Potent and Selective Ketoamide-Based Inhibitors of Cysteine Protease, Cathepsin K

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Cathepsin K, a lysosomal cysteine protease of the papain superfamily, is abundantly and selectively expressed in osteoclasts, suggesting that this enzyme is crucial for bone resorption. Prevention of osteoclast-mediated bone resorption via inhibition of cathepsin K could be an effective approach to prevent osteoporosis. Potent and selective reversible ketoamide-based inhibitors have been identified in the present study. Using a known crystal structure of a ketoamide-based inhibitor, information from residues that form the P2/P3 pocket was used in the design of inhibitors that could allow for gains in selectivity and potency. Further, incorporation of P′ selective heterocycles, along with the P2/P3 modifications, is also described. These modifications have resulted in potent and selective cathepsin K inhibitors that allow for improvements in their physiochemical properties and represent a viable lead series for the discovery of new therapies for the prevention and treatment of osteoporosis

Introduction

Bone is a mechanically optimized connective tissue providing structural integrity for movement and protection. It is composed mainly of inorganic matter with the remainder consisting of organic or extracellular matrix and water. Bone remodeling, consisting of bone formation and resorption, is a dynamic process that requires proliferation and differentiation of osteoprogenitor cells. Osteoclasts, members of the monocyte/macrophage family are key players in the dissolution of bone mineral as well as degradation of its protein matrix. Osteoclasts contain membrane-bound receptors such as $\alpha_v\beta_3$ integrins that bind to bone matrix proteins containing an RGD motif to from an extracellular, resorptive compartment bordered by the ruffled membrane. This attachment allows the establishment of an acidic environment, a process mediated by a vacuolar H^+-ATPase in the ruffled membrane, to facilitate demineralization of the bone. This is followed by the secretion of proteolytic enzymes to degrade the organic matrix. Cysteine proteases have been implicated in this osteoclast-mediated resorption of the bone matrix.[1] The expression of cathepsin K, a cysteine protease of the papain superfamily, is abundant and selective in osteoclasts, suggesting that this enzyme is crucial for bone resorption.[2a,b] Cathepsin K antisense nucleotide studies have implicated this enzyme in osteoclast-mediated bone resorption.[3] In addition, a link between mutations in human cathepsin K and pycnodysostosis, a rare osteopetrotic disease characterized by abnormal bone resorption, has been demonstrated.[4] Moreover, it has also been shown that cathepsin K-deficient mice exhibit a distinct osteopetrotic phenotype.[5] Recent work has shown that cathepsin K, and not cathepsin L, is the major protease responsible for human osteoclastic bone resorption.[6] Further, recent studies have shown that complex formation with glycosaminoglycans specifically enhances collagenase activity of cathepsin K in osteoclast, while potently inhibiting collagenase activity of other cysteine proteases such as cathepsins L and S.[6c] The irreversible cysteine protease inhibitor E-64 has been shown to attenuate osteoclastic bone resorption.[7] The effects of a small molecule inhibitor of cathepsin K have also been evaluated on bone resorption in vivo using a nonhuman primate model of postmenopausal bone loss in which the active form of cathepsin K is identical to the human ortholog. These studies suggest that selective inhibition of cathepsin K could provide an effective therapy for the treatment of osteoporosis.

Cysteine proteases make up the vast majority of lysosomal proteases. There are currently eleven members of the human cathepsin cysteine protease family. Compared to cathepsin K, the physiological role and pathological implications of many cathepsins are less well understood. Since cysteine proteases such as cathepsins B, S, and L have been implicated in immunological responses, selectivity versus these proteases is desirable.[8,9] In lieu of the fact that cathepsins K, L, S, and V have a high degree of homology, design of inhibitors that are selective for the inhibition of cathepsin K is challenging. The difficulty in designing selective cathepsin inhibitors results from the similarity in their substrate recognition as well as their common proteolytic mechanism.[11b] Various irreversible inhibitors such as epoxides, peptidyl vinyl sulfones, and acyloxymethyl ketones have been reported in the literature.[10] Since the treatment of osteoporosis would require chronic drug administration, the development of a selective reversible inhibitor is desired. This could potentially avoid antigenicity due to covalent modification of proteins via

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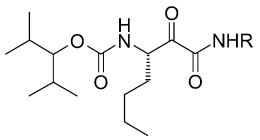
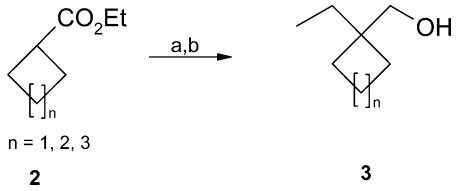


Figure 1.

Scheme 1[a]



[a] (a) LDA, THF, EtI.; (b) LAH, THF, reflux.

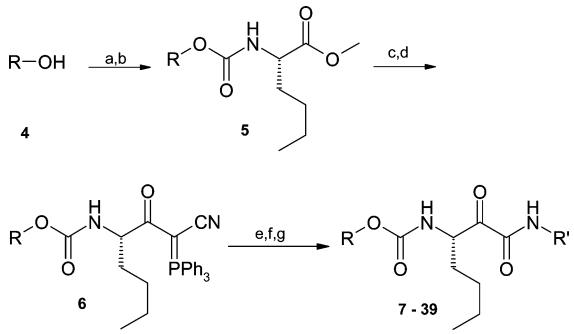
irreversible inhibition. Numerous warheads have been utilized in reversible cysteine protease inhibitors including peptidic aldehydes,[12] nitriles,[13] cyclopropenones,[14] diamino ketones,[15] and α-ketoamides.[16] There has also been a recent report on the use of noncovalent amides as cathepsin K inhibitors.[17,25b] This group was particularly interested in exploring α-ketoamides as reversible inhibitors of cathepsin K. The ketoamide class of inhibitors has been widely employed in inhibiting cysteine and serine proteases.[16,18] Herein is described its use in the inhibition of the cysteine protease, cathepsin K.[19] A report on the development of potent, selective and orally bioavailable inhibitors was recently described from this laboratory.[19a] That paper was primarily focused on the heterocyclic modifications at the prime region of ketoamide-based inhibitors keeping the P2/P3 substituent constant (Figure 1). In the present report the consequences of making changes in the P2/P3 region, along with the incorporation of prime substituents that were found to be important for selectivity, is disclosed. Further, incorporation of diverse P2/P3 groups that are potent and selective ketoamide inhibitors would allow for opportunities in improving upon physiochemical properties of this class of cathepsin K inhibitors.

Chemistry. The requisite four-, five-, and six-membered alcohols were synthesized as shown in Scheme 1. Deprotonation with LDA followed by electrophilic quench with ethyl iodide and subsequent reduction of the ester moiety afforded the desired (1-ethylcycloalkyl)-methanol derivatives **3**. The general syntheses of ketoamide inhibitors are shown in Scheme 2. The alcohols **4** were treated with phosgene to afford chloroformates, and subsequent addition of an amino ester provided carbamates **5**.[20] Hydrolysis of esters **5** to acids followed by coupling with the phosphorane afforded nitrile ylides **6**. Oxidation of the ylide **6** following the Wassermann procedure[21] and trapping the acyl nitrile with amines afforded the ketoamides **7**−**39**. The 1-alkyl-1*H*-pyrazole-5-ylamines (**13a**, **29a**−**39a**) were synthesized following literature procedures.[22−24]

Results and Discussion

Previous efforts in this laboratory identified P′ heterocyclic groups that provided potent and very selective cathepsin K inhibitors, while keeping the P2/P3 substituent constant. In our earlier report, achievement of selectivity over cathepsins L, S, and V had proven difficult which was overcome by incorporation of N-

Scheme 2[a]



[a] (a) 20% $COCl_2$ in toluene, THF, rt; (b) L-norleucine methyl ester hydrochloride, Hunig's base, THF, rt; (c) 1M LiOH/THF (1:1), rt; (d) (triphenylphosphoranylidene)acetonitrile, EDCI, DMAP, DCM, rt; (e) ozone, DCM, −78 °C, 15 min.; (f) amine, 1 M $AgNO_3$/THF (1:5), rt.

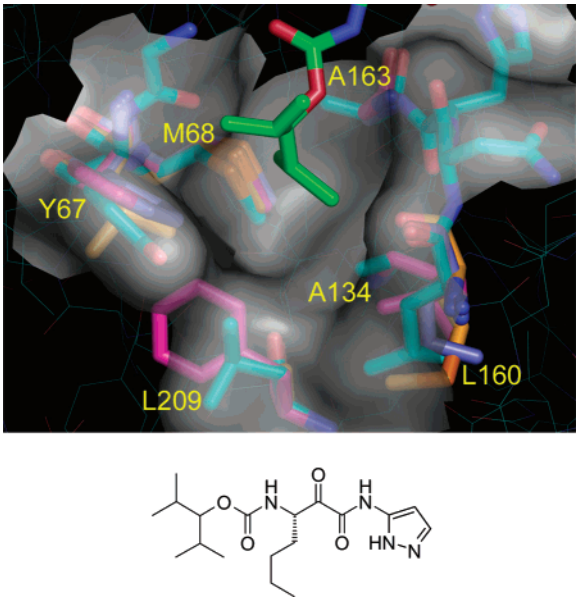


Figure 2. a. Closeup of the S2 pocket of cathepsins K, S, V, and L: S2 pocket of cathepsins K (cyan), S (magenta), V (purple), and L (orange) with the residues for cathepsin K labeled. The cathepsin K backbone and ligand (diisopropyl carbamate moiety, Figure 2b) are shown[19a] with side chains from other cathepsins colored as listed above. All of the structures used are deposited in the PDB database and have the following codes: cathepsin K (1Q6K), cathepsin S (1NPZ), cathepsin V (1FH0), and cathepsin L (1ICF). The figure was made using PYMOL (www.pymol.org).

substituted pyrazoles. Modifications in the P2/P3 region, along with P′ changes, are the focus of this paper. The S2 pocket of cathepsin K is composed of ^{67}Tyr, ^{68}Met, ^{134}Ala, ^{160}Leu, ^{163}Ala, and ^{209}Leu. The homologous residues for cathepsins L, V, and S are very similar and listed in Table 4 and shown in Figure 2a. All the S2 pockets of these enzymes have hydrophobic residues and would favor small hydrophobic residues or groups. Since the S2 pockets of cathepsins L, S, and V are formed by residues that differ slightly in steric bulk, opportunities for additional gain in potency and/or selectivity could be envisioned by making changes in the P2/P3 groups of the ketoamide-based inhibitors. Recent published reports that have disclosed selective cathepsin K inhibitors have taken advantage of steric congestion in the P2 pocket.[25] Cathepsin K inhibition data for the P2/P3 analogues, along with P′ modifications, are shown in

Table 1. Inhibitory Potencies vs Human Cathepsin K

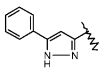
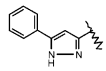
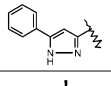
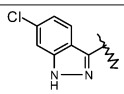
Cmpd	R	R_1	IC_{50} (nM)
7	 **7a**		36
8	 **8a**		1.6
9	 **9a**		1200
10	 **10a**		25
11	 **11a**		71
12	 **12a**		1.9
13	 **13a**		62
14	 **14a**		6.2
15	 **15a**		20
16	 **16a**		68
17	 **17a**		17
18	 **18a**		350
19	 **19a**		10
20	 **20a**		68
21	 **21a**		1.8
22	 **22a**		135
23	 **23a**		22
24	 **24a**		140
25	 **25a**		18
26	 **26a**		≥12000
27	**27a**		120
28	**28a**		1.6

Table 1. The P′ five-membered heterocycles having hydrogen bonding ability with the indole NH of ^{184}Trp were preferred in the present work. Incorporation of the P2/P3 *gem*-dimethyl group with the previously reported nonselective P′ groups afforded analogues **7**, **8**, and **10**. Since the *N*-methyl group was found to provide potent and selective cathepsin K inhibitors, this moiety was incorporated, along with the *gem*-dimethyl change to afford compound **9** (IC_{50} = 1200 nM). A dramatic loss in potency was observed. The unsubstituted pyrazole analogue **8** was the most potent inhibitor in the P2/P3 *gem*-dimethyl analogues. In agreement with our previous work, changes in the P2/P3 region of the inhibitor did not affect selectivity but they had a dramatic effect on the potency against cathepsin K. Constrained analogues of the *gem*-dimethyl moiety such as the cyclobutyl, cyclopentyl, and cyclohexyl afforded analogues **11**−**28**. In every case, the N-unsubstituted pyrazole analogues **12**, **17**, **21**, and **25** were found to be the most potent inhibitors in the constrained series. Having a heteroatom, as in the oxetane analogues **16**−**19**, did not seem to affect the general trend in potency. Incorporation of a phenyl substituent on the pyrazole that might $\pi-\pi$ stack with the indole side chain of ^{184}Trp gave varied results. There was a 3-fold and 12-fold decrease in potency in the cyclobutyl and cyclopentyl analogues, respectively (**12** vs **14** and **21** vs **23)**. Constraining the phenyl ring afforded an increase by 11-fold in the cyclohexyl series (**25** vs **28**). Incorporation of the known selective *N*-methylpyrazole group on the P′ region afforded analogues **13**, **22**, and **26**. While analogue **13** (IC_{50} = 62 nM) and **22** (IC_{50} = 135 nM) were fairly potent, there was a dramatic loss in potency in analogue **26** (IC_{50} = 12 μM). Although some decrease in potency on *N*-methyl substitution was expected (33-fold for analogue **13**, 75-fold for analogue **22** vs analogues **12** and **21**) due to a loss in hydrogen bonding to the enzyme, the complete loss in potency of analogue **26** suggests that accommodation in the S′ subsite results in shifts in binding at other subsites that is less favorable to cathepsin K.

Having identified the pyrazole as a potency enhancing P′ moiety, a selectivity study was undertaken to determine how modifications in the P2/P3 region would affect the potency/selectivity of these ketoamide inhibitors. As can be seen from Table 2, these unsubstituted pyrazole analogues did not show significant selectivity over cathepsins S, V, and L. Although exploitation of steric differences for selectivity/potency had been fruitful for some known cathepsin K inhibitors,[25] this was not found to be the case for these ketoamide inhibitors. These inhibitors contain a carbamate linker that forces the P2 substituent one atom further away compared to the corresponding dipeptides-based inhibitors. This shift seems to have a dramatic effect on the placement of the P2 group and affects the selectivity ratios.

Although, addition of a *N*-methyl group resulted in some loss in potency, it was gratifying to find that changes in the P2/P3 pocket, along with *N*-methyl pyrazole in the P′ region of ketoamide inhibitors, afforded a fairly potent and selective inhibitor **13**. Encouraged by the enhanced selectivity of analogue **13**, a series of N-substituted ketoamides were synthesized. Increase in the steric bulk at the P′ position afforded analogues

Table 2. Inhibitory Potencies (IC50) vs Human Cathepsins K, S, V, and L

Cmpd	Structure (R)	R_1	K (IC_{50} nM)	S/K*	V/K	L/K
8			1.6	1.6	----	-----
12			1.9	1.4	10	37
14			17	1.3	4.9	14
21			1.8	1.0	30	8.3
25			18	0.32	----	----
28			1.6	0.81	3.3	7.1

* Represents selectivity ratios over the other cathepsins (e.g. L/K = IC_{50} cat L/IC_{50} cat K).

29−**39**. The *N*-alkyl analogues **13**, **30**−**39** maintain relatively constant cathepsin K inhibitory activity with increases in selectivity compared to the N-unsubstituted pyrazole **12**. Increasing the steric bulk from methyl **13** to ethyl **30** to isopropyl **31** resulted in a gradual loss in selectivity against cathepsin S (Table 3). Constrained analogues of the isopropyl **31**, particularly analogues **36**−**38**, did not result in an increase in selectivity against cathepsin S. It is clear that even though the S′ pockets of cathepsins K, L, V, and S are very similar, the P′ groups have a dramatic effect on the selectivity of the compounds. This suggests that either the orientation of the P′ groups of the compounds influences the placement of the P2 groups conferring selectivity or that the P′ groups somehow confer selectivity on their own in the S′ pockets of the enzymes in a yet undefined manner. Interestingly, the unsaturated version **39** of analogue **38** resulted in a gain in selectivity over the cathepsins tested. Movement of the substituent to the adjacent nitrogen (analogue **29**, IC_{50} = 3.0) resulted in a loss in selectivity but enhanced potency compared to analogue **13**.

Conclusion

Based on the successful implementation of N-substituted pyrazoles with a fixed P2/P3 moiety for achieving potency and selectivity of ketoamide inhibitors of cathepsin K in our previous work, efforts in the present work deal with combining P2/P3 changes with N-substituted pyrazoles. Although, differences in the P2 pocket were identified from homology modeling, changes in the steric bulk in this region did not afford selective cathepsin K inhibitors. However, addition of P′ selective moieties along with P2/P3 changes did afford selective cathepsin K inhibitors. Such a change in the P2/P3 region broadens the scope for designing cathepsin K inhibitors allowing for improvements in their physiochemical properties.

Experimental Section

Chemistry. General Methods. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. Unless stated otherwise, reagents were obtained from commercial sources and were used directly. Reactions involving air- or moisture-sensitive reagents were carried out under a nitrogen atmosphere. If not specified, reactions were carried out at ambient temperature. Silica gel (EM Science, 230−400 mesh) was used for chromatographic purification unless otherwise indicated. Anhydrous solvents were obtained from Aldrich (Sure Seal). ^{1}H NMR spectra were recorded on a Varian spectrometer; chemical shifts are reported in parts per million (ppm) relative to TMS. The following abbreviations are used to describe peak patterns when appropriate: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High performance liquid chromatography (HPLC) was performed on a Beckman 126 with a Beckman 166 UV Detector (monitoring at 215 nm) with a Rainin Dynamax-60A column using a gradient consisting of 20/80 A:B to 10/90 A/B over 20 min, where A= 1% aqueous trifluoroacetic acid (TFA), B = 1% TFA in CH_3CN. Elemental analyses, performed by Atlantic Microlab, Inc. Norcross, GA, were within 0.4% of the theoretical values calculated for C, H, and N.

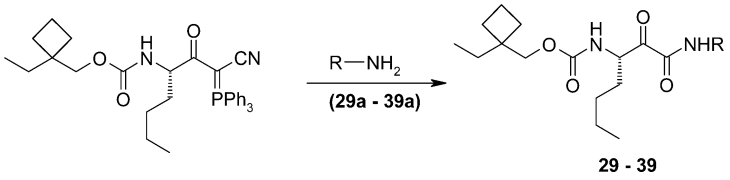
General Procedure for the Synthesis of Ethyl 1-Ethylcycloalkylcarboxylates. To a solution of diisopropylamine (6.44 mL, 46 mmol) in THF (90 mL) at −20 °C was added a 2.5 M solution (15.81 mL, 50.6 mmol) of *n*-BuLi in hexanes, and the contents were stirred for 15 min. The reaction mixture was then cooled to −78 °C for 15 min followed by the addition of ethyl cycloalkylcarboxylates (46 mmol). The reaction was then warmed to −30 °C for 15 min and then cooled back to −78 °C. Ethyl iodide (4.42 mL, 55.2 mmol) was then added, and the contents were allowed to warm to room temperature over 1 h. After stirring for another 3 h, the reaction mixture was quenched with sat. NH_4Cl (100 mL) followed by the addition of EtOAc (200 mL). After separation of the layers, the organic layer was dried with $MgSO_4$ and then concentrated under vacuum to afford the crude product which was purified with hexane:ethyl acetate (95:5) to afford the desired product in 60−75% yield as a colorless oil.

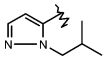
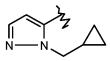
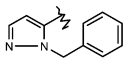
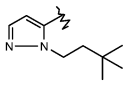
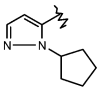
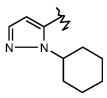
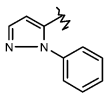
Ethyl 1-ethylcyclobutanecarboxylate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 4.13 (q, J = 7.2 Hz, 2H), 2.41 (m, 2H), 1.88−1.71 (m, 6H), 1.23 (t, J = 7.1 Hz, 3H), 0.78 (t, J = 7.3 Hz, 3H).

Ethyl 1-ethylcyclopentanecarboxylate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 4.12 (q, J = 7.1 Hz, 2H), 2.40 (m, 2H), 1.90−1.69 (m, 8H), 1.21 (t, J = 7.1 Hz, 3H), 0.80 (t, J = 7.2 Hz, 3H).

Ethyl 1-ethylcyclohexanecarboxylate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 4.12 (q, J = 7.3 Hz, 2H), 2.42 (m, 2H), 1.91−1.57 (m, 10H), 1.25 (t, J = 7.2 Hz, 3H), 0.78 (t, J = 7.2 Hz, 3H).

Table 3. Inhibitory Potencies (IC_{50}) vs Human Cathepsins K, L, S, V, H, and B



Cmpd	Structure (R)	K (IC_{50} nM)	L/K*	S/K*	V/K*	H/K*	B/K*
13	 **13a**	62	>200	65	150	>200	>200
29	 **29a**	3.4	38	1.3	8.5	3715	3715
30	 **30a**	52	>240	44	89	>240	>240
31	 **31a**	54	>230	30	65	>230	>230
32	 **32a**	76	>170	14	89	>170	>170
33	 **33a**	51	>250	33	102	>250	>250
34	 **34a**	65	130	7.2	34	>200	>200
35	 **35a**	71	160	10	43	>180	>180
36	 **36a**	45	270	14	49	>280	>280
37	 **37a**	83	130	8.5	32	>150	>150
38	 **38a**	58	150	10	33	>220	>220
39	 **39a**	63	240	33	62	≥200	≥200

* Represents selectivity ratios over the other cathepsins (e.g. L/K = IC_{50} cat L/IC_{50} cat K).

Table 4. S2 Pocket Residues of Cathepsins K, L, S, and V

K	L	S	V
^{67}Tyr	Leu	Phe	Phe
^{68}Met	Met	Met	Met
^{134}Ala	Ala	Gly	Ala
^{160}Leu	Met	Val	Leu
^{163}Ala	Gly	Gly	Gly
^{209}Leu	Ala	Phe	Ala

General Procedure for the Synthesis of (1-Ethylcycloalkyl)methanol. To a solution of ethyl 1-ethylcycloalkylcarboxylate (5.73 g, 36.7 mmol) in ether (75 mL) was added a 1.0 M solution of LAH in THF (73.4 mL, 73.4 mmol) at room temperature. The reaction contents were then heated at reflux for 6 h. After the reaction was quenched with 1 N HCl (200 mL), ether (200 mL) was added and the organic layer separated. The aqueous layer was extracted with ether (3 × 100 mL), and the combined organic layers were dried with $MgSO_4$ to afford after concentration under vacuum the crude product. Column chromatography with hexane:ethyl acetate (90:10) afforded the alcohols in 80−85% yield as a colorless oil.

(1-Ethylcyclobutyl)methanol: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 3.36 (s, 2H), 2.41 (br s, 1H), 1.78−1.21 (m, 8H), 0.80 (t, J = 7.3 Hz, 3H).

(1-Ethylcyclopentyl)methanol: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 3.68 (br s, 1H), 3.34 (s, 2H), 1.82−1.28 (m, 10H), 0.82 (t, J = 7.5 Hz, 3H).

(1-Ethylcyclohexyl)methanol: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 3.36 (s, 2H), 2.87 (br s, 1H), 1.81−1.20 (m, 12H), 0.81 (t, J = 7.5 Hz, 3H).

Methyl *N*-[(2,2-dimethylbutoxy)carbonyl]-L-norleucinate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 5.20 (d, J = 7.6 Hz, 1H), 4.40 (m, 1H), 3.83 (s, 2H), 3.78 (s, 3H), 1.87−1.65 (m, 2H), 1.40−1.29 (m, 6H), 0.95−0.84 (m, 12H).

2,2-Dimethylbutyl (1*S*)-1-[cyano(triphenylphosphoranylidene)acetyl]pentylcarbamate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.62−7.22 (m, 15H), 5.44 (d, J = 7.7 Hz, 1H), 4.88 (m, 1H), 3.73 (s, 2H), 1.89 (m, 1H), 1.68 (m, 1H), 1.32 (m, 4H), 1.23 (t, J = 7.1 Hz, 2H), 0.86−0.74 (m, 12H).

2,2-Dimethylbutyl (1*S*)-1-(oxo{[(1*R*)-1-phenylethyl]amino}acetyl)pentylcarbamate (7): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.42−7.23 (m, 5H), 7.15 (d, J = 7.0 Hz, 1H), 5.25 (m, 3H), 3.82 (s, 2H), 1.90 (m, 1H), 1.63 (m, 4H), 1.43 (m, 6H), 0.96−0.84 (m, 12H). Anal. C, H, N.

2,2-Dimethylbutyl (1*S*)-1-[oxo(1*H*-pyrazol-3-ylamino)acetyl]pentylcarbamate (8): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 12.09 (s, 1H), 10.76 (brs, 1H), 7.61 (s, 1H), 6.94 (s, 1H), 5.49 (brs, 1H), 5.30 (m, 1H), 3.99 (brs, 1H), 3.81 (s, 2H), 2.08 (m, 1H), 1.65−1.42 (m, 7H), 0.93−0.88 (m, 11H). Anal. C, H, N.

2,2-Dimethylbutyl (1*S*)-1-[[(1-methyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (9): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 8.00 (s, 1H), 5.93 (brs, 2H), 5.35 (d, J = 7.4 Hz, 1H), 5.24 (m, 1H), 3.83 (s, 3H), 3.65 (s, 2H), 2.08 (m, 1H), 1.65 (m, 1H), 1.59−1.27 (m, 12H), 0.94−0.83 (m, 6H).

2,2-Dimethylbutyl (1*S*)-1-[(isoxazol-3-ylamino)(oxo)acetyl]pentylcarbamate (10): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 9.61 (s, 1H), 8.41 (s, 1H), 7.14 (s, 1H), 5.28 (m, 2H), 3.83 (s, 2H), 1.99 (m, 1H), 1.67−1.28 (m, 7H), 0.93−0.88 (m, 12H). Anal. C, H, N.

Methyl *N*-{[(1-ethylcyclobutyl)methoxy]carbonyl}-L-norleucinate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 5.14 (d, J = 7.9 Hz, 1H), 4.32 (m, 1H), 3.97 (s, 2H), 3.70 (s, 3H), 1.81−1.43 (m, 9H), 1.32−1.18 (m, 5H), 0.87−0.77 (m, 6H).

(1-Ethylcyclobutyl)methyl (1*S*)-1-[cyano(triphenylphosphoranylidene)acetyl]pentylcarbamate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.70−7.30 (m, 15H), 5.52 (d, J = 7.7 Hz, 1H), 4.97 (m, 1H), 4.11 (s, 2H), 1.85−1.73 (m, 7H), 1.54−1.26 (m, 7H), 0.95−0.80 (m, 6H).

(1-Ethylcyclobutyl)methyl (1*S*)-1-(oxo{[(1*R*)-1-phenylethyl]amino}acetyl)pentylcarbamate (11): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.42−7.30 (m, 5H), 7.15 (d, J = 7.7 Hz, 1H), 5.25 (m, 3H), 4.04 (s, 2H), 1.94−1.71 (m, 6H), 1.63−1.30 (m, 11H), 0.98−0.82 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[oxo(1*H*-pyrazol-3-ylamino)acetyl]pentylcarbamate (12): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 11.98 (s, 1H), 10.79 (brs, 1H), 7.61 (s, 1H), 6.96 (s, 1H), 5.47 (d, J = 7.5 Hz, 1H), 5.28 (m, 1H), 4.04 (s, 2H), 1.88−1.29 (m, 13H), 0.96−0.85 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-methyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (13): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 8.05 (s, 1H), 5.88 (brs, 2H), 5.33 (m, 2H), 4.04 (s, 2H), 3.65 (s, 3H), 1.89−1.27 (m, 14H), 0.96−0.85 (m, 6H). Anal. C, H, N

(1-Ethylcyclobutyl)methyl (1*S*)-1-{oxo[(3-phenyl-1*H*-pyrazol-5-yl)amino]acetyl}pentylcarbamate (14): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.66 (s, 1H), 7.65 (s, 1H), 7.63−7.22 (m, 5H), 7.11 (s, 1H), 5.34 (brs, 1H), 5.17 (m, 1H), 4.17 (s, 2H), 2.04 (m, 1H), 1.85−1.44 (m, 7H), 1.40−1.16 (m, 6H), 0.89−0.81 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[(isoxazol-3-ylamino)(oxo)acetyl]pentylcarbamate (15): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 9.42 (s, 1H), 8.41 (s, 1H), 7.14 (s, 1H), 5.26 (brs, 2H), 4.05 (s, 2H), 2.08−1.27 (m, 14H), 0.97−0.82 (m, 6H). LC-MS ($C_{18}H_{27}N_3O_5$ + H) 366.

Methyl *N*-{[(3-ethyloxetan-3-yl)methoxy]carbonyl}-L-norleucinate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 5.23 (d, J = 8.0 Hz, 1H), 4.47 (s, 2H), 4.42 (s, 2H), 4.36 (s, 2H), 4.32 (m, 1H), 3.71 (s, 3H), 1.80−1.60 (m, 5H), 1.28 (m, 3H), 0.89−0.84 (m, 6H).

(3-Ethyloxetan-3-yl)methyl (1*S*)-1-[cyano(triphenylphosphoranylidene)acetyl]pentylcarbamate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.64−7.39 (m, 15H), 5.49 (d, J = 7.9 Hz, 1H), 4.89 (m, 1H), 4.87 (s, 2H), 4.44 (s, 2H), 4.14 (s, 2H), 1.97−1.64 (m, 4H), 1.36−1.14 (m, 4H), 0.90−0.82 (m, 6H).

(3-Ethyloxetan-3-yl)methyl (1*S*)-1-(oxo{[(1*R*)-1-phenylethyl]amino}acetyl)pentylcarbamate (16): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.42−7.30 (m, 5H), 7.17 (brs, 1H), 5.41 (d, J = 8.0 Hz, 1H), 5.12 (m, 2H), 4.53 (s, 2H), 4.43 (s, 2H), 4.21 (s, 2H), 2.08 (m, 1H), 1.81−1.73 (m, 3H), 1.59 (d, J = 6.8 Hz, 3H), 1.54−1.27 (m, 4H), 0.96−0.88 (m, 6H). Anal. C, H, N.

(3-Ethyloxetan-3-yl)methyl (1*S*)-1-[oxo(1*H*-pyrazol-5-ylamino)acetyl]pentylcarbamate (17): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 11.41 (s, 1H), 10.71 (brs, 1H), 7.51 (s, 1H), 6.87 (s, 1H), 6.16 (d, J = 7.9 Hz, 1H), 5.30 (m, 1H), 4.56 (s, 2H), 4.40 (s, 2H), 4.09 (s, 2H), 1.99 (m, 1H), 1.72−1.64 (m, 3H), 1.33−1.21 (m, 4H), 0.89−0.81 (m, 6H). Anal. C, H, N.

(3-Ethyloxetan-3-yl)methyl (1*S*)-1-[(isoxazol-3-ylamino)(oxo)acetyl]pentylcarbamate (18): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 9.70 (s, 1H), 8.33 (s, 1H), 7.06 (s, 1H), 5.43 (d, J = 7.8 Hz, 1H), 5.20 (m, 1H), 4.46 (s, 2H), 4.34 (s, 2H), 4.14 (s, 2H), 1.99 (m, 1H), 1.77−1.55 (m, 4H), 1.36−1.19 (m, 3H), 0.87−0.84 (m, 6H). LC-MS ($C_{17}H_{25}N_3O_6$ + H) 369.

(3-Ethyloxetan-3-yl)methyl (1*S*)-1-{oxo[(3-phenyl-1*H*-pyrazol-5-yl)amino]acetyl}pentylcarbamate (19): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 11.10 (s, 1H), 7.66 (s, 1H), 7.49−7.40 (m, 5H), 7.20 (s, 1H), 6.42 (d, J = 8.2 Hz, 1H), 5.42 (m, 1H), 4.52 (s, 2H), 4.47 (s, 2H), 4.11 (s, 2H), 2.13 (m, 1H), 1.81−1.64 (m, 3H), 1.37−1.26 (m, 4H), 0.96−0.88 (m, 6H). Anal. C, H, N.

Methyl *N*-{[(1-ethylcyclopentyl)methoxy]carbonyl}-L-norleucinate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 5.11 (d, J = 7.9 Hz, 1H), 4.32 (m, 1H), 3.83 (s, 2H), 3.70 (s, 3H), 1.78 (m, 1H), 1.64 (m, 5H), 1.43−1.21 (m, 10H), 0.87 (m, 6H).

(1-Ethylcyclopentyl)methyl (1*S*)-1-[cyano(triphenylphosphoranylidene)acetyl]pentylcarbamate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.71−7.53 (m, 15H), 5.51 (d, J = 7.8 Hz, 1H), 4.96 (m, 1H), 4.14 (s, 2H), 1.77−1.40 (m, 6H), 1.39−1.27 (m, 10H), 0.97−0.82 (m, 6H).

(1-Ethylcyclopentyl)methyl (1*S*)-1-(oxo{[(1*R*)-1-phenylethyl]amino}acetyl)pentylcarbamate (20): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.42−7.33 (m, 5H), 7.16 (d, J = 7.2 Hz, 1H), 5.25 (brs, 1H), 5.19 (m, 2H), 3.90 (s, 2H), 1.95 (m, 1H), 1.86−1.2 (m, 18H), 0.99−0.84 (m, 6H). Anal. C, H, N.

(1-Ethylcyclopentyl)methyl (1*S*)-1-[oxo(1*H*-pyrazol-5-ylamino)acetyl]pentylcarbamate (21): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 11.93 (s, 1H), 10.71 (brs, 1H), 7.61 (s, 1H), 6.96 (s, 1H), 5.41 (brs, 1H), 5.29 (m, 1H), 3.90 (s, 2H), 2.08 (m, 1H), 1.62−1.22 (m, 15H), 0.96−0.88 (m, 6H). Anal. C, H, N.

(1-Ethylcyclopentyl)methyl (1*S*)-1-[[(1-methyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (22): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.99 (s, 1H), 5.92 (brs, 2H), 5.31 (m, 2H), 3.89 (s, 2H), 3.64 (s, 3H), 1.95 (m, 1H), 1.61−1.40 (m, 15H), 0.96−0.84 (m, 6H). Anal. C, H, N.

(1-Ethylcyclopentyl)methyl (1*S*)-1-{oxo[(3-phenyl-1*H*-pyrazol-5-yl)amino]acetyl}pentylcarbamate (23): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 11.75 (s, 1H), 7.69 (s, 1H), 7.52−7.41 (m, 5H), 7.21 (s, 1H), 5.38 (brs, 1H), 5.24 (m, 1H), 3.91 (s, 2H), 2.08 (m, 1H), 1.65−1.22 (m, 15H), 0.97−0.85 (m, 6H). Anal. C, H, N.

Methyl *N*-{[(1-ethylcyclohexyl)methoxy]carbonyl}-L-norleucinate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 5.11 (d, J = 7.9 Hz, 1H), 4.31 (m, 1H), 3.86 (s, 2H), 3.69 (s, 3H), 1.80−1.61 (m, 2H), 1.39−1.20 (m, 15H), 0.84 (m, 4H), 0.77 (t, J = 7.5 Hz, 3H).

(1-Ethylcyclohexyl)methyl (1*S*)-1-[cyano(triphenylphosphoranylidene)acetyl]pentylcarbamate: ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.66−7.50 (m, 15H), 5.48 (d, J = 7.0 Hz, 1H), 4.91 (m, 1H), 3.88 (s, 2H), 2.03 (m, 1H), 1.71 (m, 1H), 1.56−1.25 (m, 16H), 0.91−0.79 (m, 6H).

(1-Ethylcyclohexyl)methyl(1*S*)-1-(oxo{[(1*R*)-1-phenylethyl]amino}acetyl)pentylcarbamate (24): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.39−7.32 (m, 5H), 7.18 (d, J = 7.3 Hz, 1H), 5.26

(brs, 1H), 5.19 (m, 2H), 3.92 (s, 2H), 2.01 (m, 1H), 1.59 (d, J = 6.9 Hz, 3H), 1.50−1.33 (m, 17H), 0.95−0.79 (m, 6H). Anal. C, H, N.

(1-Ethylcyclohexyl)methyl (1*S*)-1-[oxo(1*H*-pyrazol-5-ylamino)acetyl]pentylcarbamate (25): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 11.93 (s, 1H), 10.78 (brs, 1H), 7.62 (s, 1H), 6.96 (s, 1H), 5.44 (brs, 1H), 5.30 (m, 1H), 3.93 (s, 2H), 2.09 (m, 1H), 1.64−1.23 (m, 17H), 0.98−0.84 (m, 6H). Anal. C, H, N.

(1-Ethylcyclohexyl)methyl (1*S*)-1-[[(1-methyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (26): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 8.00 (s, 1H), 5.88 (brs, 2H), 5.31 (brs, 1H), 5.23 (m, 1H), 3.93 (s, 2H), 3.65 (s, 3H), 2.08 (m, 1H), 1.94 (m, 1H), 1.44−1.34 (m, 16H), 0.97−0.79 (m, 6H). Anal. C, H, N.

(1-Ethylcyclohexyl)methyl (1*S*)-1-[(isoxazol-3-ylamino)(oxo)acetyl]pentylcarbamate (27): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 9.52 (s, 1H), 8.42 (s, 1H), 7.14 (s, 1H), 5.26 (brs, 2H), 3.95 (s, 2H), 2.09 (m, 1H), 1.68−1.34 (m, 17H), 0.97−0.82 (m, 6H). Anal. C, H, N.

(1-Ethylcyclohexyl)methyl (1*S*)-1-[[(6-chloro-1*H*-indazol-3-yl)amino](oxo)acetyl]pentylcarbamate (28): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 11.01 (s, 1H), 10.90 (brs, 1H), 8.16 (d, J = 7.7 Hz, 1H), 7.48 (s, 1H), 7.12 (d, J = 7.8 Hz, 1H), 5.48 (d, J = 7.9 Hz, 1H), 5.28 (m, 1H), 5.30 (m, 1H), 3.79 (s, 2H), 2.05 (m, 1H), 1.93 (m, 2H), 1.44−1.27 (m, 14H), 0.96−0.80 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-methyl-1*H*-pyrazol-3-yl)amino](oxo)acetyl]pentylcarbamate (29): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 9.12 (s, 1H), 7.29 (brs, 1H), 6.71 (s, 1H), 5.27 (m, 2H), 4.00 (s, 2H), 3.83 (s, 3H), 2.01−1.25 (m, 14H), 0.91−0.75 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-ethyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (30): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.99 (s, 1H), 5.88 (brs, 2H), 5.32 (d, J = 7.8 Hz, 1H), 5.21 (m, 1H), 4.00 (s, 2H), 3.95 (q, J = 7.2 Hz, 2H), 1.90−1.62 (m, 7H), 1.59−1.24 (m, 10H), 0.93−0.79 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-isopropyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (31): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 8.01 (s, 1H), 5.90 (brs, 2H), 5.29 (brs, 1H), 5.21 (m, 1H), 4.19 (m, 1H), 3.99 (s, 2H), 2.03−1.25 (m, 20H), 0.89−0.77 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-isobutyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (32): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 8.00 (s, 1H), 5.89 (brs, 2H), 5.32 (brs, 1H), 5.22 (m, 1H), 4.00 (s, 2H), 3.67 (d, J = 7.3 Hz, 2H), 2.26 (m, 1H), 1.82−1.17 (m, 14H), 0.96−0.79 (m, 12H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[{[1-(cyclopropylmethyl)-1*H*-pyrazol-5-yl]amino}(oxo)acetyl]pentylcarbamate (33): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.99 (s, 1H), 5.94 (brs, 2H), 5.32 (d, J = 7.5 Hz, 1H), 5.22 (m, 1H), 4.00 (s, 2H), 3.81 (d, J = 6.8 Hz, 2H), 1.83−1.17 (m, 15H), 0.92−0.79 (m, 10H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-benzyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (34): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 8.06 (s, 1H), 7.53−7.20 (m, 5H), 5.70 (brs, 2H), 5.36 (d, J = 7.5 Hz, 1H), 5.33 (m, 1H), 5.16 (s, 2H), 4.00 (s, 2H), 1.83−1.61 (m, 7H), 1.58−1.24 (m, 7H) 0.92−0.79 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[{[1-(3,3-dimethylbutyl)-1*H*-pyrazol-5-yl]amino}(oxo)acetyl]pentylcarbamate (35): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.98 (s, 1H), 5.79 (brs, 2H), 5.32 (d, J = 7.9 Hz, 1H), 5.20 (m, 1H), 4.01 (s, 2H), 3.89 (t, J = 8.2 Hz, 2H), 1.84−1.25 (m, 16H), 0.99 (s, 9H), 0.98−0.79 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-cyclobutyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (36): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 8.00 (s, 1H), 5.84 (brs, 2H), 5.32 (d, J = 8.1 Hz, 1H), 5.19 (m, 1H), 4.46 (m, 1H), 4.00 (s, 2H), 2.68 (m, 2H), 2.44 (m, 2H), 2.04−1.23 (m, 16H), 0.92−0.77 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-cyclopentyl-1*H*-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (37): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.98 (s, 1H), 5.88 (brs, 2H), 5.33 (d, J = 7.9 Hz, 1H), 5.21 (m, 1H), 4.01 (s, 2H), 3.74 (m, 1H), 1.89−1.24 (m, 22H), 0.89−0.78 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-[[(1-cyclohexyl-1H-pyrazol-5-yl)amino](oxo)acetyl]pentylcarbamate (38): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 7.97 (s, 1H), 5.88 (brs, 2H), 5.33 (d, J = 7.7 Hz, 1H), 5.20 (m, 1H), 4.31 (m, 1H), 4.01 (s, 2H), 1.89−1.24 (m, 24H), 0.89−0.78 (m, 6H). Anal. C, H, N.

(1-Ethylcyclobutyl)methyl (1*S*)-1-{oxo[(1-phenyl-1*H*-pyrazol-5-yl)amino]acetyl}pentylcarbamate (39): ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 8.17 (s, 1H), 7.56−7.42 (m, 5H), 6.14 (brs, 2H), 5.34 (d, J = 7.7 Hz, 1H), 5.21 (m, 1H), 4.01 (s, 2H), 1.89−1.24 (m, 14H), 0.89−0.78 (m, 6H). Anal. C, H, N.

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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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