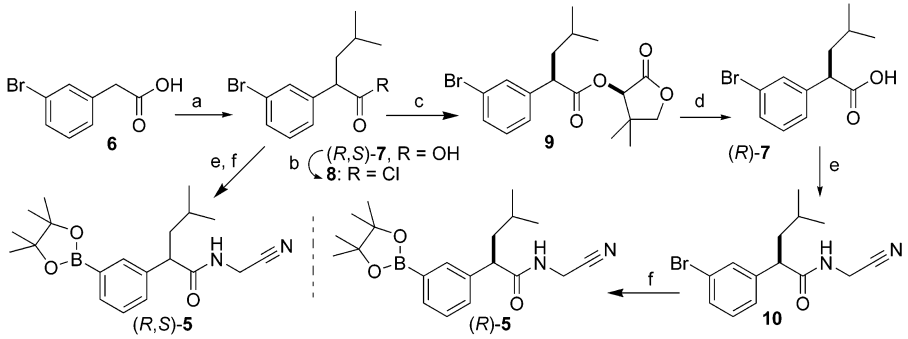
Scheme 1[a]



[a] Conditions: (a) $PdCl_2$(dppf), Na_2CO_3, DMF or 2-propanol, 85−95 °C, 2−18 h.

Scheme 2[a]



[a] Conditions: (a) LiHMDS, THF, 0 °C to rt; Me_2CHCH_2I; (b) $(COCl)_2$, DMF (cat.), MePh, rt; (c) $EtNMe_2$, 0 °C; (*R*)-pantolactone, MePh, −78 °C to 0 °C; (d) HCl, AcOH, 85 °C, 18 h; (e) PyBOP, $HCl{\cdot}H_2NCH_2CN$, Et_3N, DMF, rt; (f) diboron pinacol ester, KOAc, $PdCl_2$(dppf), DMF, 85−90 °C, 3 h.

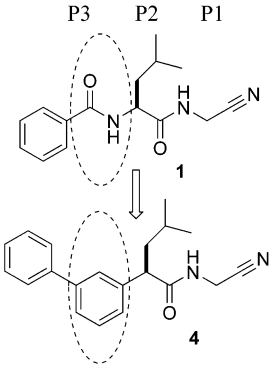


Figure 1. Replacement of one peptide bond of dipeptide **1** with a phenyl ring affords biaryl compound **4**.

and selective small biaryl molecules with good overall profiles. The purpose of the present report is to report some of the SAR and enzymology obtained in this series and the in vivo efficacy in an OVX rhesus monkey model of bone resorption.

Chemistry

Our key step, a Suzuki cross-coupling between various aryl/heteroaryl halides or triflates with the required boronic ester intermediate **5**, as outlined in Scheme 1, provided us with access to a large number of compounds that could be rapidly assembled in one final step. The racemic boronic ester (*R*,*S*)-**5** was constructed in three steps (see Scheme 2). Deprotonation of 3-bromophenyl acetic acid **6** with LiHMDS followed by alkylation with 1-iodo-2-methyl propane yielded the desired alkylated acid (*R*,*S*)-**7**. Conversion of this racemic acid (*R*,*S*)-**7** to its aminoacetonitrile amide derivative was accomplished by a standard PyBOP coupling. The palladium-mediated cross-coupling reaction of the aryl bromide with diboron pinacol ester proceeded smoothly to yield the desired racemic boronic ester (*R*,*S*)-**5**.

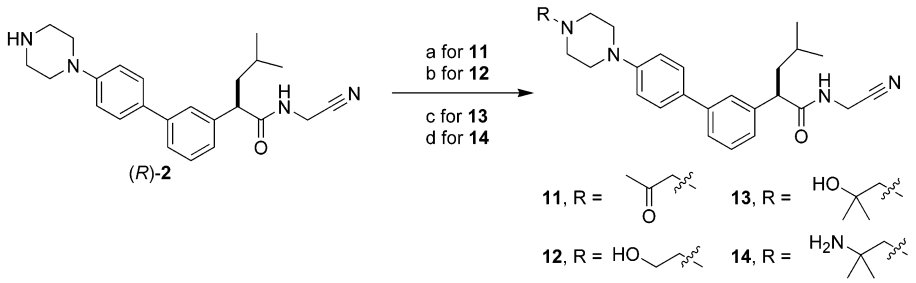
Preparation of the chiral boronic ester (*R*)-**5** represented a more challenging synthetic approach. The strategy employed involved the deracemization of the alkylated acid (*R*,*S*)-**7** by the use of a chiral protonation protocol.[19] According to this protocol, the alkylated acid (*R*,*S*)-**7** is first converted to its corresponding acid chloride **8** with oxalyl chloride. Treatment of this intermediate **8** with ethyldimethylamine generates the ketene functionality in situ which is trapped at low temperature with (*R*)-(−)-pantolactone to afford the desired chiral ester **9**. This methodology consistently afforded under carefully controlled conditions, the desired chiral pantolactone ester **9** in a 10:1 ratio in favor of the desired diastereoisomer.

Hydrolytic cleavage of the chiral ester **9** under acidic conditions yielded the required chiral acid (*R*)-**7** without compromising the integrity of the newly generated stereocenter.[19] Following hydrolysis, the chiral acid (*R*)-**7** was converted to its aminoacetonitrile amide derivative **10** by a standard PyBOP coupling. Finally, a palladium-mediated cross-coupling reaction of this chiral aryl bromide **10** with diboron pinacol ester efficiently afforded the desired boronic ester (*R*)-**5**, now ready for functionalization. The initial exploration of P3 SAR was carried out as described in Scheme 1, with Suzuki cross-couplings between either the racemic or chiral boronate esters (*R*,*S*)-**5** or (*R*)-**5** and various halogenated aryl/heteroaryl bromides.

P3 Substitution. Extended P3 explorations were also conducted by further substitution of the terminal nitrogen of the piperazine (*R*)-**2** as depicted in Scheme 3. Treatment of piperazine (*R*)-**2** with chloroacetone and triethylamine afforded the desired alkylated product **11**. Similarly, treatment of (*R*)-**2** with 2-bromoethanol or isobutylene oxide in the presence of K_2CO_3 yielded the desired alkylated products **12** and **13**, respectively. Reaction of piperazine (*R*)-**2** with 2-amino-2-methylpropyl hydrogen sulfate afforded the desired alkylated product **14**.

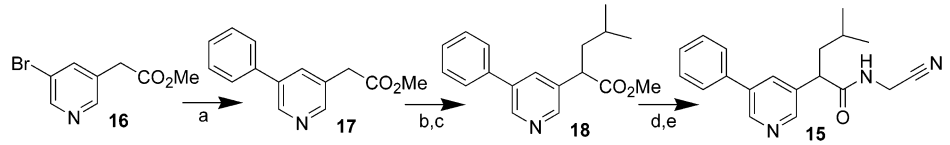
The synthesis of compound **15** (see Scheme 4) in which the internal phenyl ring is replaced with a

Scheme 3[a]



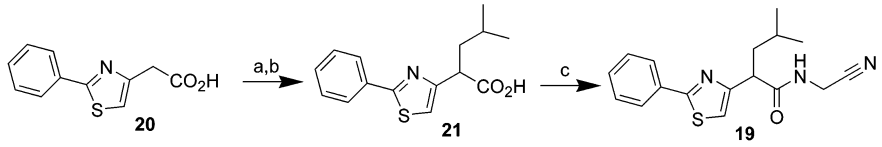
[a] Conditions: (a) chloroacetone, Et_3N, DMF, rt; (b) 2-bromoethanol, K_2CO_3, MeCN, Δ; (c) isobutylene oxide, K_2CO_3, DMF, 100 °C; (d) 2-amino-2-methylpropyl hydrogen sulfate, K_2CO_3, DMF, 100 °C.

Scheme 4[a]



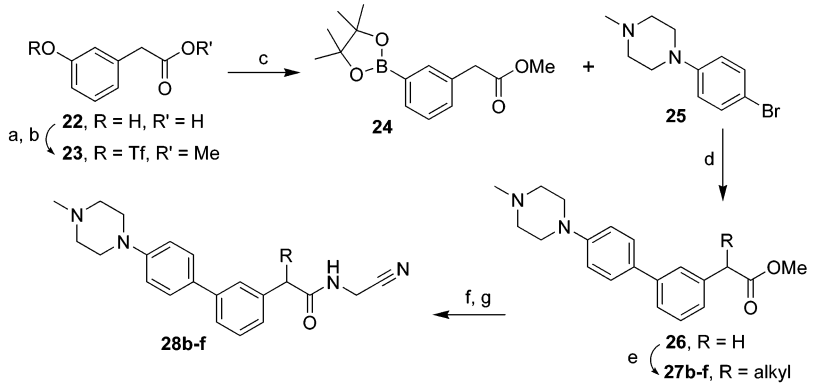
[a] Conditions: (a) $PhB(OH)_2$, $PdCl_2$(dppf), KOAc, DMF, 80 °C; (b) LDA, THF, 0 °C; 1-bromo-2-butene, −78 °C to rt; (c) H_2, 5% Pd/C*, EtOH, 40 psi, 8 h; (d) NaOH, MeOH/H_2O (1:1), 18 h; (e) PyBOP, HCl·H_2NCH_2CN, Et_3N, DMF, rt.

Scheme 5[a]



[a] Conditions: (a) LDA, THF, 0 °C; 1-bromo-2-butene, −78 °C to rt; (b) H_2, 5% Pd/C*, EtOH, 40 psi, 8 h; (c) PyBOP, HCl·H_2NCH_2CN, Et_3N, DMF, rt.

Scheme 6[a]

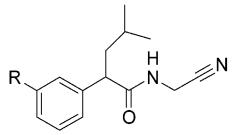


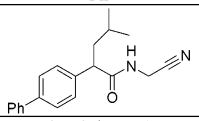
[a] Conditions: (a) pTsOH, $HC(OCH_3)_3$, MeOH, Δ; (b) $(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2, 0 °C; (c) diboron pinacol ester, KOAc, $PdCl_2$(dppf), DMF, 90 °C; (d) Na_2CO_3, $PdCl_2$(dppf), DMF, 100 °C; (e) KHMDS, THF, 0 °C to rt; RX, THF, 0 °C to rt; (f) LiOH, MeOH, H_2O; (g) PyBOP, HCl·H_2NCH_2CN, Et_3N, DMF, rt.

pyridine began with a Suzuki cross-coupling reaction between commercially available methyl (5-bromopyridin-3-yl)acetate (**16**) and phenylboronic acid to afford the desired biaryl compound **17**. α-Alkylation of ester **17** with 1-bromo-2-butene, followed by hydrogenation of the resulting double bond, yielded the desired functionalized ester **18**. This ester was saponified to its corresponding carboxylic acid derivative using NaOH and submitted to a standard PyBOP coupling reaction with aminoacetonitrile hydrochloride and triethylamine to afford the desired pyridine analogue **15**. The synthesis of the thiazole containing compound **19** (see Scheme 5) began with the α-alkylation of acid **20**[20] with LDA and 1-bromo-2-butene to afford, after hydrogenation of the generated double bond, the carboxylic acid **21**. Standard PyBOP coupling reaction of this intermediate with aminoacetonitrile hydrochloride and triethylamine afforded the desired thiazole analogue **19**.

P2 Substitution. Exploration of the P2 group in this series was carried out to determine which substituents would provide optimal cathepsin K activity. The synthetic route began with the esterification of (3-hydroxyphenyl)acetic acid (**22**) with trimethylorthoformate and tosic acid, followed by the treatment of the phenol with triethylamine and trifluoromethanesulfonic anhydride to yield the desired triflic ether **23** as indicated in Scheme 6. The triflate **23** was then converted to the corresponding boronic ester **24** by a palladium-mediated reaction with diboron pinacol ester. Boronic ester **24** was then cross-coupled under palladium-catalyzed Suzuki

Table 1. Inhibition of Human Cathepsins K, L, S and B by Biaryl Analogues



			IC_{50} (nM)[a]					
Compound	**Molecular Formula**	**R**	**Stereochem.**	**Cat K**	**Cat L**	**Cat S**	**Cat B**	**Bone Res.**
1	$C_{15}H_{19}N_3O_2$	See Figure 1	*S*	51	42	41	1903	275
4	$C_{20}H_{22}N_2O$	Ph	*R,S*	56	498	1578	>10000	3560
29	$C_{20}H_{22}N_2O$		*R,S*	790	>10000	3343	>10000	-
15	$C_{21}H_{22}F_3N_3O_3$	See Scheme 4	*R,S*	142	825	>10000	>10000	1400*
19	$C_{17}H_{19}N_3OS$	See Scheme 5	*R,S*	1463	>10000	>10000	>10000	-
30	$C_{21}H_{23}N_3O_2$	*p*-NH_2COPh	*R,S*	1015	>10000	1373	>10000	-
31	$C_{21}H_{24}N_2O_3S$	*p*-$MeSO_2Ph$	*R,S*	1227	>10000	6623	>10000	-
32	$C_{21}H_{22}N_6O$	*p*-phenyl tetrazole	*R*	2580	>10000	>10000	>10000	-
33	$C_{24}H_{29}N_3O_2$	*p*-phenyl morpholine	*R,S*	638	1593	9422	1744	-
34	$C_{24}H_{29}N_3O_2$	*m*-phenyl morpholine	*R,S*	630	>10000	>10000	>10000	6580*
35	$C_{24}H_{29}N_3O_2$	*o*-phenyl morpholine	*R,S*	614	919	3368	>10000	2810*
36	$C_{18}H_{20}N_4O$	*p*-pyrimidine	*R,S*	1036	>10000	>10000	>10000	-
37	$C_{19}H_{21}N_3O$	*o*-pyridine	*R,S*	803	5952	8039	>10000	-
38	$C_{19}H_{21}N_3O$	*m*-pyridine	*R,S*	488	5583	>10000	>10000	-
39	$C_{21}H_{22}F_3N_3O_3$	*p*-pyridine	*R,S*	150	5783	1748	>10000	-
40	$C_{25}H_{25}N_3O$	*p*-phenyl *p*-pyridine	*R*	97	8391	1365	3329	1235*

[a] Each IC_{50} value corresponds to an average of at least two independent determinations except where noted with an asterisk.

conditions with 1-(4-bromophenyl)-4-methylpiperazine (**25**) to afford the desired alkylation precursor **26**. The key intermediate **26** was then deprotonated with KHMDS and alkylated with a variety of electrophiles to yield several P2 alkylated products **27b**−**f**. All of these ester intermediates **27b**−**f** were then sequentially hydrolyzed using LiOH, and the corresponding carboxylic acids were coupled with aminoacetonitrile hydrochloride according to a standard PyBOP amide coupling protocol to afford the desired amides **28b**−**f**.

Biochemistry

Compounds were tested against human cathepsins K, L, B, and S and a humanized form of rabbit cathepsin K. Humanized rabbit cathepsin K was obtained by mutation of two active site amino acid residues of the rabbit enzyme to the corresponding human residues ($Tyr^{61} \rightarrow Asp^{61}$ and $Val^{157} \rightarrow Leu^{157}$). Both cathepsin K enzymes also contain a $Ser^{49} \rightarrow Ala^{49}$ mutation to remove a N-glycosylation site. Human cathepsins K, L, and S and humanized rabbit cathepsin K were expressed and purified as described in the Experimental Section while purified human liver cathepsin B was obtained from Sigma. Inhibitory potency was determined after a 15 min preincubation with the various enzymes prior to the addition of 2 μM of the substrate Z-Leu-Arg-AMC. Assays were performed in 96-wells plates, and IC_{50} values were determined by fitting experimental values to a four-parameter logistic model. Compounds were also tested in an in vitro bone resorption assay that evaluates degradation of bovine bone by isolated rabbit osteoclasts as described previously.[21]

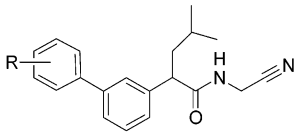
Results and Discussion

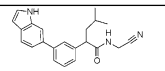
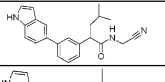
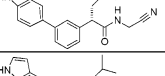
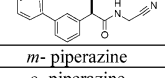
The known dipeptide nitrile **1**[18] and some of the biaryl analogues synthesized are shown in Table 1. While the original dipeptide **1** showed good potency against cathepsin K and modest selectivity against cathepsin B (40-fold), it was unselective against cathepsins L and S. Other workers have previously reported that a P2−P3 carbamate group could be replaced with a biaryl group to afford cathepsin K inhibitors with improved selectivity over their peptidic counterparts.[22] The replacement of the P2−P3 amide bond of the dipeptide nitrile **1** with a phenyl provided compound **4**, which retained activity against cathepsin K and showed a significantly improved selectivity profile against the other cathepsins (see Table 1). However, it was clear that further improvements were required, especially considering the lack of potency of the *m*-biphenyl **4** in the in vitro bone resorption assay (IC_{50} = 3560 nM).

As previously observed,[22] proper positioning of the second aryl group appeared critical as the para-linked biphenyl **29** lost considerable activity against cathepsin K. Replacement of the internal phenyl group of **4** with a either a pyridine (compound **15**) or thiazole (compound **19**) had a deleterious effect on cathepsin K potency. The pyridine compound **15**, however, showed increased activity in the in vitro bone resorption assay. This translated to a decrease in shift between the enzyme inhibition and the ability to inhibit bone resorption in vitro (60-fold shift for the biphenyl **4** vs 10-fold shift for the phenyl pyridine **15**).

Most of the remaining P3 SAR focused on modifications and substitutions to the terminal aryl ring. Para substitution off the terminal phenyl group of **4** with either a carboxamide, a methyl sulfone or a tetrazole (**30**, **31**, and **32**, respectively) proved to be detrimental to cathepsin K potency. Elaboration of the three positional isomers on the terminal phenyl group with a morpholine group revealed surprisingly little SAR to distinguish between these three spatially different compounds. All three isomers, the para **33**, the meta **34**, and the ortho **35** showed equivalent potencies against cathepsin K. When the terminal phenyl group of **4** was replaced with the three isomeric pyridines, the

Table 2. Inhibition of Human Cathepsins K, L, S and B by Basic Biaryl Analogues



				IC_{50} (nM)[a]				
Compound	**Molecular Formula**	**R**	**Stereochem.**	**Cat K**	**Cat L**	**Cat S**	**Cat B**	**Bone Res.**
41	$C_{22}H_{23}N_3O$		*R,S*	291	2710	1257	>10000	2780*
(*R,S*)-**42**	$C_{22}H_{23}N_3O$		*R,S*	52	>10000	922	>10000	249
(*S*)-**42**	$C_{22}H_{23}N_3O$		*S*	79	-	-	-	-
(*R*)-**42**	$C_{22}H_{23}N_3O$		*R*	8	-	-	-	-
43	$C_{24}H_{30}N_4O$	*m*- piperazine	*R,S*	216	>10000	>10000	>10000	4870*
44	$C_{24}H_{30}N_4O$	*o*- piperazine	*R,S*	58	338	521	1249	-
(*R,S*)-**2**	$C_{24}H_{30}N_4O$	*p*- piperazine	*R,S*	9	4804	1245	2405	162
(*R*)-2	**$C_{24}H_{30}N_4O$**	***p*- piperazine**	***R***	**3**	**3725**	**2010**	**3950**	**95**
(*R,S*)-**46**	$C_{25}H_{32}N_4O$	*p*-*N*-methyl-piperazine	*R,S*	8	4641	1695	3843	78
(*R*)-**46**	$C_{25}H_{32}N_4O$	*p*-*N*-methyl-piperazine	*R*	6	6273	3931	2599	96
47	$C_{25}H_{29}N_3O$	*p*-1,2,3,6-tetrahydropyridine	*R,S*	57	1682	652	554	267*
48	$C_{25}H_{32}N_3O$	*p*-4-piperidine	*R,S*	18	2755	1421	1075	489
49	$C_{27}H_{33}N_3O$	*p*-quinuclidine	*R*	37	2235	1991	2227	198*

[a] Each IC_{50} value corresponds to an average of at least two independent determinations except where noted with an asterisk.

potency of the compounds increased as the position of the nitrogen moved away from the point of attachment with the *p*-pyridine **39** being the most potent against cathepsin K. Insertion of an extra 1,4-disubstituted phenyl group between the pyridine and the internal phenyl group of **39** (compound **40**) did not significantly alter potency against cathepsin K and roughly maintained the observed 10-fold shift between the enzyme activity and the bone resorption assay.

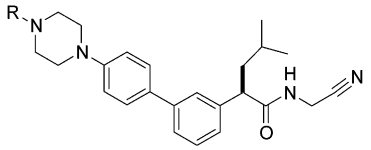
While the selectivity profiles of several of these analogues showed improvement over the original dipeptide **1**, the potencies of these compounds were still lacking, both in the cathepsin K enzyme assay, and in the in vitro bone resorption assay. Encouraged by the results obtained for the pyridine analogues **39** and **40**, we decided to focus our efforts on introducing other basic functional groups in the P3 portion of the molecule. The *m*-indole **41** (see Table 2) failed to give any improvement, but there was a considerable increase in potency against cathepsin K for the *p*-indole (*R,S*)-**42**. But while this increase in potency only equaled that of the initial biaryl **4**, it was clear that the potency of this compound in the bone resorption assay (IC_{50} = 249 nM) was a dramatic improvement over the initial biaryl **4**. The shift between the enzyme assay and the bone resorption assay was drastically reduced to 5-fold for the indole (*R,S*)-**42**.

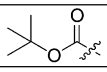
At this point it was decided to separate the two enantiomers of the racemic indole (*R,S*)-**42** to determine whether the potency of racemic (*R,S*)-**42** resided predominantly in only one of the two enantiomers. Separation by chiral chromatography afforded enantiomers (*S*)-**42** and (*R*)-**42** which showed very different potencies vs cathepsin K (IC_{50} = 79 nM and 8 nM, respectively). This 10-fold difference in potency between the enantiomers prompted us to develop an efficient enantioselective route to these compounds (see Scheme 2).

Although the substitution pattern around the terminal phenyl ring afforded similar potencies with the morpholine analogues (**33**, **34**, and **35**, see Table 1), it was observed that this trend did not hold true for the piperazine analogues. Ortho-branching of a piperazine ring onto the biaryl core afforded **44** which showed no improvement in cathepsin K activity over the previously described compounds (**2** and (*R,S*)-**42**). Shifting the piperazine to the meta position (compound **43**) resulted in a loss of activity vs cathepsin K. We were pleased, however, to discover that the para position was optimal for the piperazine substitution, yielding compound (*R,S*)-**2**, which showed a substantial increase in potency against cathepsin K (IC_{50} = 9 nM). The chiral piperazine (*R*)-**2** exhibited not only a low single digit potency against cathepsin K (IC_{50} = 3 nM) but also an improved selectivity profile vs the other cathepsins (>1200-fold for cathepsins L and B and >650-fold for cathepsin S). The increase in cathepsin K potency also translated to an increased potency in the bone resorption assay (IC_{50} = 95 nM), although the shift remains somewhat high (30-fold).

Interestingly, the racemic and chiral *N*-methylated compounds (*R,S*)-**46** and (*R*)-**46** showed very similar profiles (Cat K IC_{50} = 8 nM and 6 nM, respectively). These results suggest that further elaboration of the basic piperazine nitrogen of (*R*)-**2** should be possible. Replacement of the piperazine ring with a 1,2,3,6-tetrahydropyridine ring (compound **47**) afforded a mod-

Table 3. Inhibition of Human Cathepsins K, L, S and B by P3 Alkylated Analogues



				IC_{50} (nM)[a]				
Compound	**Molecular Formula**	**R**	**Stereochem.**	**Cat K**	**Cat L**	**Cat S**	**Cat B**	**Bone Res.**
(*R*)-**2**	$C_{24}H_{30}N_4O$	H	*R*	3	3725	2009	3950	95
50	$C_{29}H_{38}N_4O_3$		*R*	922	>10000	1298	>10000	-
11	$C_{27}H_{34}N_4O_2$		*R*	10	2163	2364	550	121*
51	$C_{28}H_{38}N_4O$		*R*	20	1667	8340	379	340
12	$C_{26}H_{34}N_4O_2$		*R*	12	140	609	3343	-
13	$C_{28}H_{38}N_4O_2$		*R*	6	9546	>10000	6369	142*
14	$C_{28}H_{39}N_5O$		*R*	16	>10000	1427	>10000	552*

[a] Each IC_{50} value corresponds to an average of at least two independent determinations except where noted with an asterisk.

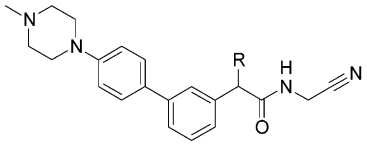
est loss in potency against cathepsin K; however, hydrogenation of the double bond (compound **48**) improved the cathepsin K potency and also improved the selectivity profile against the other cathepsins. In conclusion, these efforts led to the discovery of the para-oriented piperazine compound (*R*)-**2** which displays a range of highly desired properties (cathepsin K potency, selectivity, and potency in the assay).

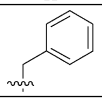
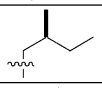
Extended P3 SAR. Following our observation that the basic secondary nitrogen of the piperazine ring can be substituted without adversely affecting potency and selectivity (i.e., compound (*R,S*)-**2** vs compound (*R,S*)-**46**, see Table 2), we decided to conduct further SAR studies on the terminal P3 portion of the molecule (see Table 3). The Boc-protected compound **50** lost considerable activity against cathepsin K (IC_{50} = 922 nM), possibly due to the loss in basicity of the terminal piperazine nitrogen. The ketone-capped alkyl piperazine **11** recovered almost all of the potency against cathepsin K and maintained a good overall selectivity profile against the other cathepsins. Alkylation of the terminal nitrogen of the piperazine ring with a bulky *tert*-butyl group (compound **51**) did not adversely affect the potency against cathepsin K; the presumed equatorial conformation of the *tert*-butyl substituent suggests a hydrogen bond between the tertiary alkylamine and an S3 residue via an axial vector. This result also supports our initial hypothesis that high potency and selectivity require the presence of a basic nitrogen in this P3 portion of the molecule. Other alkylated piperazines (**12**−**14**) showed similar cathepsin K potencies but did not have any substantial advantages over the lead unsubstituted piperazine (*R*)-**2**.

P2 Substitution. Having established some of the SAR on the P3 portion of this series, we decided to conduct SAR studies on the *N*-methylated version of our optimized compound (*R,S*)-**2** by varying the P2 substitution (see Table 4). It is interesting to note that compound **28a**, devoid of any P2 substituent, is also devoid of activity against any of the cathepsin enzymes. Introduction of a variety of groups in the P2 position (i.e., benzyl, propyl, cyclopropyl methyl, 2-methylbutyl, and cyclobutyl methyl) all resulted in a loss of cathepsin K potency relative to the isobutyl group of (*R,S*)-**46**. We therefore conclude that the isobutyl P2 substituent for this series of compounds appears to be optimal for potency against cathepsin K.

Measurement for Potential Nitrilase Activity of cathepsin K with Compound (*R*)-2. It has been previously described that peptide nitrile inhibitors of papain family cysteine proteases can undergo slow hydrolysis of the nitrile moiety to the corresponding amide which is then rapidly hydrolyzed to the acid.[23,24] As shown in Scheme 7a, the nitrilase activity of the enzyme can be favored by the addition of thiols. It was hypothesized that the reversible thioimidate ester bond formed between the enzyme and the inhibitor is attacked by thiols to form a nonenzymic thioimidate that is rapidly hydrolyzed to the amide. Because of the well-known amidase activity of the papain cysteine protease enzymes, the amide will be catalytically transformed to the corresponding acid (Scheme 7b). To test whether the present biaryl nitrile inhibitors could serve as substrates for cathepsin K and be potentially inactivated by this enzyme, 1 μM of compound (*R*)-**2** was incubated with two concentrations of cathepsin K in the presence of 1 mM β-mercaptoethanol. To identify the hydrolysis products of the reaction, the amide and the carboxylic acid analogues **3** and **52** of compound (*R*)-**2** were chemically synthesized and used as standards for RP-HPLC analysis. After a 30 min incubation, the assay mixture was analyzed by RP-HPLC for the presence of the carboxylic acid **52**. As shown in Figure 2, no detectable amount of acid **52** could be detected in the presence of either 10 nM (Figure 2B) or 1 μM (Figure 2C) enzyme. After 30 min, in the presence of 10 nM cathepsin K, 1 μM of the synthetic amide **3** was extensively converted to the acid **52** (Figure 2D), consistent with the amidase activity of cathepsin K. Amidase activity was also blocked by the addition of 1 μM of the irreversible inhibitor E64 (Figure 2E). Incubation of compound (*R*)-**2** with β-mercaptoethanol up to 24 h did not lead to the formation of any

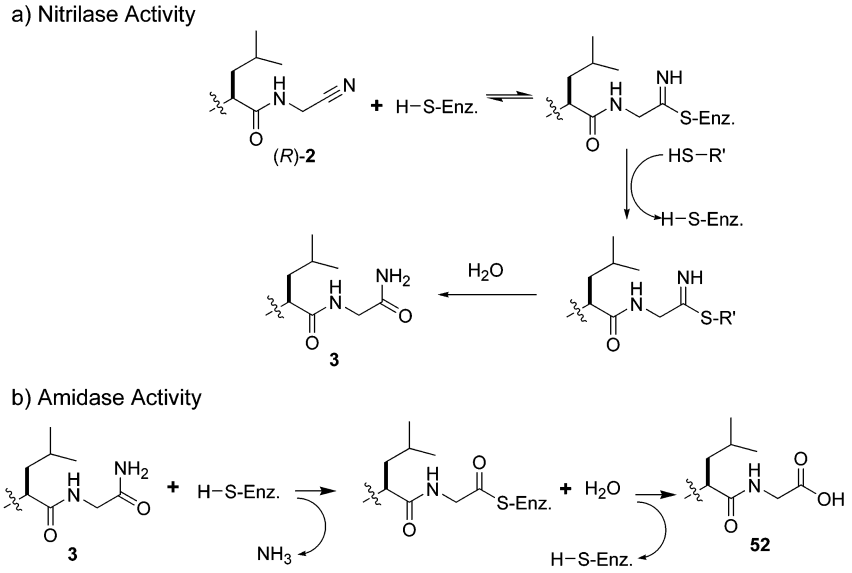
Table 4. Inhibition of Human Cathepsins K, L, S, and B by P2 Alkylated Analogues



				IC_{50} (nM)[a]			
Compound	**Molecular Formula**	**R**	**Stereochem.**	**Cat K**	**Cat L**	**Cat S**	**Cat B**
(*R*,*S*)-**46**	$C_{25}H_{32}N_4O$		*R*,*S*	8	4641	1695	3843
28a	$C_{21}H_{24}N_4O$	H	-	>10000	>10000	>10000	>10000
28b	$C_{28}H_{30}N_4O$		*R*,*S*	1405	3907	>10000	>10000
28c	$C_{24}H_{30}N_4O$		*R*,*S*	87	>10000	>10000	>10000
28d	$C_{25}H_{30}N_4O$		*R*,*S*	65	>10000	6597	3433
28e	$C_{26}H_{34}N_4O$		*R*,*S* Methyl is *S*	51	>10000	>10000	5788
28f	$C_{26}H_{32}N_4O$		*R*,*S*	62	>10000	2310	8055

[a] Each IC_{50} value corresponds to an average of at least two independent determinations.

Scheme 7



hydrolysis products. Although cysteine proteases of the papain family are known to possess nitrilase activity, this enzyme-catalyzed reaction is extremely slow when compared to the peptidase or amidase activity. It has been previously demonstrated that the nitrilase activity K_m of dipeptide nitrile inhibitors for papain corresponds to their K_i for protease inhibition.[24] Our results show that compound (*R*)-**2** which possesses good affinity for the enzyme (K_i = 5 nM) is clearly a poor substrate for this reaction since no hydrolysis products were observed at 100-fold K_i concentration and 1 mM β-mercaptoethanol. These results show that it is unlikely that the nitrile moiety of this class of nonpeptidic inhibitors could be hydrolyzed by cysteine proteases to cause the inactivation of the inhibitor in vivo.

Time-Dependency of Inhibition. Previously described cysteine protease inhibitors which form covalent bonds with the cysteine active site of the enzyme often inhibit in a time-dependent manner.[17,21,25] That is, the attainment of the equilibrium between the enzyme-bound inhibitor and the free inhibitor is slow enough to be experimentally observable (seconds time scale). Figure 3 shows the time course of the reaction when cathepsin K is incubated with the synthetic substrate Z-Leu-Arg-AMC in the presence of various concentrations of compound (*R*)-**2** (Figure 3A). The results

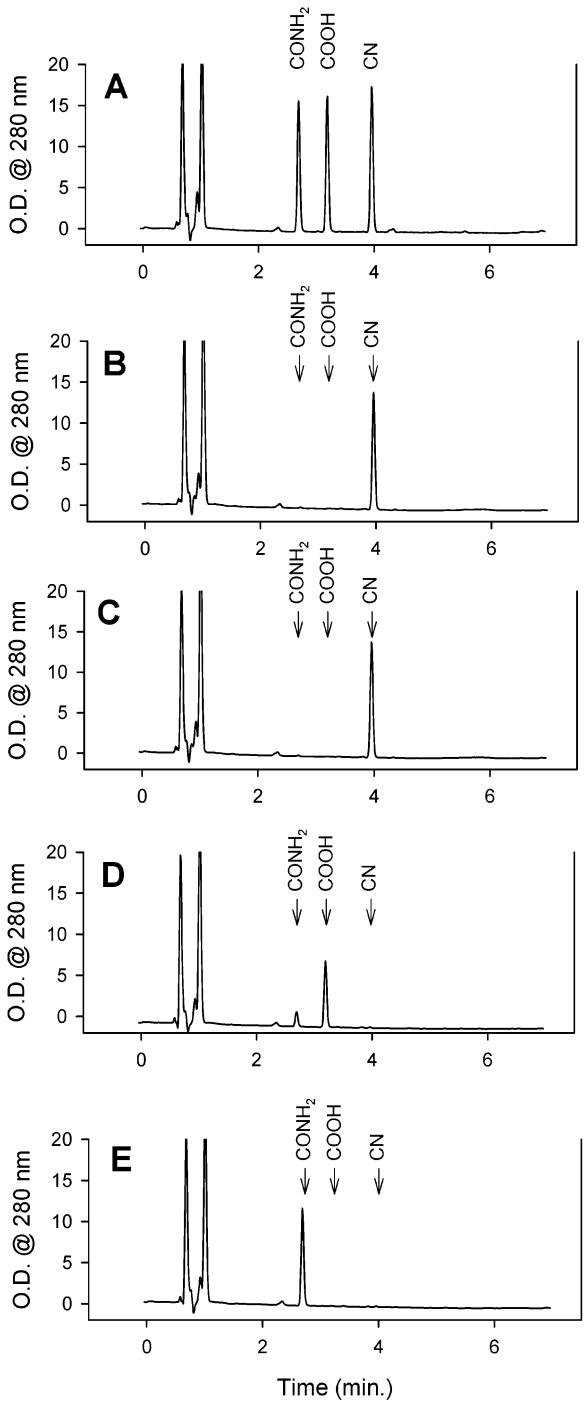


Figure 2. RP−HPLC profiles of the reaction mixture when humanized rabbit cathepsin K is incubated with compound (*R*)-**2**. (A) Coinjection of 1 μM of compound (*R*)-**2** (CN) with 1 μM of the synthetic amide **3** ($CONH_2$) and 1 μM of the synthetic acid **52** (COOH). (B) Reaction mixture when 1 μM of compound (*R*)-**2** is incubated with 10 nM humanized rabbit cathepsin K and 1 mM β-mercaptoethanol for 30 min at room temperature. (C) Same as (B) with 1 μM enzyme. (D) Reaction mixture when 1 μM of the synthetic amide **3** is incubated with 10 nM enzyme and 1 mM β-mercaptoethanol for 30 min and (E) in the presence of 1 μM E64.

obtained show that the rate of the reaction is linear for two concentrations of compound (*R*)-**2** assayed and implies a relatively rapid attainment of the equilibrium. However, the addition of a spiro-cyclopropyl ring at P1 gave a time-dependent inhibitor (compound **45**) that had a similar potency to that of compound (*R*)-**2** (IC_{50} =

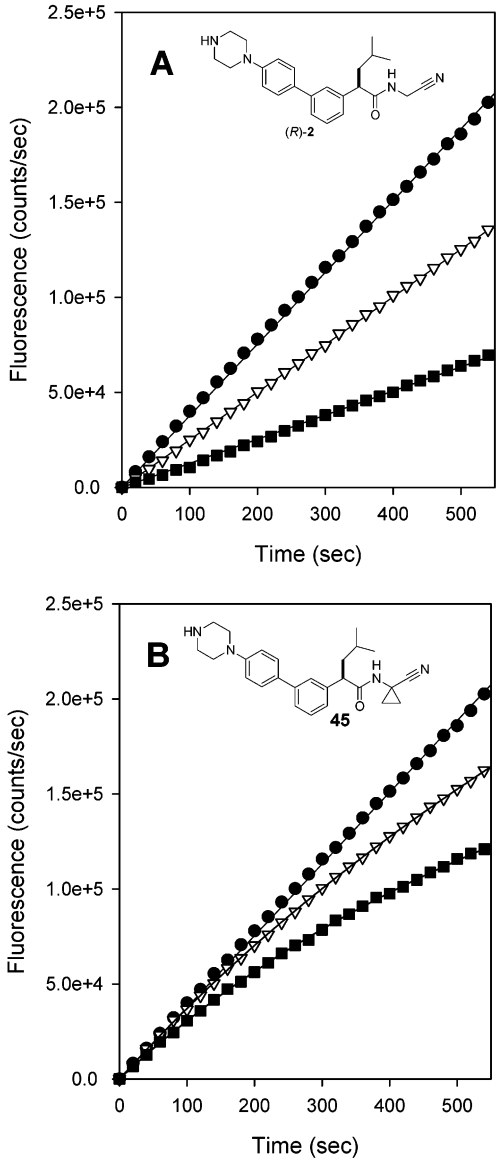


Figure 3. Progress curves for the reaction of humanized rabbit cathepsin K in the presence of compound (*R*)-**2** (A) or compound **45** (B). Progress curves were obtained after initiation of the reaction by the addition of 0.2 nM of humanized rabbit cathepsin K. Control reaction (●) without inhibitor; (A) 33 nM (▽) or 100 nM (■) of compound (*R*)-**2**; (B) 100 nM (▽) or 500 nM (■) of compound **45**.

3 nM) (Figure 3B). The curves obtained in Figure 3B (compound **45**) were fitted to eq 1 (see Experimental Methods) and the kinetic parameters k_{off} and k_{on} were calculated (see Experimental Methods) giving ($n = 2$) values of 1.4×10^{-3} s^{-1} and 1.4×10^{5} M^{-1} s^{-1}, respectively. The calculated K_i (10 nM) agrees with the IC_{50} (10 nM) measured at sub K_m concentrations of substrate (where $K_i \sim IC_{50}$). A similar observation has been reported for cathepsin S dipeptide nitrile inhibitors.[17] There, the addition of a dimethylglycine residue at P1 slows the *on*-rate and the *off*-rate of the compound by 2 to 3 orders of magnitude when compared to the P1 monosubstituted compounds.

With both compounds ((*R*)-**2** and **45**), the kinetics of the inhibition obtained were consistent with fully reversible compounds. Experiments (data not shown) in which the enzyme and the inhibitor were preincubated together (100-fold concentrated) and then diluted into

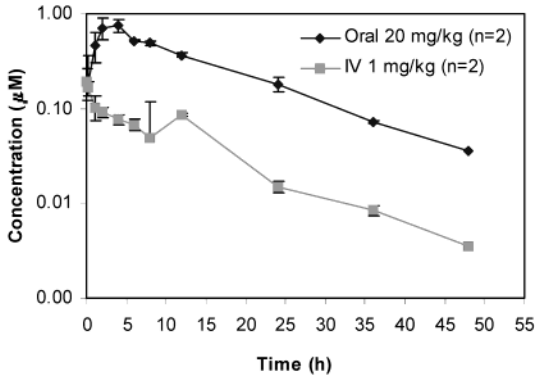


Figure 4. Mean plasma levels after intravenous administration of the HCl salt of compound (*R*)-**2** in 5% dextrose and after oral administration of the HCl salt of (*R*)-**2** as a solution in water in intact rhesus monkeys. Dosing volumes for both intravenous and oral administration were 1 mL/kg. Compound levels were determined in the plasma samples by LC/MS/MS at the times indicated.

substrate-containing buffer showed a complete recovery of the activity which is once again consistent with the reversible nature of these nitrile inhibitors.

Pharmacokinetic Profile of Inhibitor (*R*)-2. To determine if the compounds in this series possessed a suitable pharmacokinetic profile, in vivo dosing studies were performed with compound (*R*)-**2**. The results of this study are presented in Figure 4. When the HCl salt of compound (*R*)-**2** was administered orally to intact rhesus monkeys at a dose of 20 mg/kg in water, the compound was well absorbed with a bioavailability of 32%. The half-life of compound (*R*)-**2** was determined after intravenous dosing in 5% dextrose and was found to be 9.6 h in rhesus monkeys. These results clearly demonstrate that compound (*R*)-**2** possesses good pharmacokinetic properties (in contrast to most peptidic compounds) in rhesus monkeys and could be used orally for in vivo studies to test the effects of a cathepsin K inhibitor on bone resorption.

Rhesus Monkey Bone Resorption Marker Assay. In the Rhesus Monkey Bone Resorption Marker Assay (rhBRMA), estrogen-deficiency bone loss (i.e., low BMD) associated with accelerated bone resorption and formation is established by surgical oophorectomy (OVX) at least three months pretreatment. The same measurement of bone resorption markers (bone collagen-specific breakdown products), urinary N-telopeptides [uNTx] used in humans taking antiresorptive therapy, is used in the rhBMRA. A group of 21 OVX rhesus monkeys was sequentially trained over a nine-week period to chair restraint, chair restraint plus nasogastric (NG) tubing, and NG tubing plus 5 mL saline dosing. These monkeys, about three years post-OVX, aged 13.5 years, and weighing 7.4 ± 1.1 kg were used and randomized to groups ($N = 11$ vehicle; $N = 10$ compound (*R*)-**2**) by baseline uNTx/Cre and body weight. Compound (*R*)-**2** monkeys were treated po, qd for 2 days with 1% carboxymethylcellulose (vehicle) and then treated with compound (*R*)-**2** po, qd for 8 days at 20 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, the second, fifth, and eighth days of drug dosing, and the third day after the end of drug dosing. One additional urine collection was made 17 days after the last drug dose. The urine samples were frozen at −70 °C within 4 h of collection and on the day of assay, samples were thawed and measured in duplicate using the N-telopeptides (NTx) kit (Ostex International; Seattle, WA) using manufacturers' instructions. Creatinine in each urine specimen was also measured (Catalog 555-A, Sigma; St. Louis, MO). The results are expressed as uNTx/Cre (nM/mM).

As shown in Figure 5, the cathepsin K inhibitor (*R*)-**2** dosed at 20 mg/kg po, qd decreased uNTx/Cre by 75−87% relative to vehicle-treatment ($p < 0.001$). The suppression was detected after the second dose, the first sampling time. During the eight-day dosing period, the mean reduction in uNTx/Cre by compound (*R*)-**2** was 80% ($p < 0.001$). At 3 days after the last dose of compound (*R*)-**2**, uNTx/Cre remained suppressed by 58% ($p < 0.004$). However, by 17 days after the last dose, uNTx/Cre in compound (*R*)-**2**-treated monkeys had

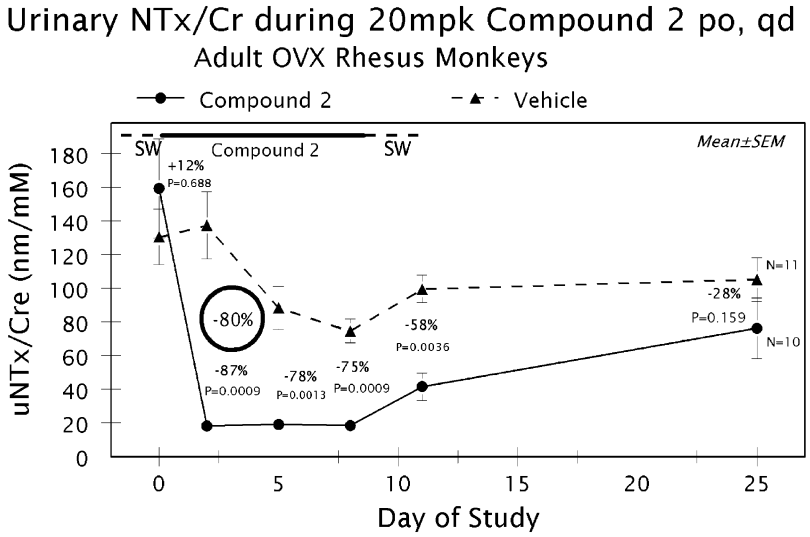


Figure 5. Compound (*R*)-**2** reduces bone resorption in OVX rhesus monkeys. Bone resorption in OVX monkeys treated with either the HCl salt of compound (*R*)-**2** (●) or vehicle (▲) was evaluated using the urine biochemical marker NTx. Data are expressed as uNTx/creatinine (nM/mM).

returned to control group levels. As indicated in the pharmacokinetic studies in intact rhesus monkeys (Figure 4), plasma levels of compound (*R*)-**2** remained at or above the bone resorption IC_{50} for a least 24 h following a 20 mg/kg oral dose. The significant suppression of uNTx/Cre levels demonstrates that inhibition of cathepsin K by compound (*R*)-**2** leads to ~80% inhibition of bone resorption markers in OVX rhesus monkeys, similar to what is observed in osteoporotic humans treated with bisphosphonates.[26] This finding 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.

Conclusion

In the present report we have shown that cathepsin K can be inhibited by nonpeptidic nitrile compounds. These biaryl compounds represent a new class of cysteine protease inhibitors. The SAR within this series indicated that the *R* configuration of an isobutyl P2 group was optimal for cathepsin K potency as was the *p*-piperazine biphenyl moiety in P3. These substitutions resulted in the optimal nitrile compound (*R*)-**2** which was selective vs cathepsins L, S, and B and sub-100 nM in an in vitro model of bone resorption. This series inhibited cathepsin K in a non-time-dependent fashion, and the potential nitrilase activity of cathepsin K (nitrile to amide conversion) was shown not to occur with compound (*R*)-**2**. Moreover, this inhibitor proved to have good pharmacokinetic properties in rhesus monkeys and showed excellent in vivo efficacy in the rhesus monkey model for inhibition of bone resorption.

Experimental Section

General. All substrates and reagents were obtained commercially and used without further purification. Proton (^{1}H NMR) magnetic resonance spectra were recorded on a Bruker 400 MHz instrument unless noted otherwise. All spectra were recorded using residual solvent ($CHCl_3$, acetone, or DMSO) as internal standard. Signal multiplicity was designated according to the following abbreviations: s = singlet, d = doublet, app d = apparent doublet, dd = doublet of doublets, t = triplet, q = quartet, dt = doublet of triplets, dq = doublet of quartets, m = multiplet, br s = broad singlet, br t = broad doublet, br t = broad triplet. Elemental analyses were provided by Oneida Research Services Inc., Whitesboro, NY. High-resolution mass spectra (HRMS−FAB$^+$) were obtained at the Biomedical Mass Spectrometry Unit, McGill University, Montreal, Quebec, Canada. HPLC chromatograms were obtained using a Waters Delta Prep 3000 HPLC with a Chiralpak AD column (5 cm, I.D. × 50 cm length, 20 μm). Rotations were measured on a Perkin-Elmer polarimeter. Reactions were carried out with continuous stirring under a positive pressure of nitrogen except where noted. Flash chromatography was carried out with silica gel 60, 230−400 mesh.

***N*^{2}-Benzoyl-*N*^{1}-(cyanomethyl)-L-leucinamide (1).**[18] HRMS (+FAB): calcd for $C_{15}H_{20}N_3O_2$ [MH$^+$] 274.15555, found 274.15553.

2-(1,1′-Biphenyl-3-yl)-*N*-(cyanomethyl)-4-methylpentanamide (4). A solution of LDA (2.2 mL, 2.0 M in THF) in THF (20 mL) was cooled to 0 °C and then treated with 1,1′-biphenyl-3-ylacetic acid (0.212 g, 1.0 mmol). The mixture was stirred for 40 min, cooled to −78 °C, and then treated with 3-bromo-2-methylpropene (135 μL, 1.3 mmol). The mixture was stirred for 1 h, treated with 1.0 M hydrochloric acid (5 mL), and then diluted with ethyl acetate (50 mL). The organic layer was separated, washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography over silica gel (33% ethyl acetate/hexanes) to provide 2-(1,1′-biphenyl-3-yl)-4-methylpentenoic acid (250 mg, 0.93 mmol). LCMS: 267.1 (M + H$^+$). A solution of 2-(1,1′-biphenyl-3-yl)-4-methylpentenoic acid (250 mg, 0.93 mmol) and Pd/C (10 mol %) in EtOH was subjected to hydrogenation under pressure (40 psi) for 8 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo to yield 2-(1,1′-biphenyl-3-yl)-4-methylpentanoic acid. A solution comprised of 2-(1,1′-biphenyl-3-yl)-4-methylpentanoic acid (251 mg, 0.91 mmol) in DMF (5 mL) was treated with aminoacetonitrile (180 mg, 2.0 mmol), PyBOP (520 mg 1.0 mmol) and triethylamine (500 μL, 3.0 mmol). The mixture was stirred for 4 h and then diluted with water (50 mL) and ethyl acetate (20 mL). The organic layer was separated, washed with an aq 1.0 M Na_2CO_3 solution, aq 1.0 M hydrochloric acid solution, water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from 30% ethyl acetate/hexanes to provide the title compound **4** (300 mg, 88% yield). LCMS: 307.3 (M + H$^+$). HRMS (+FAB): calcd for $C_{20}H_{23}N_2O$ [MH$^+$] 307.18104, found 307.18108. Anal. ($C_{20}H_{22}N_2O$) C: calcd, 78.40; found, 78.14; H: calcd, 7.24; found, 7.24; N: calcd, 9.14; found, 8.99.

2-(1,1′-Biphenyl-4-yl)-*N*-(cyanomethyl)-4-methylpentanamide (29). The title compound was synthesized according to the same procedure used for compound **4** except 1,1′-biphenyl-4-ylacetic acid was used instead of 1,1′-biphenyl-3-ylacetic acid. LCMS: 307.1 (M + H$^+$). HRMS (+FAB): calcd for $C_{20}H_{23}N_2O$ [MH$^+$] 307.18104, found 307.18108.

***N*-(Cyanomethyl)-4-methyl-2-(2-phenyl-1,3-thiazol-4-yl)pentanamide (19).** To a cold (0 °C), stirred solution of (2-phenyl-1,3-thiazol-4-yl)acetic acid[20] (1.2 g, 5 mmol) in THF was slowly added a solution of LDA (2.0 M in THF, 5.2 mL, 10.4 mmol). The reaction mixture was stirred at 0 °C for 1 h, 3-bromo-2-methylpropene (1.3 g, 10.0 mmol) was added, and the 0 °C solution was stirred for an additional 1 h. HCl (1.0 N) was added, and the mixture was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. Upon concentration in vacuo, the residue was purified by flash silica gel column chromatography (1:1 EtOAc/hexanes) to afford 0.6 g (42% yield) of 4-methyl-2-(2-phenyl-1,3-thiazol-4-yl)pent-4-enoic acid. A solution of 4-methyl-2-(2-phenyl-1,3-thiazol-4-yl)pent-4-enoic acid and 10% Pd/C (5 mol %) in EtOH was subjected to hydrogenation under pressure (40 psi) for 8 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo to yield 4-methyl-2-(2-phenyl-1,3-thiazol-4-yl)pentanoic acid. To a solution of 4-methyl-2-(2-phenyl-1,3-thiazol-4-yl)pentanoic acid (300 mg, 1.1 mmol) in DMF (10 mL) were added aminoacetonitrile HCl (184 mg, 2.0 mmol), PyBOP (520 mg, 1.0 mmol), and TEA (0.5 mL, 3.0 mmol). The mixture was stirred at room-temperature overnight. The DMF was removed in vacuo, and to the residue was added EtOAc (20 mL) and a saturated aqueous solution of $NaHCO_3$ (20 mL). The organic layer was washed with brine and dried over Na_2SO_4. Upon concentration in vacuo, the residue was purified by flash column chromatography (1/1, EtOAc/hexanes) to afford 250 mg (72% yield) of the title compound (**19**). HRMS (+FAB): calcd for $C_{17}H_{20}N_3OS$ [MH$^+$] 314.13271, found 314.13255. Anal. ($C_{17}H_{19}N_3OS$) C: calcd, 65.15; found, 65.07; H: calcd, 6.11; found, 5.96; N: calcd, 13.41; found, 13.34.

***N*-(Cyanomethyl)-4-methyl-2-(5-phenylpyridin-3-yl)-pentanamide Trifluoroacetate (15).** To a solution of bromobenzene (3.2 g, 20.0 mmol) in DMF (20 mL), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi-1,3,2-dioxaborolane (5.0 g, 20.0 mmol), and KOAc (3.5 g, 40.0 mmol) were added. The solution was degassed with nitrogen for 5 min, $PdCl_2$(dppf) (0.5 g, 0.68 mmol) was added, and the mixture was heated at 80 °C for 2 h. After the mixture was cooled to room temperature, methyl (5-bromopyridin-3-yl)acetate (**16**) (4.68 g, 20.0 mmol) and Na_2CO_3 (10 mL) were added. The reaction mixture was degassed for 5 min, $PdCl_2$(dppf) (0.5 g, 0.68 mmol) was added, and the mixture was heated in a sealed vessel overnight at 85 °C. The reaction mixture was filtered through a plug of Celite, and the DMF was evaporated in vacuo. To the residue was added a mixture of EtOAc/aq $NaHCO_3$ (150 mL/150 mL), and the

organic phase was separated and washed with brine. After drying over Na_2SO_4 and concentration in vacuo, the residue was purified by silica gel, using EtOAc as eluent, to yield 3.0 g (66% yield) of methyl (5-phenylpyridin-3-yl)acetate (**17**). Methyl (5-phenylpyridin-3-yl)acetate (**17**) was converted to methyl 4-methyl-2-(5-phenylpyridin-3-yl)pent-4-enoate using the same procedure used to generate 2-(1,1′-biphenyl-3-yl)-4-methylpentanoic acid (intermediate in the synthesis of **38**). A solution of methyl 4-methyl-2-(5-phenylpyridin-3-yl)pent-4-enoate and 10% Pd/C (10 mol %) in EtOH was subjected to hydrogenation under pressure (40 psi) for 8 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo to yield methyl 4-methyl-2-(5-phenylpyridin-3-yl)pentanoate (**18**). Methyl 4-methyl-2-(5-phenylpyridin-3-yl)pentanoate (**18**) was saponified following general procedure **4** to yield 4-methyl-2-(5-phenylpyridin-3-yl)pentanoic acid. 4-Methyl-2-(5-phenylpyridin-3-yl)pentanoic acid (300 mg, 1.0 mmol) was converted to the title compound **15** using PyBOP (520 mg, 1.0 mol), aminoacetonitrile hydrochloride (184 mg, 2 mmol), and TEA (0.3 mL, 2.0 mmol) in DMF (5 mL). The usual workup procedure afforded 250 mg (82% yield) of *N*-(cyanomethyl)-4-methyl-2-(5-phenylpyridin-3-yl)-pentanamide trifluoroacetate (**15**). HRMS (+FAB): calcd for $C_{19}H_{22}N_3O$ [MH^+] 308.17629, found 308.17639. Anal. ($C_{21}H_{22}F_3N_3O_3$) C: calcd, 59.85; found, 57.69; H: calcd, 5.26; found, 5.38; N: calcd, 13.52; found, 9.75.

2-(3-Bromophenyl)-4-methylpentanoic Acid ((*R*,*S*)-7). To 3-bromophenyl acetic acid **6** (60.0 g, 279 mmol) dissolved in dry THF (400 mL) at 0 °C under a dry nitrogen atmosphere was added LiHMDS dropwise (1.06 M solution in THF, 552 mL, 586 mmol), and the slurry was stirred with a mechanical stirrer for 2 h until it became a solution. 1-Iodo-2-methylpropane (33.7 mL, 293 mmol) was added dropwise and the reaction mixture stirred 2 days. The reaction was quenched with ice followed by addition of citric acid (30 g), the product was extracted with EtOAc (2×), and the organic phase washed with brine and dried over Na_2SO_4. The solvent was removed in vacuo and the product purified by trituration in hexanes (250 mL) to afford 2-(3-bromophenyl)-4-methylpentanoic acid ((*R*,*S*)-**7**) (41.3 g, 55%). Anal. ($C_{12}H_{15}BrO_2$) C: calcd, 53.32; found, 53.24; H: calcd, 5.59, found: 5.61.

(2*R*)-2-(3-Bromophenyl)-4-methylpentanoic Acid ((*R*)-7). To a solution of 2-(3-bromophenyl)-4-methylpentanoic acid ((*R*,*S*)-**7**) (60.0 g, 221.4 mmol) in toluene (1.2 L) was added DMF (350 μL, 4.43 mmol), followed by oxalyl chloride (23.2 mL, 265.7 mmol) gradually at room temperature, and the reaction mixture was stirred for 2.25 h. The solution was cooled to 0 °C, dimethylethylamine (60 mL, 553.5 mmol) was added, and the reaction mixture stirred for 2.5 h. The reaction mixture was cooled to −78 °C, and a solution of (*R*)−(−)-pantolactone (34.6 g, 265.7 mmol) in toluene (1.0 L) was added gradually, followed by warming to 0 °C and stirring for another 2.5 h. Water was added and saturated with NaCl, and the organic layer was separated, washed with aq 10% HCl, water, aq 50% sat. solution of $NaCO_3$, and brine, and dried over $MgSO_4$. The solvent was removed in vacuo to afford crude (3*R*)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl (2*R*)-2-(3-bromophenyl)-4-methylpentanoate (**9**) (80.0 g, 10:1 diastereoisomeric ratio) that was used as such in the next step. An analytically pure sample of **9** was prepared by flash chromatography over silica gel (EtOAc/hexanes, 2/8) for analysis. $[\alpha]_D = +7.9°$ (c = 1.48, MeOH). HRMS (+FAB): calcd for $C_{18}H_{23}BrO_4$ [MH^+] 383.07797, found 383.08566. The (3*R*)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl (2*R*)-2-(3-bromophenyl)-4-methylpentanoate (**9**) (80.0 g, 221.4 mmol) was dissolved in acetic acid (640 mL), a solution of HCl (aq, 2.0 M) was added, and the reaction mixture was heated to 85 °C for 48 h. Approximately 50% of the solvent was distilled off, and water was added along with seeding from a previous reaction. Filtration afforded the desired (2*R*)-2-(3-bromophenyl)-4-methylpentanoic acid ((*R*)-**7**) (47.0 g, 78% yield) which retained the initial 10:1 diastereoisomeric ratio of the starting ester **9**. Anal. ($C_{20}H_{26}BrNO_2$) C: calcd, 61.23; found, 61.48; H: calcd, 6.68; found, 6.42; N: calcd, 3.57; found, 3.85.

(2*R*)-*N*-(Cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*)-5). To the (2*R*)-2-(3-bromophenyl)-4-methylpentanoic acid ((*R*)-**7**) (52.5 g, 193.7 mmol), PyBOP (105.83 g, 203.4 mmol), and aminoacetonitrile hydrochloride (35.84 g, 287.5 mmol) was added dry DMF (1.2 L) under an atmosphere of dry nitrogen followed by Et_3N (84.0 mL, 600.5 mmol), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was poured into aq sat. $NaHCO_3$, extracted with Et_2O, and the organic layer washed with aq sat. $NaHCO_3$ (3×), brine, and dried over $MgSO_4$. The solvent was concentrated in vacuo to afford a crude oil which was purified by flash chromatography over silica gel (EtOAc/hexanes, 2/8, then 3/7) to yield the desired (2*R*)-2-(3-bromophenyl)-*N*-(cyanomethyl)-4-methylpentanamide (**10**) as a pale yellow oil (59.9 g, quantitative yield). $[\alpha]_D = -47.7°$ (c = 1.49, MeOH). HRMS (+FAB): calcd for $C_{14}H_{17}BrN_2O$ [MH^+] 309.0524, found 309.06034.

To (2*R*)-2-(3-bromophenyl)-*N*-(cyanomethyl)-4-methylpentanamide (**10**) (21.7 g, 70.23 mmol), diboron pinacol ester (21.4 g, 84.27 mmol), and KOAc (20.68 g, 210.69 mmol) was added dry DMF (400 mL) under an atmosphere of dry nitrogen, followed by $PdCl_2$(dppf) (1.723 g, 2.11 mmol), and the reaction was heated at 85 °C for 1 h. More $PdCl_2$(dppf) (0.03 equiv) was added and the reaction mixture heated at 90 °C for another 2 h. The reaction mixture was poured into water and extracted with C_6H_6/Et_2O, and the organic phase was washed with water, brine, and dried over Na_2SO_4. The solvent was concentrated in vacuo to afford a crude oil which was purified by flash chromatography over silica gel (EtOAc/hexanes, 3/7, then 35/65) to yield the desired (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*)-**5**) (20.0 g, 80% yield, 9:1 ratio of enantiomers). Chiral HPLC: AD column; eluents 2-propanol/hexanes, 5/95; 1.0 mL/min. UV detector 220 nm. t_R: (*R*)-**5** 10.12 min, (*S*)-**5** 8.11 min.

General Procedure 1 (Suzuki Cross-Coupling). To (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5** or (*R*)-**5**) (1 equiv), aryl or heteroaryl bromide/iodide/triflate (1.1−1.5 equiv), and $PdCl_2$(dppf) (0.03 equiv) was added DMF or 2-propanol (5−10 mL/mmol) under a dry nitrogen atmosphere followed by a degassed aqueous 2.0 M solution of Na_2CO_3 (2.5−3.0 equiv). The reaction mixture was stirred at 80−85 °C for 0.5−1 h, more $PdCl_2$(dppf) (0.03 equiv) was added, and the reaction was heated at 80−95 °C until complete consumption of (*R*)-**5** as detected by TLC analysis (2−18 h). Upon completion of the reaction, water was added, the product was extracted with Et_2O (3×), and the combined organic phases washed with brine and dried over Na_2SO_4 or $MgSO_4$. The solvent was removed in vacuo, and the product was purified by flash chromatography over silica gel (EtOAc/hexanes, or CH_2Cl_2/MeOH/aq sat. NH_4OH).

3′-(1-{[(Cyanomethyl)amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-4-carboxamide (30). Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 4-bromobenzamide to afford title compound 3′-(1-{[(cyanomethyl)-amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-4-carboxamide (**30**) (145 mg, 59% yield, purified by flash chromatography, EtOAc/hexanes, 8/2). HRMS (+FAB): calcd for $C_{21}H_{24}N_3O_2$ [MH^+] 350.18685, found 350.18695. Anal. ($C_{21}H_{23}N_3O_2$) C: calcd, 72.18; found, 70.83; H: calcd, 6.63; found, 6.60; N: calcd, 12.03; found, 11.68.

***N*-(Cyanomethyl)-4-methyl-2-[4′-(methylsulfonyl)-1,1′-biphenyl-3-yl]pentanamide (31).** Following general procedure 1, (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 4-bromophenyl methyl sulfone to afford title compound *N*-(cyanomethyl)-4-methyl-2-[4′-(methylsulfonyl)-1,1′-biphenyl-3-yl]pentanamide (**31**) (265 mg, 98% yield, purified by flash chromatography, EtOAc/hexanes, 1/1). HRMS (+FAB): calcd for $C_{21}H_{25}N_2O_3S$ [MH^+] 385.15859, found 385.15870. Anal. ($C_{21}H_{24}N_2O_3S$)

C: calcd, 65.60; found, 64.53; H: calcd, 6.29; found, 6.84; N: calcd, 7.29; found, 6.65.

(2*R*)-*N*-(Cyanomethyl)-4-methyl-2-[4′-(1*H*-tetraazol-5-yl)-1,1′-biphenyl-3-yl]pentanamide (32). Following general procedure 1, (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*)-**5**) was submitted to a Suzuki cross-coupling reaction with 5-(4-bromophenyl)-1*H*-tetrazole to afford title compound (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(1*H*-tetraazol-5-yl)-1,1′-biphenyl-3-yl]pentanamide (**32**) (130 mg, 26% yield, purified by flash chromatography, CH_2Cl_2/MeOH/aq sat. NH_4OH, 80/18/2). HRMS (+FAB): calcd for $C_{21}H_{23}N_6O$ [MH^+] 375.19333, found 375.19333. Anal. ($C_{21}H_{22}N_6O$) C: calcd, 67.36; found, 64.81; H: calcd, 5.92; found, 6.59; N: calcd, 22.44; found, 17.79.

***N*-(Cyanomethyl)-4-methyl-2-[4′-(4-morpholinyl)[1,1′-biphenyl]-3-yl]pentanamide (33).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 4-(4-bromophenyl)morpholine (prepared by the bromination of commercially available *N*-phenylmorpholine[27] to afford the title compound *N*-(cyanomethyl)-4-methyl-2-[4′-(4-morpholinyl)-[1,1′-biphenyl]-3-yl]pentanamide (**33**) (172 mg, 63%, purified by flash chromatography, EtOAc/hexanes, 4/6). MS (-ESI) *m*/*z* 390.3 $(M - H)^-$. HRMS (+FAB): calcd for $C_{24}H_{29}KN_3O_2$ [$M + K^+$] 430.18968, found 430.18959. Anal. ($C_{24}H_{29}N_3O_2$) C: calcd, 73.63; found, 72.04; H: calcd, 7.47; found, 7.55; N: calcd, 10.73; found, 9.52.

***N*-(Cyanomethyl)-4-methyl-2-[3′-(4-morpholinyl)[1,1′-biphenyl]-3-yl]pentanamide (34).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 3-morpholin-4-ylphenyl trifluoromethanesulfonate[28] to afford the title compound *N*-(cyanomethyl)-4-methyl-2-[3′-(4-morpholinyl)-[1,1′-biphenyl]-3-yl]pentanamide (**34**) (155 mg, 51% yield, purified by flash chromatography, EtOAc/hexanes, 4/6). MS (-ESI) *m*/*z* 390.4 $(M - H)^-$. HRMS (+FAB): calcd for $C_{24}H_{29}KN_3O_2$ [$M + K^+$] 430.18968, found 430.18980. Anal. ($C_{24}H_{29}N_3O_2$) C: calcd, 73.63; found, 70.17; H: calcd, 7.47; found, 7.80; N: calcd, 10.73; found, 9.82.

***N*-(Cyanomethyl)-4-methyl-2-[2′-(4-morpholinyl)[1,1′-biphenyl]-3-yl]pentanamide (35).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 2-morpholin-4-ylphenyl trifluoromethanesulfonate[29] to afford the title compound *N*-(cyanomethyl)-4-methyl-2-[2′-(4-morpholinyl)-[1,1′-biphenyl]-3-yl]pentanamide (**35**) (180 mg, 59%, purified by flash chromatography, EtOAc/hexanes, 4/6). MS (-ESI) *m*/*z* 390.4 $(M - H)^-$. HRMS (+FAB): calcd for $C_{24}H_{30}N_3O_2$ [MH^+] 392.23380, found 392.23362. Anal. ($C_{24}H_{29}N_3O_2$) C: calcd, 73.63; found, 72.44; H: calcd, 7.47; found, 7.36; N: calcd, 10.73; found, 10.04.

***N*-(Cyanomethyl)-4-methyl-2-(3-pyrimidin-5-ylphenyl-)pentanamide (36).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 5-bromopyrimidine to afford the title compound *N*-(cyanomethyl)-4-methyl-2-(3-pyrimidin-5-ylphenyl)pentanamide (**36**) (185 mg, 86% yield, purified by flash chromatography, EtOAc/hexanes, 6/4, then 7/3). HRMS (+FAB): calcd for $C_{18}H_{21}N_4O$ [MH^+] 309.17154, found 309.17150. Anal. ($C_{18}H_{20}N_4O$) C: calcd, 70.11; found, 69.95; H: calcd, 6.54; found, 6.66; N: calcd, 18.17; found, 18.32.

***N*-(Cyanomethyl)-4-methyl-2-(3-pyridin-2-ylphenyl)-pentanamide (37).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 2-bromopyridine to afford the title compound *N*-(cyanomethyl)-4-methyl-2-(3-pyridin-2-ylphenyl)-pentanamide (**37**) (92 mg, 55% yield, purified by flash chromatography, EtOAc/hexanes, 1/9). HRMS (+FAB): calcd for $C_{19}H_{22}N_3O$ [MH^+] 308.17629, found 308.17639. Anal. ($C_{19}H_{21}N_3O$) C: calcd, 74.24; found, 72.68; H: calcd, 6.89: found, 7.39; N: calcd, 13.67; found, 13.72.

***N*-(Cyanomethyl)-4-methyl-2-(3-pyridin-3-ylphenyl)-pentanamide (38).** To a cold (0 °C), stirred solution of (3-pyridin-3-ylphenyl)acetic acid (1.2 g, 5.6 mmol.) in THF (50 mL) was slowly added a solution of LDA (1.6 M in THF, 8.0 mL, 13.0 mmol). The reaction mixture was stirred at 0 °C for 1 h. After cooling to −78 °C, 3-bromo-2-methylpropene (1.3 g, 10.0 mmol) was added, and the solution was warmed to room temperature and stirred for an additional 1 h. The reaction mixture was evaporated, dissolved in water (10 mL), and acidified with 6.0 N HCl to pH 5. The crude product was purified by preparative HPLC to give the desired compound 4-methyl-2-(3-pyridin-3-ylphenyl)pent-4-enoic acid (1.3 g, 60% yield) as a white solid. This so-obtained compound was dissolved in EtOH (20 mL), Pd/C (5 mol %) was added, and the reaction mixture was subjected to hydrogenation under pressure (40 psi) for 8 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo to yield the desired 4-methyl-2-(3-pyridin-3-ylphenyl)pentanoic acid in quantitative yield (1.0 g). To a solution of this 4-methyl-2-(3-pyridin-3-ylphenyl)pentanoic acid acid (1.0 g, 2.3 mmol) in DMF (10 mL) at room temperature were added aminoacetonitrile hydrochloride (211 mg, 2.3 mmol), PyBOP (1.2 g, 2.3 mmol), and TEA (0.5 mL, 4.0 mmol). The reaction was left stirring overnight. The DMF was evaporated in vacuo and the residue was treated with a mixture of EtOAc/aq $NaHCO_3$ (20 mL/20 mL). The organic phase was washed with brine and dried over Na_2SO_4. The crude product was purified on preparative HPLC to afford the desired product *N*-(cyanomethyl)-4-methyl-2-(3-pyridin-3-ylphenyl)pentanamide (**38**) (600 mg, 61%) as a white solid. HRMS (+FAB): calcd for $C_{19}H_{22}N_3O$ [MH^+] 308.17629, found 308.17639. Anal. ($C_{19}H_{21}N_3O$) C: calcd, 74.24; found, 51.57; H: calcd, 6.89; found, 4.78; N: calcd, 13.67; found, 8.83.

***N*-(Cyanomethyl)-4-methyl-2-(3-pyridin-4-ylphenyl)-pentanamide trifluoroacetate (39).** A solution of *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) (71 mg, 0.2 mmol), 4-bromopyridine (47 mg, 0.3 mmol), $PdCl_2$(dppf) (13 mg, 0.018 mmol), and Na_2CO_3 (2.0 M, aqueous, 0.5 mL, 1.0 mmol) in dry DMF (4 mL) was heated to 80−85 °C for 18 h. Water was added and the product extracted with Et_2O (2×) and dried over Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified by flash chromatography over silica gel (EtOAc/hexanes, 1/1) to afford the desired compound (**39**). HRMS (+FAB): calcd for $C_{19}H_{22}N_3O$ [MH^+] 308.17629, found 308.17639.

(2*R*)-*N*-(Cyanomethyl)-4-methyl-2-[4′-(4-pyridinyl)[1,1′-biphenyl]-3-yl]pentanamide (40). To 4-bromopyridine hydrochloride (6.61 g, 33.96 mmol), 4-methoxyphenylboronic acid (5.16 g, 33.96 mmol), and $PdCl_2$(dppf) (835 mg, 1.02 mmol) was added DMF (200 mL) under a dry nitrogen atmosphere followed by an aq solution of Na_2CO_3 (2.0 M, 67.9 mL). The reaction mixture was stirred at 85 °C for 1.25 h, more $PdCl_2$(dppf) (835 mg, 1.02 mmol) was added, and the reaction was heated another 1 h. Water was added, the product extracted with Et_2O, and the organic phase dried over Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified by flash chromatography over silica gel (EtOAc/hexanes, 55/45) to afford the desired 4-(4-methoxyphenyl)-pyridine (5.77 g, 92% yield). To 4-(4-methoxyphenyl)pyridine (2.15 g, 11.62 mmol) in dry DMF (15 mL) was added NaSMe (3.26 g, 46.48 mmol) at room temperature, and the reaction mixture was heated at 130 °C for 5 h. After being cooled to room temperature, the reaction was carefully poured into water and the product extracted with Et_2O, and the organic phase was dried over $MgSO_4$. The solvent was removed in vacuo, and the product was purified by flash chromatography over silica gel (EtOAc/hexanes, 8/2 then 100% EtOAc) to afford the desired product 4-pyridin-4-ylphenol (875 mg, 44% yield). A portion of the so obtained 4-pyridin-4-ylphenol (275 mg, 1.61 mmol) was converted to the triflate ether 4-pyridin-4-ylphenyl trifluoromethanesulfonate (302 mg, 62% yield).[29] Following

general procedure 1, (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*)-**5**) was submitted to a Suzuki cross-coupling reaction with 4-pyridin-4-ylphenyl trifluoromethanesulfonate to afford title compound (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(4-pyridinyl)[1,1′-biphenyl]-3-yl]pentanamide (**40**) (359 mg, 94% yield, purified by flash chromatography, 100% EtOAc). MS (+ESI) *m*/*z* 384.0 $(M + H)^+$. HRMS (+FAB): calcd for $C_{25}H_{26}N_3O$ $[MH^+]$ 384.20759, found 384.20765. Anal. ($C_{25}H_{25}N_3O$) C: calcd, 78.30; found, 77.94; H: calcd, 6.57; found, 6.95; N: calcd, 10.96; found, 11.00.

***N*-(Cyanomethyl)-2-[3-(1*H*-indol-6-yl)phenyl]-4-methylpentanamide (41).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 6-bromoindole to afford title compound *N*-(cyanomethyl)-2-[3-(1*H*-indol-6-yl)phenyl]-4-methylpentanamide (**41**) (190 mg, 79% yield, purified by flash chromatography, EtOAc/hexanes, 3/7). HRMS (+FAB): calcd for $C_{22}H_{23}KN_3O$ $[M + K^+]$ 384.14782, found 384.14799. Anal. ($C_{22}H_{23}N_3O$) C: calcd, 76.49; found, 74.93; H: calcd, 6.71; found, 7.51; N: calcd, 12.16; found, 11.17.

***N*-(Cyanomethyl)-2-[3-(1*H*-indol-5-yl)phenyl]-4-methylpentanamide ((*R*,*S*)-42).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 5-bromoindole to afford title compound *N*-(cyanomethyl)-2-[3-(1*H*-indol-5-yl)phenyl]-4-methylpentanamide ((*R*,*S*)-**42**) (50 mg, 21% yield, purified by flash chromatography, EtOAc/hexanes, 3/7). MS (-ESI) *m*/*z* 344.3 $(M - H)^-$. HRMS (+FAB): calcd for $C_{22}H_{24}N_3O$ $[MH^+]$ 346.19194, found 346.19204. Anal. ($C_{22}H_{23}N_3O$) C: calcd, 76.49; found, 72.62; H: calcd, 6.71; found, 7.62; N: calcd, 12.16; found, 10.39.

(2*R*)-*N*-(Cyanomethyl)-2-[3-(1*H*-indol-5-yl)phenyl]-4-methylpentanamide ((*R*)-42) and (2*S*)-*N*-(Cyanomethyl)-2-[3-(1*H*-indol-5-yl)phenyl]-4-methylpentanamide ((*S*)-42). Racemic *N*-(cyanomethyl)-2-[3-(1*H*-indol-5-yl)phenyl]-4-methylpentanamide ((*R*,*S*)-**42**) was separated by chiral HPLC (Chiralpak AD column, mobile phase EtOH/hexanes, 2/8) to afford at 8.44 min (2*R*)-*N*-(cyanomethyl)-2-[3-(1*H*-indol-5-yl)-phenyl]-4-methylpentanamide ((*R*)-**42**) ($[\alpha]^{23}_D$ −54.6 [c = 1.20, $CHCl_3$]) and at 9.7 min (2*S*)-*N*-(cyanomethyl)-2-[3-(1*H*-indol-**5**-yl)phenyl]-4-methylpentanamide ((*S*)-**42**) ($[\alpha]^{23}_D$ +41.0 [c = 1.00, $CHCl_3$]).

***N*-(Cyanomethyl)-4-methyl-2-(3′-piperazin-1-yl-1,1′-biphenyl-3-yl)pentanamide methanesulfonate (43).** Boc-piperazine (7.89 g, 42.39 mmol), 1,3-dibromobenzene (13.0 g, 57.22 mmol), dicyclohexyl[3-(2-methyl-1,3-dioxolan-2-yl)phenyl]phosphine (1.56 g, 2.54 mmol), $Pd_2(dba)_3$ (0.8 g, 0.85 mmol), and *t*-BuOK (5.71 g, 50.87 mmol) in 25 mL toluene were heated to 105 °C overnight. The reaction was cooled to room temperature, partitioned between ether and water, and extracted with ether (2 × 75 mL). The combined ether layers were washed with water (2 × 75 mL) and dried over $MgSO_4$. The organic extracts were concentrated in vacuo, and the residue obtained was purified by flash column chromatography (5−15% EtOAc/hexanes) to yield 2.60 g of *tert*-butyl 4-(3-bromophenyl)-piperazine-1-carboxylate as a yellow oil. *tert*-Butyl 4-(3-bromophenyl)piperazine-1-carboxylate (1.0 g, 2.81 mmol), *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) (400 mg, 1.12 mmol), $PdCl_2$(dppf) (28 mg, 0.034 mmol), and Na_2CO_3 (2.0 M aqueous, 2.81 mL, 5.61 mmol) in 10 mL of DMF were heated to 80 °C overnight. The reaction was cooled to room temperature and partitioned between ethyl acetate and water and extracted with EtOAc (3 × 75 mL). The combined ethyl acetate layers were dried over $MgSO_4$ and concentrated in vacuo. The residue obtained was purified by flash chromatography (21−37% EtOAc/hexanes) to yield 399 mg of *tert*-butyl 4-[3′-(1-{[(cyanomethyl)amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-3-yl]piperazine-1-carboxylate as a white foamy solid.

tert-Butyl 4-[3′-(1-{[(Cyanomethyl)amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-3-yl]piperazine-1-carboxylate (335 mg, 0.68 mmol) and methanesulfonic acid (0.18 mL, 2.73 mmol) in 5 mL of anhydrous THF under nitrogen were stirred at room temperature for 5 h. TLC analysis indicated complete consumption of starting material. The reaction was diluted with 50 mL ether, and the solution was then decanted off. This was repeated six times. The combined organics were concentrated in vacuo, and the residue was then dried in vacuo to yield 407.7 mg of the title compound **43** as a brown solid. LC/MS (es): 391.07 $(M + H)^+$.

***N*-(Cyanomethyl)-4-methyl-2-[2′-(1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide (44).** To *N*-(2-hydroxyphenyl)-piperazine (2.00 g, 11.22 mmol) and DMAP (2.06 g, 16.83 mmol) in MeCN (40 mL) at 0 °C was added di-*tert*-butyl dicarbonate (2.94 g, 13.17 mmol) dissolved in MeCN (20 mL), and the reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was poured into water, the product extracted with Et_2O and washed with brine, and the organic phase was dried over $MgSO_4$. The solvent was removed in vacuo, and the crude product was purified by flash chromatography over silica gel (EtOAc/hexanes, 1/9, then 15/85) to afford the desired *tert*-butyl 4-(2-hydroxyphenyl)piperazine-1-carboxylate (1.05 g, 34% yield).

A portion of the so obtained *tert*-butyl 4-(2-hydroxyphenyl)-piperazine-1-carboxylate (405 mg, 1.46 mmol) was converted to the triflate ether *tert*-butyl 4-(2-{[(trifluoromethyl)sulfonyl]-oxy}phenyl)piperazine-1-carboxylate (550 mg, 100% yield).[30] Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with *tert*-butyl 4-(2-{[(trifluoromethyl)sulfonyl]oxy}-phenyl)piperazine-1-carboxylate to afford the desired *tert*-butyl 4-[3′-(1-{[(cyanomethyl)amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-2-yl]piperazine-1-carboxylate (562 mg, 70%, purified by flash chromatography, EtOAc/hexanes, 3/7). Removal of the Boc protecting group of 4-[3′-(1-{[(cyanomethyl)amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-2-yl]piperazine-1-carboxylate (562 mg, 1.15 mmol) was accomplished following general procedure **2** to afford the title compound *N*-(cyanomethyl)-4-methyl-2-[2′-(1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide (**44**) (293 mg, 65%, purified by flash chromatography, CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1). MS (+ESI) *m*/*z* 391.1 $(M + H)^+$. HRMS (+FAB): calcd for $C_{24}H_{31}N_4O$ $[MH^+]$ 391.24196, found 391.24971. Anal. ($C_{24}H_{30}N_4O$) C: calcd, 73.81; found, 71.56; H: calcd, 7.74; found, 7.42; N: calcd, 14.35; found, 13.37.

***N*-(Cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*,*S*)-2).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 1-(4-bromophenyl)piperazine hydrochloride to afford title compound *N*-(cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*,*S*)-**2**) (155 mg, 57% yield, purified by flash chromatography, CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1). MS (+ESI) *m*/*z* 391.2 $(M + H)^+$. HRMS (+FAB): calcd for $C_{24}H_{31}N_4O$ $[MH^+]$ 391.24979, found 391.24971. Anal. ($C_{24}H_{30}$-N_4O) C: calcd, 73.81; found, 70.89; H: calcd, 7.74; found, 7.44; N: calcd, 14.35; found, 13.83.

(2*R*)-*N*-(Cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)-[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-2). Following general procedure 1, (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*)-**5**) was submitted to a Suzuki cross-coupling reaction with 1-(4-bromophenyl) piperazine hydrochloride to afford title compound (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)-[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-**2**) (773 mg, 55% yield, purified by flash chromatography, CH_2Cl_2/MeOH/aq sat. NH_4-OH, 90/9/1). MS (+ESI) *m*/*z* 391.2 $(M + H)^+$. HRMS (+FAB): calcd for $C_{24}H_{31}N_4O$ $[MH^+]$ 391.24196, found 391.24196. Anal. ($C_{24}H_{30}N_4O$) C: calcd, 73.81; found, 73.87; H: calcd, 7.74; found, 7.64; N: calcd, 14.35; found, 14.28.

General Procedure 2 (Boc Deprotection). To a solution of a Boc-protected amino derivative in dry THF (1−2 mL/mmol) under dry nitrogen atmosphere was added 2 equiv of $MeSO_3H$ dropwise, and the reaction mixture was stirred for

approximately 1 h. Another 1 equiv of $MeSO_3H$ was added, and the reaction mixtures were stirred overnight (in certain cases, another 1 or 2 equiv was required to drive the reaction to completion). The reaction mixture was carefully quenched using an aq sat. solution of $NaHCO_3$, the product was extracted with EtOAc (2×), and the organic phase was dried over Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1).

(2*R*)-*N*-(1-Cyanocyclopropyl)-4-methyl-2-(4′-piperazin-1-yl-1,1′-biphenyl-3-yl)pentanamide (45). Compound **45** was prepared according to the general PyBOP coupling procedure 4 except 1-amino-1-cyclopropane-carbonitrile hydrochloride was used instead of aminoacetonitrile hydrochloride and coupled with (2*R*)-2-{4′-[4-(*t*-butoxycarbonyl)piperazin-1-yl]-1,1′-biphenyl-3-yl}-4-methylpentanoic acid, followed by the general Boc deprotection procedure 2 to afford the desired (2*R*)-*N*-(1-cyanocyclopropyl)-4-methyl-2-(4′-piperazin-1-yl-1,1′-biphenyl-3-yl)pentanamide (**45**). HRMS (+FAB): calcd for $C_{24}H_{33}N_4O_2$ [MH^+] 417.26544, found 417.26554.

1-(4-Bromophenyl)-4-methylpiperazine (25). To a stirred suspension of 1-(4-bromophenyl)piperazine (10 g, 1 equiv) in DMF (120 mL) was added NaH (60% in oil, 4.3 g, 3 equiv) in three portions. After being stirred at room temperature for 1 h, the mixture was cooled to −78 °C and a solution of methyl iodide (2.24 mL, 1 equiv) in DMF (5 mL) was added dropwise over 15 min. The mixture was stirred at −78 °C for 15 min and then warmed to room temperature where stirring was continued for 1 h. The reaction mixture was poured into H_2O and extracted with EtOAc (4×). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 5% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford the title compound **25** (6.5 g, 71% yield).

***N*-(Cyanomethyl)-4-methyl-2-[4′-(4-methyl-1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*,*S*)-46).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 1-(4-bromophenyl)-4-methylpiperazine (**25**) to afford title compound *N*-(cyanomethyl)-4-methyl-2-[4′-(4-methyl-1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*,*S*)-**46**) (85 mg, 30% yield, purified by flash chromatography, CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1). MS (+ESI) *m*/*z* 405.1 $(M + H)^+$. HRMS (+FAB): calcd for $C_{25}H_{33}N_4O$ [MH^+] 405.26544, found 405.26535.

(2*R*)-*N*-(Cyanomethyl)-4-methyl-2-[4′-(4-methyl-1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-46). Following general procedure 1, (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*)-**5**) was submitted to a Suzuki cross-coupling reaction with 1-(4-bromophenyl)-4-methylpiperazine to afford title compound (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(4-methyl-1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-**46**) (733 mg, 48%, purified by flash chromatography, CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1). MS (+ESI) *m*/*z* 405.1 $(M + H)^+$. HRMS (+FAB): calcd for $C_{25}H_{32}KN_4O$ [$M + K^+$] 443.22132, found 443.22139. Anal. ($C_{25}H_{32}N_4O$) C: calcd, 74.22; found, 73.80; H: calcd, 7.97; found, 7.94; N: calcd, 13.85; found, 13.70.

***N*-(Cyanomethyl)-4-methyl-2-[4′-(1,2,3,6-tetrahydro-4-pyridinyl)[1,1′-biphenyl]-3-yl]pentanamide (47).** Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*,*S*)-**5**) was submitted to a Suzuki cross-coupling reaction with 4-(4-bromophenyl)-1,2,3,6-tetrahydropyridine to afford the title compound *N*-(cyanomethyl)-4-methyl-2-[4′-(1,2,3,6-tetrahydro-4-pyridinyl)[1,1′-biphenyl]-3-yl]pentanamide (**47**) (70 mg, 36% yield, purified by flash chromatography, CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1). MS (+ESI) *m*/*z* 388.2 $(M + H)^+$. HRMS (+FAB): calcd for $C_{25}H_{30}N_3O$ [MH^+] 388.23889, found 388.23891.

***N*-(Cyanomethyl)-4-methyl-2-[4′-(4-piperidinyl)[1,1′-biphenyl]-3-yl]pentanamide (48).** To a solution of 2-(3-bromophenyl)-4-methylpentanoic acid (*R*,*S*)-**7** (3.15 g, 12.91 mmol) in Et_2O (30 mL) at 0 °C was added diazomethane dropwise until a light yellowish color persisted. After stirring an additional 15 min, acetic acid was added to quench any remaining diazomethane, and the reaction mixture was concentrated in vacuo to afford a crude oil which was purified by flash chromatography over silica gel (EtOAc/hexanes, 1/9) to yield the desired methyl 2-(3-bromophenyl)-4-methylpentanoate (3.32 g, 90% yield). To methyl 2-(3-bromophenyl)-4-methylpentanoate (3.3 g, 11.58 mmol), diboron pinacol ester (3.35 g, 13.90 mmol), and KOAc (3.31 g, 34.74 mmol) in dry DMF (80 mL) under an atmosphere of dry nitrogen was added $PdCl_2$(dppf) (285 mg, 0.347 mmol), and the reaction was heated to 85 °C for 3 h. The reaction mixture was poured into water, extracted with Et_2O, and washed with brine, and the organic layer was dried over Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified by flash chromatography over silica gel (EtOAc/hexanes, 1/9) to afford the desired methyl 4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanoate (2.57 g, 67% yield). To 4-(4-bromophenyl)-4-piperidinol (1.00 g, 3.90 mmol) was added TFA (15 mL), and the reaction was stirred at room temperature for 6 h. The solvent was concentrated in vacuo, aq sat. $NaHCO_3$ was added, the product was extracted with EtOAc, and the organic layer was dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by flash chromatography over silica gel (CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1) to afford the desired product 4-(4-bromophenyl)-1,2,3,6-tetrahydropyridine (618 mg, 67% yield). To 4-(4-bromophenyl)-1,2,3,6-tetrahydropyridine (3.40 g, 14.28 mmol) and DMAP (3.48 g, 28.56 mmol) in MeCN (50 mL) at 0 °C under an atmosphere of dry nitrogen was added di-*tert*-butyl dicarbonate (4.68 g, 21.42 mmol), and the reaction mixture was stirred at that temperature for 1 h. The reaction was poured into aq sat. $NaHCO_3$ and extracted with Et_2O, and the organic layer was dried over Na_2SO_4. The solvent was removed in vacuo and the product purified by flash chromatography over silica gel (EtOAc/hexanes, 1/9) to afford the desired product *tert*-butyl 4-(4-bromophenyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (3.84 g, 80% yield). To *tert*-butyl 4-(4-bromophenyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (2.62 g, 7.74 mmol), methyl 4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanoate (2.57 g, 7.74 mmol), and $PdCl_2$(dppf) (190 mg, 0.232 mmol) was added dry DMF (80 mL) under a nitrogen atmosphere followed by a degassed aqueous 2.0 M solution of Na_2CO_3 (11.6 mL, 23.2 mmol). The reaction mixture was stirred at 85 °C for approximately 1 h, more $PdCl_2$(dppf) (190 mg, 0.232 mmol) was added, and the reaction was heated at 85 °C for another 3 h. Water was added to the reaction, product extracted with Et_2O (3×), and the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by flash chromatography over silica gel (EtOAc/hexanes, 2/8) to afford the desired product *tert*-butyl 4-{3′-[1-(methoxycarbonyl)-3-methylbutyl]-1,1′-biphenyl-4-yl}-3,6-dihydropyridine-1(2*H*)-carboxylate (2.28 g, 63% yield). To *tert*-butyl 4-{3′-[1-(meth[oxycarbonyl)-3-methylbutyl]-1,1′-biphenyl-4-yl}-3,6-dihydropyridine-1(2*H*)-carboxylate (2.135 g, 4.61 mmol) in EtOH/EtOAc (50 mL, 1:1) under an atmosphere of dry nitrogen was added 10% Pd/C (250 mg), the reaction vessel was degassed with hydrogen, and the reaction mixture stirred for 6.5 h. After the reaction vessel was thoroughly degassed with dry nitrogen, CH_2Cl_2 was added, and the reaction mixture was filtered on a pad of Celite. The solvent was removed in vacuo and the crude product purified by flash chromatography over silica gel (EtOAc/hexanes, 15/85) to afford the desired product *tert*-butyl 4-{3′-[1-(methoxycarbonyl)-3-methylbutyl]-1,1′-biphenyl-4-yl}-piperidine-1-carboxylate (2.07 g, 97% yield). To *tert*-butyl 4-{3′-[1-(methoxycarbonyl)-3-methylbutyl]-1,1′-biphenyl-4-yl}-piperidine-1-carboxylate (1.22 mg, 2.63 mmol) in THF/MeOH (40 mL, 1:1) was added an aqueous solution of NaOH (1.0 M, 2.9 mL, 2.9 mmol), and the reaction mixture was stirred for 6 h at room temperature. More NaOH (aq, 1.0 M, 2.9 mL, 2.9 mmol) was added, and the reaction mixture was stirred another 18 h. The mixture was concentrated down, and aq 10% HCl was added gradually until the solution reached pH = 4.

The solvent was removed in vacuo and the crude product purified by flash chromatography over silica gel (EtOAc/hexanes, 3/7 + 1% AcOH) to afford the desired product 2-{4′-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-1,1′-biphenyl-3-yl}-4-methylpentanoic acid (1.02 g, 86% yield). To 2-{4′-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-1,1′-biphenyl-3-yl}-4-methylpentanoic acid (1.02 g, 2.22 mmol), PyBOP (1.22 g, 2.33 mmol), and aminoacetonitrile hydrochloride (411 mg, 4.44 mmol) was added dry DMF (50 mL) under an atmosphere of dry nitrogen followed by Et_3N (0.96 mL, 6.88 mmol), and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was poured into aq sat. $NaHCO_3$ and extracted with Et_2O (3×), and the organic layer was washed with brine and dried over $MgSO_4$. The solvent was concentrated in vacuo to afford a crude oil which was purified by flash chromatography over silica gel (EtOAc/hexanes, 4/6) to yield the desired product *tert*-butyl 4-[3′-(1-{[(cyanomethyl)amino]-carbonyl}-3-methylbutyl)-1,1′-biphenyl-4-yl]piperidine-1-carboxylate (915 mg, 84% yield). Following general procedure 2, *tert*-butyl 4-[3′-(1-{[(cyanomethyl)amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-4-yl]piperidine-1-carboxylate was deprotected to afford the title compound *N*-(cyanomethyl)-4-methyl-2-[4′-(4-piperidinyl)[1,1′-biphenyl]-3-yl]pentanamide (**48**) (535 mg, 74% yield) following purification by flash chromatography (CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1). MS (+ESI) *m*/*z* 390.9 $(M + H)^+$. HRMS (+FAB): calcd for $C_{25}H_{32}N_3O$ $[MH^+]$ 390.25454, found 390.25436.

(2*R*)-2-[4′-(1-Azabicyclo[2.2.2]oct-4-yl)-1,1′-biphenyl-3-yl]-*N*-(cyanomethyl)-4-methylpentanamide (49). To 4-phenylquinuclidine (230 mg, 1.23 mmol) in AcOH (8 mL) was added Br_2 (1.25 mL, 4.6 mmol), and the reaction mixture was refluxed for 3 h. The reaction mixture was poured into aq sat. NaOH, the crude product extracted with EtOAc (3×), and the organic layer dried over Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1) to afford the desired 4-(4-bromophenyl)quinuclidine (201 mg, 61% yield). Following general procedure 1, (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*)-**5**) was submitted to a Suzuki cross-coupling reaction with 4-(4-bromophenyl)quinuclidine to afford title compound (2*R*)-2-[4′-(1-azabicyclo[2.2.2]oct-4-yl)-1,1′-biphenyl-3-yl]-*N*-(cyanomethyl)-4-methylpentanamide (**49**) (127 mg, 41%, purified by flash chromatography, CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1). HRMS (+FAB): calcd for $C_{27}H_{34}N_3O$ $[MH^+]$ 416.27019, found 416.27005. Anal. ($C_{27}H_{33}N_3O$) C: calcd, 78.03; found, 75.55; H: calcd, 8.00; found, 7.73; N: calcd, 10.11; found, 9.62.

***tert*-Butyl 4-[3′-(1-{[(Cyanomethyl)amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-4-yl]piperazine-1-carboxylate (50).** To 1-(4-bromophenyl)piperazine hydrochloride (103.15 g, 371.59 mmol), DMAP (4.54 g, 37.159 mmol), and Et_3N (155 mL, 1.115 mol) in MeCN (1.5 L) at room temperature was gradually added di-*tert*-butyl dicarbonate (121.65 g, 557.39 mmol) dissolved in a minimal amount of MeCN, and the reaction mixture was stirred for 5.5 h. The suspension was filtered, EtOAc was added, and the organic phase was washed with an aq 10% citric acid solution, water (2×), and brine and dried over $MgSO_4$. The solvent was concentrated in vacuo to afford the desired crude product *tert*-butyl 4-(4-bromophenyl)-piperazine-1-carboxylate (118.75 g, 94% yield) that was used as such in the following step. Following general procedure 1, *N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R,S*)-**5**) was submitted to a Suzuki cross-coupling reaction with *tert*-butyl 4-(4-bromophenyl)piperazine-1-carboxylate to afford the title compound *tert*-butyl 4-[3′-(1-{[(cyanomethyl)amino]carbonyl}-3-methylbutyl)-1,1′-biphenyl-4-yl]piperazine-1-carboxylate (**50**) (231 mg, 74% yield, purified by flash chromatography, EtOAc/hexanes, 35/65). HRMS (+FAB): calcd for $C_{29}H_{38}KN_4O_3$ $[M + K^+]$ 529.25810, found 529.25828.

(2*R*)-*N*-(Cyanomethyl)-4-methyl-2-{4′-[4-(2-oxopropyl)-piperazin-1-yl]-1,1′-biphenyl-3-yl}pentanamide (11). To a solution of (2*R*)-*N*-(cyanomethyl)-4-methyl-2-(4′-piperazin-1-yl-1,1′-biphenyl-3-yl)pentanamide hydrochloride ((*R*)-**2**) (100 mg, 0.234 mmol) in DMF (3 mL) was added Et_3N (82 μL, 0.589 mmol) followed by chloroacetone (22 μL, 0.281 mmol). The reaction was stirred at room-temperature overnight. Et_2O and H_2O were added, and the aqueous layer was extracted with Et_2O (3×). The combined organic extracts were washed with brine (1×), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to afford the title compound (2*R*)-*N*-(cyanomethyl)-4-methyl-2-{4′-[4-(2-oxopropyl)piperazin-1-yl]-1,1′-biphenyl-3-yl}pentanamide (**11**) (92 mg, 88% yield). Anal. ($C_{27}H_{34}N_4O_2$) C: calcd, 72.62; found, 70.64; H: calcd, 7.67; found, 7.54; N: calcd, 12.55; found, 12.53.

(2*R*)-2-[4′-(4-*tert*-Butylpiperazin-1-yl)-1,1′-biphenyl-3-yl]-*N*-(cyanomethyl)-4-methylpentanamide (51). A mixture of 1,4-dibromobenzene (1.8 g, 50 mmol) and *tert*-butylpiperazine dihydrobromide (3.04 g, 10 mmol)[30] was dissolved in 1:1 dioxane/toluene (60 mL). This solution was degassed, charged with potassium *tert*-butoxide (5.61 g, 50 mmol), $Pd_2(dba)_3$ (275 mg, 0.30 mmol), and (2′-dicyclohexylphosphanylbiphenyl-2-yl)dimethylamine (DavePhos)[31] (210 mg, 0.60 mmol) and sealed. The reaction mixture was heated at 80 °C overnight. 1:1 Ethyl acetate/THF (200 mL) was added. The mixture was washed with 1:1 water/brine (200 mL) and brine (100 mL), filtered through a plug of $MgSO_4$/DARCO/silica gel, evaporated to dryness, and purified by flash chromatography (0−50% ethyl acetate/dichloromethane, final elution of product with 5% methanol/dichloromethane) to give 0.68 g (23% yield) of 1-(4-bromophenyl)-4-*tert*-butylpiperazine. A solution of (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pentanamide ((*R*)-**5**) (0.356 g, 1.00 mmol), 1-(4-bromophenyl)-4-*tert*-butylpiperazine (0.297 g, 1.00 mmol), and 2.5 M sodium carbonate (1.25 mL) in DMF (5 mL) in a Schlenk tube was degassed and charged with $PdCl_2(dppf)$·(44 mg, 0.06 mmol). The tube was sealed and heated at 85 °C overnight. After being cooled, the reaction mixture was partitioned between 4:1 ethyl acetate/THF and brine/water (2:3, 100 mL), the organic phase was washed with saturated aqueous Na_2CO_3 (50 mL) and brine (50 mL), filtered through a plug of $MgSO_4$/DARCO/silica, concentrated in vacuo, and purified by flash chromatography (0−70% ethyl acetate/CH_2Cl_2 followed by elution with 5−7% methanol/CH_2Cl_2). The product was then precipitated from CH_2Cl_2/ether and hexanes, yielding 55 mg (13% yield) of the title compound **51**. HRMS (+FAB): calcd for $C_{28}H_{39}N_4O$ $[MH^+]$ 447.31239, found 447.312387.

(2*R*)-*N*-(Cyanomethyl)-2-{4′-[4-(2-hydroxyethyl)-1-piperazinyl][1,1′-biphenyl]-3-yl}-4-methylpentanamide (12). To (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-**2**) (105 mg, 0.269 mmol) in MeCN (10 mL) were added 2-bromoethanol (25 μL, 0.282 mmol) and K_2CO_3 (40 mg, 0.289 mmol), and the reaction mixture was heated to 80 °C for 3.5 h. More 2-bromoethanol (10 mL, 0.141 mmol) and K_2CO_3 (20 mg, 0.145 mmol) were added, and the reaction mixture was refluxed for 3 h. Water and brine were added, and the product was extracted with EtOAc (2×) and dried over $MgSO_4$. The solvent was removed in vacuo, and the crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1) to afford the desired (2*R*)-*N*-(cyanomethyl)-2-{4′-[4-(2-hydroxyethyl)-1-piperazinyl][1,1′-biphenyl]-3-yl}-4-methylpentanamide (**12**) (96 mg, 82% yield). MS (+ESI) *m*/*z* 435.1 $(M + H)^+$. HRMS (+FAB): calcd for $C_{26}H_{35}N_4O_2$ $[MH^+]$ 435.27600, found 435.27594. Anal. ($C_{26}H_{34}N_4O_2$) C: calcd, 71.86; found, 70.89; H: calcd, 7.89; found, 7.66; N: calcd, 12.89; found, 12.46.

(2*R*)-*N*-(Cyanomethyl)-2-{4′-[4-(2-hydroxy-2-methylpropyl)-1-piperazinyl][1,1′-biphenyl]-3-yl}-4-methylpentanamide (13). To (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-**2**) (135 mg, 0.346 mmol), isobutylene oxide (50 mg, 0.692 mmol), and K_2CO_3 (48 mg, 0.346 mmol) was added DMF (8 mL), and the reaction mixture was heated to 100 °C for 1 h. Additional aliquots of isobutylene oxide (250 mg, 3.47 mmol) and K_2CO_3 (96 mg,

0.692 mmol) were added along with MeOH to dissolve the solids, and the reaction mixture was heated for another 2 h. Water and brine were added, and the product was extracted with EtOAc (3×) and dried over Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified by flash chromatography over silica gel (100% EtOAc) to afford the desired (2*R*)-*N*-(cyanomethyl)-2-{4′-[4-(2-hydroxy-2-methylpropyl)-1-piperazinyl][1,1′-biphenyl]-3-yl}-4-methylpentanamide (**13**) (113 mg, 71% yield). MS (+ESI) *m/z* 384.0 $(M + H)^+$. HRMS (+FAB): calcd for $C_{28}H_{39}N_4O_2$ $[MH^+]$ 463.30730, found 463.30723. Anal. ($C_{28}H_{38}N_4O_2$) C: calcd, 72.69; found, 71.77; H: calcd, 8.28; found, 8.13; N: calcd, 12.11; found, 11.72.

(2*R*)-2-{4′-[1-(2-Amino-2-methylpropyl)piperidin-4-yl]-1,1′-biphenyl-3-yl}-*N*-(cyanomethyl)-4-methylpentanamide (14). To (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-**2**) (284 mg, 0.666 mmol), 2-amino-2-methylpropyl hydrogen sulfate (451 mg, 2.67 mmol), and K_2CO_3 (734 mg, 5.32) was added dry DMF (6 mL), and the reaction mixture was heated to 70 °C for 18 h. Additional aliquots of 2-amino-2-methylpropyl hydrogen sulfate (450 mg, 2.66 mmol) and K_2CO_3 (730 mg, 5.29 mmol) were added, and the reaction mixture was heated for another 48 h. The reaction mixture was poured into water, extracted with EtOAc (3×), dried over $MgSO_4$, concentrated in vacuo, and purified by flash chromatography over silica gel (CH_2Cl_2/MeOH/saturated aqueous NH_4OH, 90/9/1) to afford (2*R*)-2-{4′-[1-(2-amino-2-methylpropyl)piperidin-4-yl]-1,1′-biphenyl-3-yl}-*N*-(cyanomethyl)-4-methylpentanamide (**14**) as a foamy solid (11.5 mg, 4%). HRMS (+FAB): calcd for $C_{28}H_{40}N_5O$ $[MH^+]$ 462.32329, found 462.32340.

Methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]-acetate (26). To a solution of (3-hydroxyphenyl)acetic acid (**22**) (20.0 g, 131.6 mmol) and *p*-TsOH (2.5 g, 14.5 mmol) in MeOH (100 mL) was added trimethylorthoformate (29 mL, 263.2). The solution was refluxed for 4 h, cooled to room temperature, and stirred overnight. The reaction mixture was concentrated in vacuo, and the residue obtained was dissolved in Et_2O and washed with saturated aqueous $NaHCO_3$ (4×). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to yield methyl (3-hydroxyphenyl)acetate (23.0 g, quantitative yield) which was used as such in the next reaction. To a solution of methyl (3-hydroxyphenyl)acetate (23.0 g, 138.6 mmol) in CH_2Cl_2 (700 mL) was added Et_3N (31 mL, 221.7). The resultant yellow solution was cooled to 0 °C followed by the dropwise addition of trifluoromethanesulfonic anhydride (35 mL, 207.9 mmol) over 20 min. The reaction mixture was stirred at 0 °C for 1 h and then diluted with a 1:1 mixture of Et_2O/EtOAc (700 mL). The mixture was washed with H_2O (2 × 300 mL) and brine (1 × 300 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography (gradient elution: 7% EtOAc/hexanes to 20% EtOAc/hexanes) to afford methyl (3-{[(trifluoromethyl)sulfonyl]oxy}phenyl)-acetate (**23**) (33.7 g, 82% yield). A solution of methyl (3-{-[(trifluoromethyl)sulfonyl]oxy}phenyl)acetate (**23**) (33.0 g, 110.7 mmol), diboron pinacol ester (33.7 g, 132.9 mmol), KOAc (32.5 g, 332.1 mmol), and Pd(Cl_2)dppf (1.8 g, 2.20 mmol) in DMF (500 mL) was heated at 70 °C for 1.5 h. A further portion of Pd(Cl_2)dppf catalyst (1.8 g, 2.20 mmol) was then added, and the mixture was heated at 90 °C for 1.5 h. The reaction was cooled to room temperature, diluted with a 1:1 mixture of C_6H_6/Et_2O (1 L), and washed with H_2O (1 × 500 mL) and brine (2 × 300 mL). The combined aqueous layers were extracted with Et_2O (2 × 400 mL), and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (gradient elution: 5% EtOAc/hexanes to 30% EtOAc/hexanes) to afford methyl [3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]acetate (**24**) (27.5 g, 90% yield). To a solution of methyl [3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]acetate (**24**) (10.2 g, 36.96 mmol) and 1-(4-bromophenyl)-4-methylpiperazine (**25**) (6.3 g, 36.96 mmol) in DMF (200 mL) was added an aqueous solution of Na_2CO_3 (2.0 M, 37 mL, 110.88 mmol). The solution was degassed with N_2 for 30 min followed by the addition of Pd(Cl_2)dppf (605 mg, 0.741 mmol). After heating the reaction mixture at 100 °C for 1 h, a further portion of Pd(Cl_2)dppf catalyst (605 mg, 0.741 mmol) was then added, and the mixture was heated at 100 °C for a further 1.5 h. The reaction mixture was then cooled to room temperature, and H_2O was added. The mixture was extracted with EtOAc (10×), and the combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 3% MeOH in CH_2Cl_2 to 5% MeOH in CH_2Cl_2) to afford the title compound (**26**) (6 g, 75% yield).

General Procedure 3 (P2 Alkylation). To a cold (0 °C), stirred solution of methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]acetate (**26**) (1 equiv) in THF (10 mL/mmol of **26**) was added KHMDS (0.5 M in toluene, 1.2 equiv). The resultant solution was stirred at 0 °C for 20 min, warmed to room temperature, and stirred for an additional 2 h. The solution was then cooled to 0 °C, and the alkyl bromide or iodide (1.3 equiv) was added. After being stirred at 0 °C for 10 min, the reaction mixture was warmed to room temperature and stirred until the reaction was complete by TLC analysis (2 h to overnight). H_2O and EtOAc were added, and the aqueous layer was extracted with EtOAc (3×). The combined organic extracts were dried ($MgSO_4$), concentrated in vacuo, and the resultant residue was purified by flash chromatography to afford the desired alkylated compounds **27b**−**f**.

General Procedure 4 (Methyl Ester Hydrolysis and PyBOB Coupling). To a stirred solution of alkylated methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]acetate (**27a**−**f**) (1 equiv) in a 3:1 mixture of MeOH/H_2O (25−50 mL/mmol of starting material) was added an aqueous solution of LiOH (2.0 M, 10 equiv). The reaction mixture was stirred at room temperature until ester hydrolysis was complete by TLC analysis (10 min to 48 h). The reaction mixture was concentrated under reduced pressure and neutralized to pH 7 with 10% aqueous HCl. Upon neutralization, the desired acid precipitated out, filtered, and washed with H_2O. The solid was placed in a round-bottom flask, and any excess H_2O was removed by codistillation with toluene. The desired acid was then used as such in the next peptide coupling reaction. To a cold (0 °C), stirred solution of the acid (1 equiv), aminoacetonitrile hydrochloride (3 equiv), and PyBOB (1.5 equiv) in DMF (10 mL/mmol of acid) was added Et_3N (4 equiv). The resultant suspension was stirred at room-temperature overnight. Saturated aqueous $NaHCO_3$ was added followed by EtOAc. The aqueous layer was extracted with EtOAc (3×), and the combined organic extracts were washed with brine (1×), dried ($MgSO_4$), and concentrated under reduced pressure. The resultant residue was purified by flash chromatography to afford the desired amides (**28a**−**f**).

***N*-(Cyanomethyl)-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]acetamide (28a).** Following general procedure 4, methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]acetate (**26**) (134 mg, 0.43 mmol) was converted to the title compound **28a** (150 mg, 100% yield, flash chromatography using gradient elultion: 3% MeOH in CH_2Cl_2 to 5% MeOH in CH_2Cl_2). HRMS (+FAB): calcd for $C_{21}H_{25}N_4O$ $[MH^+]$ 349.20284, found 349.20289. Anal. ($C_{21}H_{24}N_4O$) C: calcd, 72.39; found, 68.93; H: calcd, 6.94; found, 6.82; N: calcd, 16.08; found, 14.92.

***N*-(Cyanomethyl)-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]-3-phenylpropanamide (28b).** Following general procedure 3, methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]acetate (**26**) (300 mg, 0.93 mmol) was alkylated with benzyl bromide to yield, after flash chromatography (5% MeOH in CH_2Cl_2), methyl 2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]-3-phenylpropanoate (**27b**) (88 mg, 23% yield). Methyl 2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]-3-phenylpropanoate (**27b**) (78 mg, 0.19 mmol) was converted to the title compound **28b** (40 mg, 49% yield) following general procedure 4 (flash chromatography using 5% MeOH in CH_2Cl_2). HRMS (+FAB): calcd for $C_{28}H_{31}N_4O$ $[MH^+]$ 439.24979, found 439.24998.

***N*-(Cyanomethyl)-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]pentanamide (28c).** Following general procedure 3, methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-

yl]acetate (**26**) (300 mg, 0.93 mmol) was alkylated with 1-iodopropane to yield, after flash chromatography (5% MeOH in CH_2Cl_2), methyl 2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]pentanoate (**27c**) (137 mg, 40% yield). Methyl 2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]pentanoate (**27c**) (137 mg, 0.37 mmol) was converted to the title compound *N*-(cyanomethyl)-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]-pentanamide (**28c**) (56 mg, 38% yield) following general procedure 4 (flash chromatography using gradient elultion: 3% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2). HRMS (+FAB): calcd for $C_{24}H_{31}N_4O$ [MH^+] 391.24979, found 391.24994. Anal. ($C_{24}H_{30}N_4O$) C: calcd, 73.81; found, 50.65; H: calcd, 7.74; found, 6.24; N: calcd, 14.35; found, 9.91.

***N*-(Cyanomethyl)-3-cyclopropyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]propanamide (28d).** Following general procedure **3**, methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]acetate (**26**) (300 mg, 0.93 mmol) was alkylated with (bromomethyl)cyclopropane to yield, after flash chromatography (5% MeOH in CH_2Cl_2), methyl 3-cyclopropyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]propanoate (**27d**) (198 mg, 57% yield). Methyl 3-cyclopropyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]propanoate (**27d**) (198 mg, 0.52 mmol) was converted to the title compound *N*-(cyanomethyl)-3-cyclopropyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]-propanamide (**28d**) (79 mg, 38% yield) following general procedure 4 (flash chromatography using gradient elultion: 5% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2). MS (+ESI) *m*/*z* 404.2 $(M + H)^+$. HRMS (+FAB): calcd for $C_{25}H_{30}KN_4O$ [$M + K^+$] 441.20567, found 441.20585. Anal. ($C_{25}H_{30}N_4O$) C: calcd, 74.59; found, 73.72; H: calcd, 7.51; found, 7.46; N: calcd, 13.92; found, 13.72.

(4S)-*N*-(Cyanomethyl)-4-methyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]hexanamide (28e, mixture of 2 diastereoisomers). Following general procedure 3, methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]acetate (**26**) (300 mg, 0.93 mmol) was alkylated with (*S*)−(+)-1-bromo-2-methylbutane to yield, after flash chromatography (5% MeOH in CH_2Cl_2), methyl (4*S*)-4-methyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]hexanoate (**27e**) (105 mg, 29% yield). Methyl (4*S*)-4-methyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]-hexanoate (**27e**) (75 mg, 0.19 mmol) was converted to the title compound **28e** (36 mg, 767% yield) following general procedure 4 (flash chromatography using gradient elultion: 3% MeOH in CH_2Cl_2 to 5% MeOH in CH_2Cl_2). HRMS (+FAB): calcd for $C_{26}H_{35}N_4O$ [MH^+] 419.28109, found 419.28094. Anal. ($C_{26}H_{34}N_4O$) C: calcd, 74.61; found, 65.80; H: calcd, 8.19; found, 8.48; N: calcd, 13.39; found, 14.32.

***N*-(Cyanomethyl)-3-cyclobutyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]propanamide (28f).** Following general procedure 3, methyl [4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]acetate (**26**) (300 mg, 0.93 mmol) was alkylated with (bromomethyl)cyclobutane to yield, after flash chromatography (5% MeOH in CH_2Cl_2), methyl 3-cyclobutyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]propanoate (**27f**) (22 mg, 6% yield). Methyl 3-cyclobutyl-2-[4′-(4-methylpiperazin-1-yl)-1,1′-biphenyl-3-yl]propanoate (**27f**) (21 mg, 0.05 mmol) was converted to the title compound **28f** (20 mg, 91% yield) following general procedure 4 (flash chromatography using 5% MeOH in CH_2Cl_2). HRMS (+FAB): calcd for $C_{26}H_{33}N_4O$ [MH^+] 417.26544, found 417.26554.

4-Methyl-2-(4′-piperazin-1-yl-biphenyl-3-yl)pentanoic Acid Carbamoylmethylamide (3). To the hydrochloride salt of (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)-[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-**2**) (1.04 g, 2.44 mmol) in CH_2Cl_2 (50 mL) was added TFA (380 μL, 4.88 mmol) dropwise, and the reaction was stirred at room temperature for 5 h. The acid was carefully quenched with a solution of aq sat. $NaHCO_3$, the product extracted with EtOAc (3×) and dried over Na_2SO_4, and solvent was removed in vacuo. Purification of the crude product by flash chromatography (CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1) afforded the desired compound 4-methyl-2-(4′-piperazin-1-yl-biphenyl-3-yl)pentanoic acid carbamolmethylamide (**3**) (502 mg, 51% yield). HRMS (+FAB): calcd for $C_{24}H_{33}N_4O_2$ [MH^+] 409.26035, found 409.26050.

***N*-[(2*R*)-4-Methyl-2-(4′-piperazin-1-yl-1,1′-biphenyl-3-yl)pentanoyl]glycine (52).** To the hydrochloride salt of (2*R*)-*N*-(cyanomethyl)-4-methyl-2-[4′-(1-piperazinyl)[1,1′-biphenyl]-3-yl]pentanamide ((*R*)-**2**) (1.37 g, 3.2 mmol) in 1,4-dioxane (8 mL) and MeOH (2 mL) was added dropwise a solution of HCl (4.0 M, in 1,4-dioxane, 2.4 mL, 9.6 mmol) under an atmosphere of dry nitrogen, and the reaction was stirred at room temperature for 1.5 h. A further aliquot of HCl (4.0 M, in 1,4-dioxane, 2.4 mL, 9.6 mmol) was added, and the reaction mixture was stirred for a total of 6 h. The acid was carefully quenched with a solution of aq sat. $NaHCO_3$, brine was added, the product was extracted with EtOAc (2×) and dried over Na_2SO_4, and the solvent was removed in vacuo. Purification of the crude product by flash chromatography (CH_2Cl_2/MeOH/aq sat. NH_4OH, 90/9/1) afforded the desired intermediate methyl *N*-[(2*R*)-4-methyl-2-(4′-piperazin-1-yl-1,1′-biphenyl-3-yl)pentanoyl]glycinate (750 mg, 55% yield). To methyl *N*-[(2*R*)-4-methyl-2-(4′-piperazin-1-yl-1,1′-biphenyl-3-yl)pentanoyl]glycinate (750 mg, 1.77 mmol) in MeOH (20 mL) was added dropwise an aq solution of LiOH (2.0 M, 4.4 mL, 8.8 mmol), and the reaction was stirred for 3.5 h. The reaction mixture was carefully neutralized with an aq solution of 10% HCl, and the solvent was removed in vacuo. The crude product was rinsed with water and then EtOAc and dissolved in MeOH, and the solvent was removed in vacuo to afford the desired product *N*-[(2*R*)-4-methyl-2-(4′-piperazin-1-yl-1,1′-biphenyl-3-yl)pentanoyl]glycine (**52**) (520 mg, 72% yield). HRMS (+FAB): calcd for $C_{24}H_{33}N_4O_2$ [MH^+] 410.24437, found 410.24418.

Enzyme Expression and Purification. Recombinant human cathepsin K was expressed in *P. pastoris* essentially as described,[32] activated by diafiltration at pH 4.5, and purified by ion exchange chromatography on a 20 mL Pharmacia Source 15S column equilibrated with 50 mM sodium acetate, pH 4.5, 1 mM EDTA, 1 mM dithiothreitol. Cathepsin K was eluted from the column with a linear gradient of 0 to 1.0 M NaCl in the same buffer. Humanized rabbit cathepsin K (rabbit cathepsin K with S163A, Y175D, and V274L mutations introduced; numbering from the initiation methionine) was expressed in *P. pastoris* and purified following the same protocol used for human cathepsin K. A human cathepsin L cDNA was isolated by PCR, cloned into the expression vector pPIC9, and transformed into *P. pastoris* strain KM71. Human cathepsin S, cloned as described,[33] was subcloned into the expression vector pPIC9 and transformed into *P. pastoris* strain KM71. Fermentation of high expressing clones for cathepsins L and S was carried out according to the supplier's protocol (Invitrogen). Recombinant cathepsin L was activated by diafiltration and purified on a Source 15S column as described for cathepsin K. Recombinant cathepsin S was activated by diafiltration at pH 4.5 and purification was carried out as described for cathepsin K except that a 0−0.5 M linear NaCl gradient was used.

Enzyme Inhibition. To measure enzyme activity, assays were carried out in 50 mM MES pH 5.5 containing 2.5 mM DTT, 2.5 mM EDTA, and 10% DMSO. IC_{50} values of compounds were determined for human cathepsins K, L, B, and S and for humanized rabbit cathepsin K using 2 μM of Z-Leu-Arg-AMC as substrate. Prior to the addition of substrate, different concentrations of the inhibitor ranging from 100 μM to 0.2 nM were preincubated for 15 min with each enzyme (0.2−1 nM) to allow the establishment of the enzyme−inhibitor complex. Substrate was then added and the enzyme activity measured from the increase of fluorescence at 460 nm ($\lambda_{ex.}$ = 355 nm). The final volume of the reaction was 100 uL. Assays were performed in 96-well plate format and the plate read using a Spectramax (Molecular Devices) plate reader. The percent inhibition of the reaction was calculated from a control reaction containing only vehicle. IC_{50} curves were generated by fitting percent inhibition values to a four parameter logistic model (SOFTMAX PRO, Molecular Devices).

Nitrilase Assay. Detection for nitrile hydrolysis was evaluated by RP-HPLC analysis. The reaction was carried out in 50 mM MES pH 5.5 containing 2.5 mM DTT, 2.5 mM EDTA, and 1 mM β-mercaptoethanol. Reaction products were ana-

lyzed by RP-HPLC using a linear gradient from 25% acetonitrile to 60% acetonitrile 0.1% TFA on a C18 column (Zorbax RX-C18 4.6 × 150 mm). The chromatogram was developed in 6 min at a flow rate of 2 mL/min. Under those conditions, the retention times for compound (*R*)-**2**, the synthetic acid **52**, and the amide **3** were 3.9, 3.2, and 2.7 min, respectively.

Kinetic Analysis. The measurement of enzyme activity to determine kinetic parameters was performed in 50 mM MES pH 5.5 containing 2.5 mM DTT, 2.5 mM EDTA, and 10% DMSO. Z-Leu-Arg-AMC (2 μM) was used as substrate, and enzymatic activity was measured at room temperature in 1 mL stirred cells using a Quantamaster spectrofluorometer (Photon Technology International) with excitation and emission wavelengths of 355 nm of 460 nm, respectively. Product formation was measured at different inhibitor concentrations following initiation of the reaction by the addition of the enzyme (0.2 nM final concentration). Formation of product (*P*) with time for an enzyme inhibited by a time-dependent inhibitor is described by the following equation[34]

$$P = v_s t - (v_s - v_0)(1 - e^{-k_{obs}})/k_{obsd} \quad (1)$$

where v_s is the rate of the reaction at steady-state, v_0 is the initial velocity of the reaction, and k_{obsd} is the apparent first-order rate constant characterizing the establishment of the steady-state velocity. Experimental values were fitted by nonlinear regression to eq 1, and fitted parameters v_s, v_0, and k_{obsd} were used to estimate pre-steady-state kinetic parameters k_{on} and k_{off} (eqs 3 and 4) and the dissociation constant K_i (eq 2) using the following relationships.[34]

$$K_i = [I]/((v_o/v_s) - 1) \quad (2)$$

$$k_{on} = k_{obsd}/([I] + K_i) \quad (3)$$

$$k_{off} = k_{on}K_i \quad (4)$$

Supporting Information Available: ^{1}H NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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