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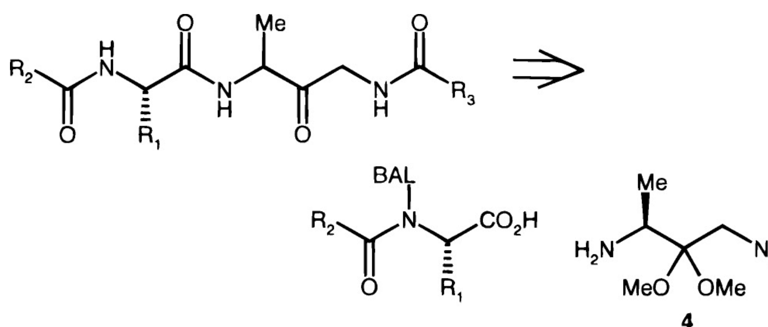

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Solid-Phase Synthesis of a Combinatorial Array of 1,3-Bis(acylamino)-2-butanones, Inhibitors of the Cysteine Proteases Cathepsins K and L

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To more rapidly prepare members of the 1,3-bis(acylamino)-2-butanone class of cysteine protease inhibitors, a solid-phase synthesis was developed. 1-Azido-3-amino-2,2-dimethoxybutane (**4**), which has the two amino groups differentiated and the ketone protected as a a ketal, served as a surrogate for the 1,3-diamino-2-butanone core. Amine (**4**) was coupled to the BAL-resin-linked carboxylic acids derived from α-amino acid esters. Evaluation of a small combinatorial array by measuring inhibition constants ($K_{i,app}$s) against cathepsins K, L, and B provided some structure−activity relationship trends with respect to selectivity and potency. Novel, potent inhibitors of cathepsins K and L were identified.

Introduction

The realization that many of members of the cysteine protease family mediate signal transduction[1] coupled with the observations that some of these enzymes are selectively expressed in specialized tissues[2] and have high sequence specificity[3] has overturned the previously conceived role of this class of enzymes as being limited to housekeeping by degrading proteins within lysosomes. Some examples of cysteine proteases that have specialized functions include calpain,[4] which regulates responses associated with rises in intracellular calcium concentration; caspases,[5] which regulate apoptosis; cathepsins S and L,[6] which regulate MHC-II-mediated antigen presentation; cathepins S and K,[7] which degrade elastin and collagen at sites of inflammation; and cathepsin K,[8] which degrades collagen at sites of bone remodeling. Selective inhibitors of these enzymes may be useful as tools to elucidate their role(s) and potentially treat many cardiovascular, pulmonary, autoimmune, and bone disease states. We have previously published the design, enzymology, and X-ray cocrystal structures of 1,3-bis-(acylamino)-2-propanones as part of a study aimed at identifying inhibitors of cathepsin K.[9] To accelerate the preparation of analogues of this class of compounds to further optimize inhibitory potency against cathepsin K and identify novel inhibitors of other cysteine proteases, a solid-phase synthesis was developed. A combinatorial array was prepared by using this methodology, and the resulting array members were evaluated for inhibitory potency against the cysteine proteases cathepsins K, L, and B. The structure−activity relationships (SARs) which emerged from these data are discussed herein. The method employs a strategy involving protection of the ketone as a ketal which was found to suffer epimerization at the α-carbon under the acidic cleavage conditions even though the ketone was shown to be stable under the same conditions.

Solid-phase organic synthesis, pioneered by Merrifield,[10] revolutionized peptide science by eliminating the need to purify synthetic intermediates. The utilization of automation and split/mix[11] and encoding strategies transformed the field by further increasing productivity. In recent years, medicinal chemists have turned to solid-phase methods to synthesize pharmaceutically relevent non-peptides and are steadily increasing the scope and efficiency of solid-phase chemistry. We wanted to develop a solid-phase strategy for synthesizing unsymmetrical 1,3-bis(acylamino)-2-propanones (**I**) with or without substitutents at the 3-position (Scheme 1, R_4). Therefore, a synthetic equivalent of the ketone core (Scheme 1, **I**, boxed) was devised that could be derived from α-amino acids. Though cognizant of the possibility of using stoichiometry and relative reactivity to differentiate primary vs secondary amines to acylating agents,[12] we chose instead to protect one of the amines as an azide since large excesses of reagents are often used to drive reactions to completion in solid-phase synthesis. Also, in order to eliminate possible complications of imine formation (i.e., cross-linking) between an unprotected ketone and the intermediate amines, we conservatively opted to protect the ketone as a dimethyl ketal. We considered using the ketal to link the template to the resin as described previously by Leznoff,[13] but instead we chose to link the template via nitrogen using a 3,5-dimethoxybenzyl linker (BAL) described by Barany.[14]

To evaluate this strategy, a 3 × 6 × 1 × 1-sized array (Scheme 1) was targeted as a model in which variable amino acids (R_1) and carboxylic acids (R_2) were coupled to form amides, and R_4 was held constant as a methyl group (derived

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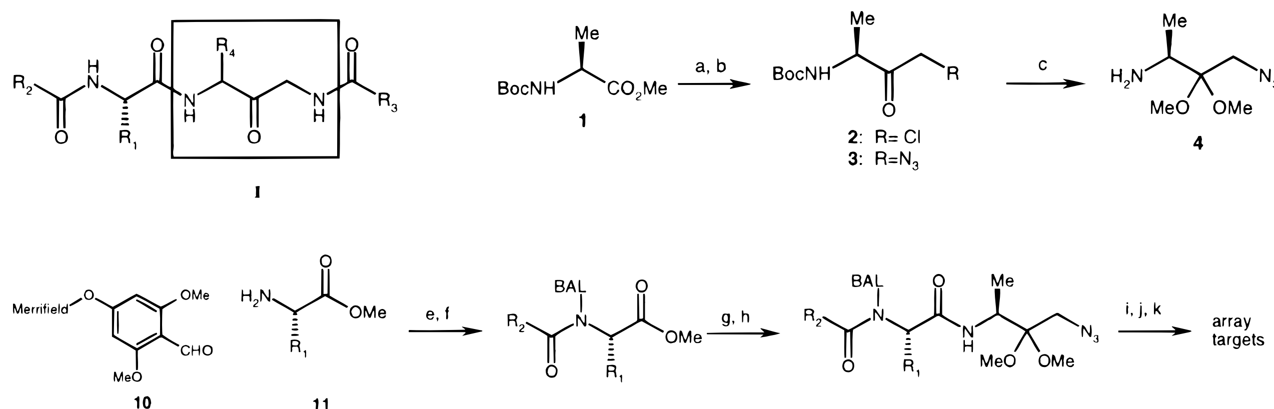
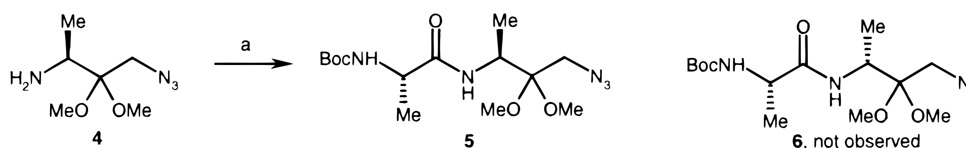
Scheme 1. Ketone Core (Boxed), Scheme for the Preparation of Ketone Core Synthetic Equivalents from α-Amino Esters, and Scheme for the Solid-Phase Synthesis of Unsymmetrical 1,3-Bis(acylamino)-2-Butanones[a]



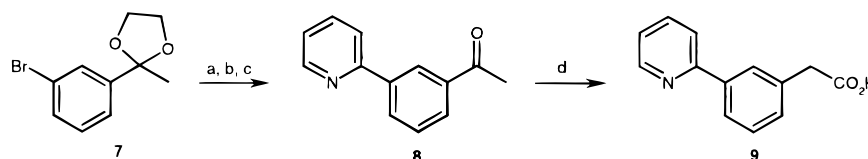
[a] Reagents and conditions: (a) ICH_2Cl, LDA, THF; (b) NaN_3, KF, DMF; (c) TsOH, MeOH, $(MeO)_3CH$, PhMe, △; (e) $NaBH(OAc)_3$, DMF, AcOH; (f) R_2CO_2H, EDC, NMP; (g) $KOSiMe_3$, THF; (h) **4**, EDC, NMP; (i) $SnCl_2$, PhSH, Et_3N, THF; (j) **9**, EDC, NMP; (k) TFA, Me_2S, H_2O.

Scheme 2. Determination of Enantiomeric Purity of Amine **4**[a]



[a] Reagents: (a) L-Boc-Leu-OH, EDC, NMP.

Scheme 3. Synthesis of 3-(2-Pyridyl)-phenylacetic Acid[a]



[a] Reagents: (a) *n*-BuLi, (*i*-PrO)$_3$B, THF; (b) $PdCl_2(PPh_3)_2$, 2-bromopyridine, 3 M K_3PO_3, DMF; (c) aq HCl/THF/MeOH; (d) S_8, morpholine, aq HCl.

from Boc-alanine methyl ester). We fixed the second amide (R_3) to 3-(2-pyridyl)-phenylacetyl, an aza-unsubstituted analogue of the previously described 3-biphenyl-4-methylvaleryl.[9b] This peptidomimetic was designed to interact favorably with tryptophans found on the primed sides of the active sites of cathepsins K and L, and it also provided a handle for additional water solubility by replacing a relatively polar pyridine in place of a hydrophobic phenyl group.

Results and Discussion

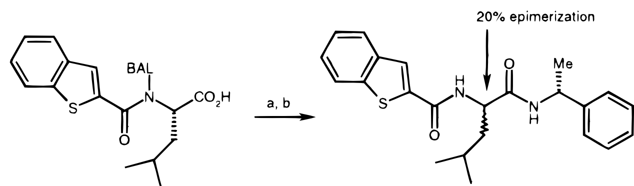
Starting with Boc-alanine methyl ester (**1**), the Kowalski and Haque[15] reaction was exploited to make Boc-alanine-α-chloromethyl ketone (**2**).[16] Nucleophilic displacement of the chloride with sodium azide provided Boc-alanine-α-azidomethyl ketone (**3**), and ketalization with concomitant deprotection of the primary amine gave our desired 1,3-diamino-2-butanone surrogate core: 1-azido-3-amino-2,2-dimethoxybutane (**4**, Scheme 1). To determine whether racemization had taken place, amine **4** was coupled to Boc-L-alanine and Boc-D-alanine forming amides **5** and **6** (the enantiomer of the potential epimerization product) (Scheme 2). Since compounds **5** and **6** had a distinct proton NMR spectrum, these data are consistent with a lack of epimerization at the methine carbon of **4**.

3-(2-Pyridyl)-phenylacetic acid (**9**) was prepared using the Kindler modification of the Willgerodt reaction[17] from 3-(2-pyridyl)-acetophenone (**8**), which in turn was synthesized by Suzuki coupling of 2-bromopyridine with 3-boronic acid-acetophenone 1,3-dioxolane, derived from the known 3-bromoacetophenone 1,3-dioxolane (**7**),[18] followed by acidic hydrolysis (Scheme 3).

The synthesis of this initial array made use of IRORI radio frequency encoded combinatorial chemistry technology (REC),[19] a commercially available array synthesis system comprised of miniature glass-encased radio frequency (RF) tags that uniquely label individual microreactors (MicroKans). Discrete compounds are synthesized by pooling and sorting MicroKans rather than pooling and splitting individual resin beads. Therefore, the efficiency advantages of split/pool synthesis[11] are realized, and at the same time, multimilligram quantities of each array member can be obtained.

Eighteen MicroKans with 18 unique IRORI RF tags were loaded with BAL aldehyde resin **10**.[14] The microreactors were then sorted into three pools (six microreactors per pool), and three different α-amino methyl esters (**11**), derived from leucine, phenylalanine, and valine, were attached by reductive amination using the racemization-free conditions reported by Ellman.[20] The MicroKans were then sorted into six pools (three microreactors each, in which each had a different α-amino ester attached). Then, each pool was reacted with one of six different carboxylic acids which were coupled to the amines with EDC again as described previously.[15] The

Scheme 4. Determination of Epimeric Purity in the Amide-Forming Step on a Model System[a]



[a] Reagents: (a) (*S*)-phenethyl amine, EDC, NMP; (b) TFA, Me_2S, H_2O.

microreactors were combined into a single pool, and the esters were converted to carboxylic acids using postassium trimethylsilyloxide.[21] 1-Azido-3-amino-2,2-dimethoxypropane (**4**) was coupled to form amides with EDC. To complete the synthesis of the array, the azides were reduced to primary amines with $SnCl_2$/PhSH.[22] The magic angle spinning (MAS) NMR spectra of the resin-bound products showed that the ketal moiety was stable to the azide reducing conditions. 3-(2-Pyridyl)-phenylacetic acid was then coupled to the immobilized amines with EDC, and final cleavage from the resin with concomitant hydrolysis of the ketals with TFA/Me_2S/H_2O gave the desired ketones. These compounds were purified by preparative reverse-phase HPLC in order to quantitate the amount of material going into the enzyme assay. Proton NMR spectra of the products indicated the presence of isomeric products consistent with the formation of diasteromers in ratios between 1.1:1 to 1.7:1.

To clarify the sources of epimerization in this reaction sequence we undertook studies to determine the cause of the diastereomeric mixtures. We first considered that the amino acid was being epimerized during the EDC coupling reaction. Therefore, a representive azide intermediate (R_1 = benzothiophene, R_2 = isobutyl) was cleaved with TFA/Me_2S/H_2O from the resin; however, the expected product decomposed under these reaction conditions probably due to the instability of the azido functionality. Therefore, (*S*)-phenethylamine was coupled to a representative carboxylic acid (R_1 = benzothiophene, R_2 = isobutyl) using EDC as a model system. After cleavage from the resin, it was apparent from the ^{1}H NMR that some epimerization had indeed taken place to the extent of approximately 20% (Scheme 4).

It was not clear if the observed partial epimerization arose from the carbodiimide coupling conditions or if the step involving conversion of the methyl ester to the carboxylic acid introduced some epimerization from enolization. To differentiate these possibilities, the carboxylic acid (R_1 = benzothiophene, R_2 = isobutyl) and (*S*)-phenethylamine were subjected to the HATU/collidine peptide segment coupling reagents reported by Carpino in which little epimerization is typically observed.[23] Indeed, under these conditions, no epimerization was present by ^{1}H NMR of the cleaved product. Therefore, the partial epimerization of the amino acid was not due to enolization from the potassium trimethylsilyloxide, but rather arose from subjection to the carbodiimide reagent.

The extent of epimerization could not be accounted for from the amine coupling step alone. We suspected that epimerization at C-3 of the butanone occurred in the cleavage step. Therefore, a representative ketone (R_1 = benzothiophene, R_2 = isobutyl) was prepared as an enantiomerically pure standard by coupling (3*S*)-3*N*-Boc-3-amino-1-amino-butan-2-ol (**12**)[24] and 3-(2-pyridyl)phenylacetic acid with HBTU. Removal of the Boc group, coupling the primary amine to Boc-leucine with HBTU, removal of the Boc group, coupling to 2-benzothiophene carboxylic acid with HBTU, and oxidation of the alcohol to the ketone with Dess−Martin periodinane[25] gave the desired product (Scheme 5).

The standard was then subjected to TFA/Me_2S/H_2O and was found to be configurationally stable under these cleavage conditions. Next, the dimethyl ketal was prepared by refluxing the ketone in MeOH, trimethylorthorformate, and toluenesulfonic acid, and based on its ^{1}H NMR spectrum, it appeared to be one diastereomer. Subjection of the ketal to the above cleavage conditions gave epimerized product to the extent of 20%. Therefore, the oxonium ion that is formed as an intermediate from the ketal is more subject to epimerization than the ketone using the reaction conditions typically employed to cleave amides from BAL resin. Attempts to further optimize the process by identifying reaction conditions that cleave amides from BAL resin and also hydrolyze dimethyl ketals while maintaining chiral integrity[26] were not made.

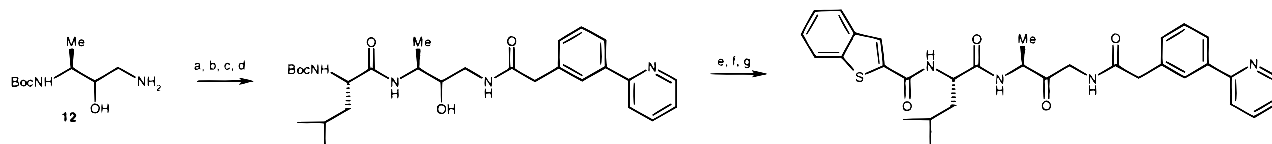
Evaluation of the combinatorial array (diastereomeric purities based on ^{1}H NMR ranging from 1.7 to 1.1:1.0, cf. Experimental Section for particular ratios) against cathepsins K and L revealed a rank order of preference of the amino acid side chain moiety (R_2): Cathepsin K prefers inhibitors derived from leucine (Table 1, row 1) vs phenylalanine (Table 1, row 2) by approximately 20-fold. On the other hand, cathepsin L prefers inhibitors derived from phenylalanine vs leucine by up to 5-fold. These findings can be rationalized based on the size and depth of the respective hydrophobic S2 pockets of cathepsins K and L: Ala 214 and Leu 69 in cathepsin L are substituted by Leu 209 and Tyr 67, respectively, in cathepsin K; thus, the larger benzyl group of phenylalanine better fills the larger pocket of cathepsin L vs the isobutyl side chain of leucine. Similar trends in selectivity have been noted with a class of irreversible inhibitors, the vinyl sulfones.[27]

Valine-derived array members were not potent inhibitors of either cathepsin K or L. Examination of an X-ray cocrystal structure of 1,3-bis-(Cbz-Leu-NH)-2-propanone[9a] bound to cathepsin K revealed that a valine side chain should be sterically accommodated by the enzyme, but due to the shortened length of the chain, the S2 pocket should not be sufficiently filled to result in high-affinity binding.

None of the members of the array were potent inhibitors of cathepsin B. This lack of binding is probably due to steric clashes between the 3-(2-pyridyl)-phenylacetyl moiety and the insertion loop present on the S′ side of cathepsin B but not present in cathepsins K or L.

Furthermore, SARs of the acyl group (R_1) attached to the amino group of the amino acid gave some interesting results: Inhibitors with acetamide (Table 1, column 1) were much less potent vs aryl amides (Table 1, columns 2−6) against both cathepsins K and L, probably due to a loss of an aromatic−aromatic or hydrophobic interaction with Tyr 67 in cathepsin K or Leu with 69 in cathepsin L, respectively.

Scheme 5. Synthesis of (3*S*)-3*N*-[(Benzothiophene-carbonyl)-L-leucinyl]-amino-1*N*-3-[(2-pyridyl)phenylacetyl]-1-aminobutan-2-one[a]



[a] Reagents: (a) HCl, dioxane; (b) 2-pyridylphenyl acetic acid, HBTU; (c) HCl, dioxane; (d) Boc-leucine, HBTU; (e) HCl, dioxane; (f) 2-benzothiophene carboxylic acid, HBTU; (b) Dess−Martin periodiname.

Table 1. $K_{i,app}$s (nM) for Cathepsins K, L, and B

R2	R1= Me	F3C– (phenyl)	MeO, MeO (phenyl)	N (quinoline)	N (quinoline)	S (benzothiophene)
	200 (K) >1000 (L) >1000 (B)	8.4 (K) 230 (L) >1000 (B)	7.4 (K) 150 (L) >1000 (B)	18 (K) 130 (L) >1000 (B)	1.7 (K) 130 (L) >1000 (B)	1.3 (K) 90 (L) >1000 (B)
Ph	>1000 (K) >1000 (L) >1000 (B)	90 (K) 120 (L) >1000 (B)	25 (K) 53 (L) >1000 (B)	170 (K) 36 (L) >1000 (B)	170 (K) 110 (L) >1000 (B)	16 (K) 18 (L) >1000 (B)
	>1000 (K) >1000 (L) >1000 (B)	430 (K) >1000 (L) >1000 (B)	>1000 (K) >1000 (L) >1000 (B)	>1000 (K) >1000 (L) >1000 (B)	830 (K) >1000 (L) >1000 (B)	300 (K) >1000 (L) >1000 (B)

Both the 2-quinoline and the 2-benzothiophene moieties gave enhanced potency (5−10-fold) against cathepsin K vs unfused aromatic groups; however, against cathepsin L, the fused and unfused aromatic groups had approximately the same activities. Also, an interesting 10-fold selectivity was observed between regioisomeric 2-quinoline and 5-quinoline in leucine-derived inhibitors (Table 1, row 1) against cathepsin K, but selectivity was not observed against cathepsin L.

To confirm the presumed simple competitive nature of inhibition of these compounds against cathepsin K, diastereomerically pure (3*S*)-3*N*- [(benzothiophenecarbonyl)-L-leucinyl]-amino-1*N*-3-(2-pyridylphenylacetyl)-1-amino-butan-2-one was subjected to further detailed kinetic analysis. Figure 1 shows a Lineweaver−Burke plot using the substrate Cbz-Leu-Arg-AMC. The lines are drawn based on fitting of all the data to a simple competitive velocity equation. Fitting the data to a more complex expression allowing for an uncompetitive term indicated that the extra term was not significant and the compound is best described as a purely competitive inhibitor of cathepsin K.

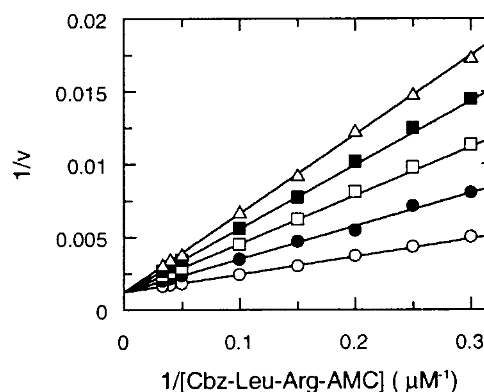


Figure 1. Lineweaver Burke plot for the compound (3*S*)-3*N*-[(benzothiophene-carbonyl)-L-leucinyl]-amino-1*N*-3-[(2-pyridyl)-phenylacetyl]-1-amino-butan-2-one. Compound concentrations are ○ = 0 nM, ● = 2 nM, □ = 4 nM, ■ = 6 nM, and △ = 8 nM.

Conclusion

A strategy for the synthesis of unsymmetrical 1,3-bis-(acylamino)-2-butanones on the solid phase was developed using 1-azido-3-amino-2,2-dimethoxybutane as a surrogate for 1,3-diamino-2-butanone. The four variable sites on this template are potentially exploitable to generate combinatorial libraries comprised of tens of thousands of components. Such a library might find particular use in identifying leads against novel cysteine proteases. In this study, we focused on analyzing a small array of purified compounds to elucidate SAR trends. Problems with epimerizations were identified that somewhat limit the utility of this methodology. Past literature indicates that conditions might be found which reduce or eliminate this problem.[26] Alternate resin linkers with different cleavage conditions might resolve this problem, also. The most apparent solution, however, is a strategy where the resin linker and the ketone protective group are one, thus ensuring that the reaction conditions will be optimal for reduced epimerization while still producing resin cleavage.

Even with this limitation, enzymological evaluation of the array revealed some interesting SARs for relative inhibition of cathepsins K and L. We anticipate that this methodology along with continued expansion of the types and improvement of the quality of reactions that can be run on the solid phase will facilitate additional SAR studies and provide sources of novel lead compounds on cysteine protease targets.

Experimental Section

General. Amino acid derivatives and HBTU were purchased from Novabiochem Intl. Carboxylic acids, EDC, tin-(II) chloride, triethylamine, thiophenol, HATU, collidine, TFA, and methyl sulfide were purchased from Aldrich Chemical Co., Inc. Merrifield resin (lot no. 150-300M-CMS 2-236A, 2 mequiv/g) was purchased from Polymer Science. All solvents were HPLC grade and used as purchased without purification with the following exceptions: THF and DMF were Aldrich anhydrous (99.8%) grade. ^{1}H NMR spectra were obtained in $CDCl_3$ and recorded on a Bruker AM-400 (400 MHz) spectrometer. Mass spectra (MS) were obtained and recorded on HP1050 spectrometer. MAS NMR spectra were obtained and recorded on Varian 300 spectrometer. HPLC was conducted using a 20 mm × 50 mm YMC reversed-phase column on Gilson 215.

(*S*)-3-(1-Chloro-2-oxo-butyl)-carbamic Acid *tert*-Butyl Ester (2).[10] Lithium diisopropyl amide (250 mL, 500 mmol, 2.0 M in THF) was added dropwise to a solution of *N*-Boc-alanine methyl ester (**1**, 20.0 g, 100 mmol) and iodochloromethane (30 mL, 400 mmol) in THF (200 mL) at −78 °C, maintaining a temperature less than −70 °C over a 3 h period. The reaction mixture was stirred an additional 20 min upon completion of the addition. A 1:1 mixture of acetic acid and THF (110 mL) was added, maintaining a temperature less than −70 °C over a 3 h period. The reaction mixture was then stirred an additional 15 min and then was diluted with EtOAc (400 mL). The organics were extracted with water (200 mL), saturated aqueous sodium bicarbonate (200 mL), and brine (200 mL), dried with magnesium sulfate, filtered, concentrated in vacuo, and chromatographed (silica gel, 15% EtOAc:hexanes) to yield the title compound as an oil (17.2 g, 79%). ^{1}H NMR ($CDCl_3$, 400 MHz): 5.12 (bs, 1H), 4.51 (m, 1H), 4.27 (s, 2H), 1.44 (s, 9H), 1.37 (d, J = 6.8 Hz, 3H). IR: 2984, 2880, 1732, 1701, 1492, 1359, 1288, 1150, 1050 cm^{-1}.

(*S*)-3-(1-Azido-2-oxo-butyl)-carbamic Acid *tert*-Butyl Ester (3). Sodium azide (6.1 g, 92.2 mmol) and potassium fluoride (6.76 g, 116.6 mmol) was added to a solution of 3-(1-chloro-2-oxo-butyl)-carbamic acid *tert*-butyl ester (17.1 g, 77.7 mmol) in DMF (300 mL), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with EtOAc (200 mL) and was extracted with water (100 mL). The combined organics were dried with magnesium sulfate, filtered, concentrated in vacuo, and chromatographed (silica gel, 15% EtOAc:hexanes) to yield the title compound as a white solid (15.0 g, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.12 (AB, $\Delta\delta$ =19.9 Hz, 2H), 1.44 (s, 9H), 1.28 (d, J = 6.8 Hz). IR: 2980, 2888, 2104, 1732, 1700, 1507, 1368, 1285, 1250 cm^{-1}.

(*S*)-3-Azido-2,2-dimethoxy-butylamine (4). Toluenesulfonic acid monohydrate (1.6 g, 8.4 mmol) was added to a solution of (*S*)-3-(1-azido-2-oxo-butyl)-carbamic acid *tert*-butyl ester (1.4 g, 6.1 mmol) and trimethyl orthoformate (5.2 g, 49.1 mmol) in MeOH (10 mL), and the solution was refluxed overnight. The reaction mixture was concentrated in vacuo, and then 1 N NaOH (9 mL) was added, concentrated in vacuo, and chromatographed (silica gel, 0.8% NH_4OH, 2% MeOH, CH_2Cl_2) to yield the title compound as an oil (0.34 g, 31%). ^{1}H NMR ($CDCl_3$, 400 MHz): 3.45 (s, 2H), 3.32 (s, 3H), 3.26 (m, 1H), 1.97 (BS, 1H), 1.17 (d, J = 6.7 Hz, 3H).

3-(2-Pyridyl)-acetophenone (8). 3-Bromoacetophenone 1,3-dioxolane (**7**)[16] (77 g, 0.316 mol) in 1.5 L of dry THF was cooled to −78 °C. *n*-Butyllithium (1.9 M in THF, 200 mL, 0.38 mol) was added dropwise over 30 min, and the reaction was stirred at −78 °C for 1 h. Triisopropyl borate (109 mL, 0.475 mol) was added in one portion, and the reaction was allowed to warm to room temperature overnight. The reaction was then carefully quenched with 10 mL of water, and the solvent was concentrated in vacuo leaving a thick white oil.

The oil was redissolved in 750 mL of DMF, and to this solution was added 2-bromopyridine (33.4 mL, 0.35 mol), 2 M K_3PO_4 (500 mL), tetrabutylammonium iodide (11 g, 0.032 mol), and $PdCl_2(PPh_3)_2$ (2.2 g, 0.0032 mol). This mixture was heated to 90 °C and was stirred for 16 h, cooled to room temperature, poured into 2 L of water, and extracted with TBME (3 × 500 mL). The TBME extracts were combined, washed with 0.5 M copper sulfate solution (500 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo to a red oil.

To the oil, dissolved in a mixture of THF (100 mL) and methanol (300 mL), was added 1 N HCl (200 mL), and the reaction was stirred for 4 h at room temperature. The solution was then concentrated to about 300 mL and extracted with hexanes (1 × 300 mL). The aqueous layer was then made basic with 1 M NaOH (ca. 250 mL) and extracted with TBME (3 × 300 mL). The TBME extracts were combined, dried with $MgSO_4$, filtered, and concentrated to yield the

title compound (52 g, 83% yield) in sufficient purity for the next step. A small sample was purified via flash chromatography (silica gel; 15% ethyl acetate in hexane). ^{1}H NMR ($CDCl_3$, 400 MHz): ^{1}H NMR ($CDCl_3$, 360 MHz): δ 7.35−7.24 (m, 5H), 6.35−6.33 (d, 1H, J = 7.74 Hz), 5.06 (s, 2H), 4.81−4.75 (m, 1H), 4.16 (br s, 1H), 4.12 (AB, J_{AB} = 10.3, $\Delta\delta_{AB}$ = 0.097, 2H), 3.40 (s, 3H), 1.60−1.30 (m, 6H), 0.92 (d, 9H, J = 6.2 Hz), 0.90 (d, 3H, J = 6.47 Hz). IR (KBr): 3301, 1731, 1694, 1646 cm^{-1}. Anal. Calcd for $C_{22}H_{34}N_2O_5$: C, 65.00; H, 8.43; N, 6.89. Found: C, 65.09; H, 8.61; N, 6.93.

3-(2-Pyridyl)-phenylacetic Acid (9). Sulfur (9.3 g, 0.29 mol) was added to 3-(2-pyridyl)-acetophenone (**8**, 52 g, 0.264 mol) and morpholine (28 mL, 0.32 mol) in a 500 mL three-necked flask with thermometer, condensor, and 1 N NaOH bubbler to trap the liberated H_2S. The reaction mixture was heated to 115° for 18 h. The mixture was cooled to room temperature, and 6 N HCl (225 mL) was added slowly via an addition funnel. This mixture was then heated at reflux for 3 h, cooled to 70 °C, and filtered through a pad of Celite, and the Celite pad was washed with 100 mL of water. The solution was cooled to room temperature, and extracted with CH_2Cl_2 (2 × 50 mL). The pH of the aqueous layer was then carefully adjusted to pH 11 with 6 N NaOH (275 mL), and the solution was concentrated to a thick paste in vacuo, adding *n*-butanol (3 × 200 mL) to aid in the removal of the morpholine. The crude residue was dissolved in 50 mL of water, the pH carefully adjusted to pH 5 using 2 N HCl, and the solution extracted with ethyl acetate (3 × 200 mL). The organic layers were combined, dried with $MgSO_4$, filtered, and concentrated, and the crude oil was recrystallized from hot acetone to give the title compound (35 g, 62% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): 8.75 (d, J = 4.7 Hz, 1H), 7.91 (s, 1H), 7.80−7.72 (m, 1H), 7.70−7.66 (m, 2H), 7.40−7.32 (m, 2H), 7.30−7.26 (m, 1H), 3.70 (s, 2H). Anal. Calcd for $C_{13}H_{11}NO_2$: C, 73.23; H, 5.20; N, 6.57. Found: C, 72.92; H, 5.33; N, 6.46; mp = 107−108 °C.

Merrifield Polystyrene-methylene-4-oxy-2,6-dimethoxy-benzaldehyde. Sodium hydride (60% suspension in oil, 2 g, 53 mmol) was added portionwise to a solution of 4-hydroxy-2,6-dimethoxy-benzaldehyde (10 g, 56 mmol) in DMF (530 mL). The reaction was agitated by bubbling argon for 0.5 h. Then Merrifield resin (8 g, 16 mmol, polychloromethylstyrene/1% divinylbenzene, 2.0 mmol/g, Polymer Labs) was added, and the reaction mixture was heated to 50 °C and was agitated by bubbling argon overnight. The resin was then filtered, washed repeatedly with DMF (3×) then CH_2Cl_2 (2×), and dried in vacuo. MAS ^{1}H NMR ($CDCl_3$): 10.31, 7.16−6.03, 3.72, 1.53.

Merrifield Polystyrene-BAL-leucine Methyl Ester (Representative Example). Merrifield polystyrene-methylene-4-oxy-2,6-dimethoxy-benzaldehyde (PS BAL aldehyde, 24 mg, 0.05 mmol) in an IRORI MicroKan was immersed in a solution of leucine methyl ester (0.15 M), $NaBH(OAc)_3$ (0.15 M) in 1% AcOH, and DMF (20 mL) and was stirred at room temperature for 2 days. The MicroKan was then filtered, rinsed repeatedly with DMF (7×), MeOH (3×), and CH_2Cl_2 (2×), and then dried in vacuo. MAS ^{1}H NMR ($CDCl_3$): 7.05−6.13, 3.58, 3.30, 1.50, 0.90, 0.83.

Merrifield Polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucine Methyl Ester (Representative Example). Merrifield polystyrene-BAL-leucine methyl ester carried from the last reaction in an IRORI MicroKan was immersed in a solution 2-benzothiophene-carboxylic acid (0.38 M) and EDC (0.38 M) in NMP (24 mL) and was agitated by rocking at room temperature overnight. The MicroKan was washed repeatedly with DMF (7×), CH_2Cl_2 (7×), and MeOH (3×) and was dried in vacuo. MAS ^{1}H NMR ($CDCl_3$): 7.82−6.08, 3.70, 3.45, 0.92, 0.85.

Merrifield Polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucine (Representative Example). Merrifield polystyrene- BAL-*N*- (2-benzothiophene-carbonyl)-leucine methyl ester carried from the last reaction in an IRORI MicroKan was immersed in a solution of potassium trimethylsilyloxide (0.39 M) in THF (24 mL) at room temperature and was agitated overnight. The MicroKan was washed repeatedly with DMF (3×) and CH_2Cl_2 (2×) and then was dried in vacuo. MAS ^{1}H NMR ($CDCl_3$): 7.83−6.05, 3.72, 1.01.

Merrifield Polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucinyl-amino-2,2-dimethoxy-butyl-3-azide (Representative Example). Merrifield polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucine carried over from the last reaction in an IRORI MicroKan was immersed in a solution of (*S*)-3-azido-2,2-dimethoxy-butylamine (**4**, 0.38 M) and EDC (0.38 M) in DMF (24 mL) and was agitated for 24 h. The MicroKan was washed repeatedly with DMF (3×), and CH_2Cl_2 (2×) and then was dried in vacuo. MAS ^{1}H NMR ($CDCl_3$): 7.84−6.02, 3.71, 3.49, 3.37, 3.24, 3.16, 1.47, 1.00.

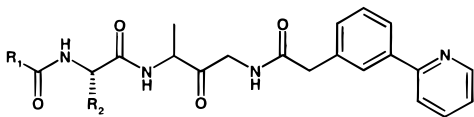
Merrifield Polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucinyl-amino- 2,2-dimethoxy-butyl-3-amine (Representative Example). Merrifield polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucinyl-amino-2,2-dimethoxy-butyl-3-azide carried over from the last reaction in an IRORI MicroKan was immersed in a solution of $SnCl_2$ (0.21 M) and thiophenol (0.84 M) in triethylamine (1.05 M) in THF (24 mL) and was agitated overnight. The MicroKan was washed repeatedly with THF (3×), DMF (2×), and CH_2Cl_2 (3×), and then was dried in vacuo. MAS ^{1}H NMR ($CDCl_3$): 7.85, 6.02, 3.71, 3.20, 1.12, 0.91.

Merrifield Polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucinyl-amino-3*N*-amino-(3-(2-pyridyl)-phenyl-acetyl)-2,2-dimethoxy-butane (Representative Example). Merrifield polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucinyl-amino-2,2-dimethoxy-butyl-3-amine carried over from the last reaction in an IRORI MicroKan was immersed in a solution of 3-(2-pyridyl)-phenylacetic acid (0.35 M) and EDC (0.35 M) in NMP (24 mL) and was agitated overnight. The MicroKan was washed repeatedly with DMF (3×), CH_2Cl_2 (3×), and MeOH (3×) and then was dried in vacuo. MAS ^{1}H NMR ($CDCl_3$): 8.60−6.06, 4.01, 3.66, 3.37, 3.19, 1.91, 1.08, 1.00.

***N*-(2-Benzothiophene-carbonyl)-leucinyl-amino-3*N*-amino-(3-(2-pyridyl)-phenylacetyl)-butanone (Representative Example).** Merrifield polystyrene-BAL-*N*-(2-benzothiophene-carbonyl)-leucinyl-amino-3*N*-amino-(3-(2-pyridyl)-phenylacetyl)-2,2-dimethoxy-butane carried over from the last reaction in an IRORI MicroKan was immersed in a solution of 18:1:1 TFA/Me_2S/H_2O (10 mL) and was agitated

overnight. The MicroKan was washed repeatedly with CH_2Cl_2 (3×), and the combined solutions were evaporated in vacuo.

All compounds were purified by reversed-phase HPLC using a Gilson 215. HPLC conditions: Samples were dissolved in DMSO and injected at CH_3CN/H_2O (10:90) on a preparative C-18 reversed-phase HPLC column, ramp 10:90−90:10 CH_3CN/H_2O over 10 min, UV detection at both 214 and 254 nm.



R_1 = 2-Benzothiophene; R_2 = Isobutyl. Diasteromeric Ratio = 1.32:1. ^{1}H NMR (400 MHz, $CDCl_3$): 8.79−7.26 δ (m, 13H), 7.19 (d, J = 8.0 Hz, 1H, d1), 7.12 (d, J = 8.0 Hz, 1H, d2), 6.84 (b, 1H), 4.63 (m, 1H), 4.50 (qd, J = 7.1 Hz, J = 7.1 Hz, 1H, d1), 4.44 (qd, J = 7.1 Hz, J = 7.1 Hz, 1H, d2), 4.15 (m, 2H), 3.67 (s, 2H, d2), 3.63 (s, 2H, d1), 1.71 (m, 3H), 1.29 (m, 3H), 0.93 (m, 6H). IR: 3285, 3000−3100, 2800−3000, 1736, 1666, 1649, 1632, 1538, 835, 799, 763, 721 cm^{-1}. ESMS: 571.3 (M + H$^+$). Isolated yield: 4.8 mg, 35%.

R_1 = Me; R_2 = Isobutyl. Diasteromeric Ratio = 1.54:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80−7.35 (m, 8H), 7.05 (d, J = 6.0 Hz, 1H, d2), 6.88 (d, J = 6.3 Hz, 1H, d1), 6.51 (br, 1H), 6.05 (d, J = 8.1 Hz, 1H, d2), 5.97 (d, J = 8.1 Hz, 1H, d1), 4.44 (m, 1H), 4.38 (m, 1H), 4.15 (m, 2H), 3.70 (s, 2H, d2), 3.68 (s, 2H, d1), 1.98 (s, 3H, d2), 1.97 (s, 3H, d1), 1.55 (m, 2H), 1.45 (m, 1H), 1.31 (d, J = 7.2 Hz, 3H, d2), 1.30 (d, J = 7.6 Hz, 3H, d1), 0.89 (m, 6H). IR: 3284, 3000−3100, 2800−3000, 1737, 1671, 1587, 1537, 835, 801, 770, 721 cm^{-1}. ESMS: 453.3 (M + H$^+$). Isolated yield: 1.9 mg, 17%.

R_1 = Me; R_2 = Benzyl. Diasteromeric Ratio = 1.35:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.62−7.10 (9m, 13H), 6.78 (d, J = 6.6 Hz, 1H, d1), 6.67 (d, J = 6.6 Hz, 1H, d2), 6.66 (m, 1H), 6.34 (d, J = 7.5 Hz, 1H, d1), 6.21 (d, J = 7.4 Hz, d2), 4.57 (m, 1H), 4.37 (9qd, J = 7.1 Hz, J = 7.1 Hz, 1H, d2), 4.35 (qd, J = 7.1 Hz, J = 7.1 Hz, 1H, d1), 4.09 (d, J = 5.1 Hz, 2H, d2), 4.01 (t, J = 4.8 Hz, 2H, d1), 3.66 (s, 2H, d2), 3.62 (s, 2H, d1), 2.96 (s, 2H), 1.92 (s, 3H), 1.21 (d, J = 7.1 Hz, 3H, d2), 1.10 (d, J = 7.2 Hz, 3H, d1). IR: 3300−3273, 3000−3100, 2800−3000, 1732, 1654−1642, 1538, 756, 720 cm^{-1}. ESMS: 487.2 (M + H$^+$). Isolated yield: 3.5 mg, 30%.

R_1 = Me; R_2 = Isopropyl. Diasteromeric Ratio = 1.2:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90−7.40 (m, 8H), 6.95 (d, J = 6.5 Hz, 1H, d1), 6.74 (d, J = 6.5 Hz, 1H, d2), 6.62 (b, 1H), 6.21 (d, J = 8.5 Hz, 1H, d1), 6.16 (d, J = 8.3 Hz, 1H, d2), 4.51 (qd, J = 7.1 Hz, J = 7.1 Hz, 1H, d1), 4.45 (qd, J = 7.1 Hz, J = 7.1 Hz, 1H, d2), 4.16 (m, 3H), 3.69 (s, 2H, d2), 3.68 (s, 2H, d1), 2.02 (m, 1H), 2.00 (s, 3H, d2), 1.99 (s, 3H, d1), 2.02 (m, 1H), 1.31 (d, J = 7.2 Hz, 3H, d2), 1.30 (d, J = 7.2 Hz, 3H, d1), 0.89 (m, 6H). IR 3284, 3280, 3000−3100, 2800−3000, 1732, 1673, 1641, 1632, 1544, 799, 766, 721 cm^{-1}. ESMS: 439.3 (M + H$^+$). Isolated yield: 2.6 mg, 25%.

R_1 = 2-Benzothiophene; R_2 = Benzyl. Diasteromeric Ratio = 1.47:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (m, 21H), 4.81 (m, 1H), 4.44 (m, 1H), 4.10 (m, 2H), 3.67 (s, 2H, d2), 3.63 (s, 2H, d1), 3.12 (m, 2H), 1.11 (d, J = 7.0 Hz, 3H, d2), 1.06 (d, J = 7.0 Hz, 3H, d1). IR: 3288, 3000−3100, 2800−3000, 1736, 1677, 1543, 835, 800, 761 cm^{-1}. ESMS: 605.2 (M + H$^+$). Isolated yield: 7.5 mg, 52%.

R_1 = 2-Benzothiophene; R_2 = Isopropyl. Diasteromeric Ratio = 1.46:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88−6.80 (m, 16H), 4.57 (qd, J = 7.0 Hz, J = 7.0 Hz, 1H, d1), 4.50 (qd, J = 7.0 Hz, J = 7.0 Hz, 1H, d2), 4.42 (m, 1H), 4.17 (b, 2H), 3.68 (s, 2H, d2), 3.65 (s, 2H, d1), 2.15 (m, 1H), 1.32 (d, J = 7.0 Hz, 3H, d2), 1.30 (d, J = 6.9 Hz, 3H, d1), 0.90 (m, 6H). IR: 3204, 3000−3100, 2800−3000, 1743, 1677, 1540, 836, 800, 773 cm^{-1}. ESMS: 557.2 (M + H$^+$). Isolated yield: 3.9 mg, 29%.

R_1 = 3,4-Dimethoxy-phenyl; R_2 = Isobutyl. Diasteromeric Ratio = 1.28:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90−6.62 (m, 14H), 4.62 (m, 1H), 4.50 (qd, J = 6.9 Hz, J = 6.9 Hz, 1H, d1), 4.42 (qd, J = 6.9 Hz, J = 6.9 Hz, 1H, d2), 4.16 (m, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.68 (s, 2H, d2), 3.65 (s, 2H, d1), 2.61 (m, 3H), 1.29 (d, J = 7.1 Hz, 3H), 0.90 (m, 6H). IR: 3300, 3000−3100, 2800−3000, 1730, 1679, 1541, 1605, 1585, 1508, 834, 800, 769, 1271, 1203, 1181, 1132 cm^{-1}. ESMS: 575.2 (M + H$^+$). Isolated yield: 3.6 mg, 26%.

R_1 = 3,4-Dimethoxy-phenyl; R_2 = Benzyl. Diasteromeric Ratio = 1.20:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80−6.70 (m, 17H), 6.60 (b, 2H), 4.77 (m, 1H), 4.41 (qd, J = 7.1 Hz, J = 7.1 Hz, 1H, d1), 4.39 (qd, J = 7.1 Hz, J = 7.1 Hz, 1H, d2), 4.11 (d, J = 5.2 Hz, 2H, d1), 4.05 (d, J = 5.2 Hz, 2H, d2), 3.88 (s, 3H), 3.86 (s, 3H), 3.69 (s, 2H, d2), 3.65 (s, 2H, d1), 3.17 (m, 2H), 1.23 (d, J = 7.1 Hz, 3H, d2), 1.13 (d, J = 7.1 Hz, 3H, d1). IR: 3278, 3000−3100, 2800−3000, 1730, 1655, 1535, 1603, 1584, 1505, 799, 755, 720, 1286, 1202, 1179, 1130 cm^{-1}. ESMS: 609.3 (M + H$^+$). Isolated yield: 2.7 mg, 18%.

R_1 = 3,4-Dimethoxy-phenyl; R_2 = Isopropyl. Diasteromeric Ratio = 1.32:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74−6.50 (m, 13H), 6.35 (b, 1H), 4.72 (m, 1H), 4.47 (m, 1H), 4.20 (m, 2H), 3.92 (s, 3H), 3.91 (s, 3H), 3.69 (s, 2H, d2), 3.67 (s, 2H, d1), 2.35 (m, 1H), 1.35 (d, J = 7.0 Hz, 3H, d2), 1.34 (d, J = 7.0 Hz, 3H, d1), 1.04 (m, 6H). IR: 3307, 3000−3100, 2800−3000, 1730, 1678, 1537, 1604, 1587, 1506, 800, 770, 723, 1286, 1206, 1183, 1134 cm^{-1}. ESMS: 561.3 (M + H$^+$). Isolated yield: 0.9 mg, 7%.

R_1 = 2-Quinoline; R_2 = Isobutyl. Diasteromeric Ratio = 1.31:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92−7.41 (m, 15 H), 7.19 (d, J = 6.5 Hz, 1H, d1), 6.96 (d, J = 6.5 Hz, 1H, d2), 6.63 (bd, J = 4.0 Hz, 1H), 4.63 (m, 1H), 4.54 (qd, J = 7.0 Hz, J = 7.0 Hz, 1H, d1), 4.50 (qd, J = 7.0 Hz, J = 7.0 Hz, H, d2), 4.19 (m, 2H), 3.69 (s, 2H, d2), 3.67 (s, 2H, d1), 1.31 (d, J = 8.1 Hz, 3H, d2), 1.30 (d, J = 7.3 Hz, 3H, d1), 0.96 (m, 6H). IR: 3295, 3000−3100, 2800−3000, 1737, 1673, 1529, 1563, 1501, 837, 800, 776, 722 cm^{-1}. ESMS: 566.2 (M + H$^+$). Isolated yield: 2.6 mg, 19%.

R_1 = 2-Quinoline; R_2 = Benzyl. Diasteromeric Ratio = 1.41:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.86−7.10 (m, 20H), 6.92 (bd, J = 7.6 Hz, 1H, d1), 6.84 (bd, J = 7.6 Hz,

1H, d2), 6.71 (b, 1H, d1), 6.64 (b, 1H, d2), 4.81 (td, $J = 7.0$ Hz, $J = 7.0$ Hz, 1H), 4.43 (m, 1H), 4.13 (d, $J = 5.2$ Hz, 2H, d1), 4.06 (d, $J = 5.2$ Hz, 2H, d2), 3.68 (s, 2H, d2), 3.65 (s, 2H, d1), 3.24 (m, 2H), 1.20 (d, $J = 7.7$ Hz, 3H, d2), 1.14 (d, $J = 7.7$ Hz, 3H, d1). IR: 3301, 3000−3100, 2800−3000, 1734, 1672, 1524, 1563, 1500, 846, 832, 798, 775, 720 cm^{-1}. ESMS: 600.2 (M + H^+). Isolated yield: 3.6 mg, 25%.

R_1 = 2-Quinoline; R_2 = Isopropyl. Diasteromeric Ratio = 1.41:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90−7.45 (m, 14H), 7.04 (bd, $J = 6.5$ Hz, 1H, d2), 6.98 (bd, $J = 6.5$ Hz, 1H, d2), 6.83 (bd, $J = 6.5$ Hz, 1H), 6.68 (b, 1H), 4.53 (m, 1H), 4.40 (m, 1H), 4.19 (m, 2H), 3.69 (s, 2H, d2), 3.66 (s, 2H, d1), 2.34 (m, 1H), 1.32 (d, $J = 7.4$ Hz, 3H, d2), 1.31 (d, $J = 7.2$ Hz, 3H, d1), 1.08 (m, 6H). IR: 3290, 3000−3100, 2800−3000, 1731, 1667, 1526, 1564, 1501, 798, 775 cm^{-1}. ESMS: 552.3 (M + H^+). Isolated yield: 4.0 mg, 30%.

R_1 = 6-Quinoline; R_2 = Isobutyl. Diasteromeric Ratio = 1.20:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.05−7.26 (m, 16H), 6.76 (b,1H), 4.70 (td, $J = 7.8$ Hz, $J = 7.8$ Hz, 1H, d1), 4.46 (qd, $J = 7.1$ Hz, $J = 7.1$ Hz, 1H, d2), 4.18 (m, 2H), 3.68 (s, 2H, d2), 3.64 (s, 2H, d1), 1.74 (m, 3H), 1.32 (d, $J = 7.2$ Hz, 3H), 0.97 (m, 6H); IR: 3276, 3255, 3000−3100, 2800−3000, 1733, 1671, 1540, 832, 799, 772, 720 cm^{-1}. ESMS: 566.2 (M + H^+). Isolated yield: 4.0 mg, 29%.

R_1 = 6-Quinoline; R_2 = Benzyl. Diasteromeric Ratio = 1.68:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.10−7.12 (m, 20H), 7.01 (d, $J = 6.9$ Hz, 1H, d1), 6.84 (d, $J = 6.9$ Hz, 1H, d2), 6.76 (m, 1H), 4.88 (m, 1H), 4.48 (qd, $J = 7.1$ Hz, $J = 7.1$ Hz, 1H, d1), 4.47 (qd, $J = 7.1$ Hz, $J = 7.1$ Hz, 1H, d2), 4.12 (m, 2H), 3.64 (s, 2H, d2), 3.63 (s, 2H, d1), 3.61 (m, 2H), 1.31 (d, $J = 7.2$ Hz, 3H, d2), 1.15 (d, $J = 7.2$ Hz, 3H, d1). IR: 3283, 3255, 3000−3100, 2800−3000, 1736, 1676, 1649, 1538, 1497, 832, 799, 766, 721 cm^{-1}. ESMS: 600.3 (M + H^+). Isolated yield: 4.2 mg, 29%.

R_1 = 6-Quinoline; R_2 = Isopropyl. Diasteromeric Ratio = 1.20:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.05−7.41 (m, 14H), 7.01 (bd, $J = 9.0$ Hz, 1H, d1), 6.99 (bd, $J = 9.0$ Hz, 1H, d2), 6.86 (bd, $J = 6.5$ Hz, 1H, d1), 6.68 (bd, $J = 6.5$ Hz, 1H, d2), 6.47 (b,1H), 4.46 (m, 2H), 4.18 (m, 2H), 3.72 (s, 2H, d2), 3.68 (s, 2H, d1), 2.23 (m, 1H), 1.37 (d, $J = 7.1$ Hz, 3H, d2), 1.36 (d, $J = 7.1$ Hz, 3H, d1), 1.02 (m, 6H). IR: 3276, 3283, 3000−3100, 2800−3000, 1736, 1682, 1545, 1498, 1465, 1433, 840, 801, 723 cm^{-1}. ESMS: 552.2 (M + H^+). Isolated yield: 1.0 mg, 8%.

R_1 = 4-Trifluoromethylphenyl; R_2 = Isobutyl. Diasteromeric Ratio = 1.45:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88−7.39 (m, 12H), 7.17 (bd, $J = 7.0$ Hz, 1H, d1), 7.07 (bd, $J = 8.0$ Hz, 1H, d2), 7.05 (bd, $J = 7.0$ Hz, 1H, d1), 6.97 (bd, $J = 8.0$ Hz, 1H, d2), 6.70 (b, 1H), 4.68 (td, $J = 8.5$ Hz, $J = 8.5$ Hz, 1H, d1), 4.66 (td, $J = 8.5$ Hz, $J = 8.5$ Hz, 1H, d2), 4.16 (m, 2H), 3.69 (s, 2H, d2), 3.66 (s, 2H, d1), 1.71 (m, 3H), 1.30 (d, $J = 7.1$ Hz, 3H), 0.91 (m, 6H). IR: 3284, 3255, 3000−3100, 2800−3000, 1742, 1657, 1538, 1583, 863, 773, 722, 1329 cm^{-1}. ESMS: 583.2 (M + H^+). Isolated yield: 3.5 mg, 25%.

R_1 = 4-Trifluoromethylphenyl; R_2 = Benzyl. Diasteromeric Ratio = 1.13:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.76−7.12 (m, 17H), 7.10 (bd, $J = 7.4$ Hz, 1H, d1), 6.99 (bd, $J = 7.4$ Hz, 1H, d2), 6.75 (bd, $J = 6.8$ Hz, 1H, d2), 6.67 (bd, $J = 6.8$ Hz, 1H, d1), 6.47 (b, 1H), 4.82 (td, $J = 7.4$ Hz, $J = 7.4$ Hz, 1H, d1), 4.78 (td, $J = 7.4$ Hz, $J = 7.4$ Hz, 1H, d2), 4.44 (qd, $J = 6.8$ Hz, $J = 6.8$ Hz, 2H), 4.40 44 (qd, $J = 6.8$ Hz, $J = 6.8$ Hz, 2H), 4.06 (m, 2H), 3.69 (s, 2H, d2), 3.64 (s, 2H, d1), 3.16 (m, 2H), 1.19 (d, $J = 8.1$ Hz, 3H, d2), 1.14 (d, $J = 7.2$ Hz, 3H, d1). IR: 3293, 3255, 3000−3100, 2800−3000, 1741, 1651, 1638, 1536, 1581, 862, 775, 752, 1330 cm^{-1}. ESMS: 617.1 (M + H^+). Isolated yield: 5.9 mg, 40%.

R_1 = 4-Trifluoromethylphenyl; R_2 = Isopropyl. Diasteromeric Ratio = 1.10:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95−7.46 (m, 13H), 6.96 (m, 1H), 6.78 (m, 1H), 4.60 (qd, $J = 7.0$ Hz, $J = 7.0$ Hz, 1H, d1), 4.57 (qd, $J = 7.0$ Hz, $J = 7.0$ Hz, 1H, d1), 4.54 (m, 1H), 4.17 (m, 2H), 3.70 (s, 2H, d2), bd, $J = 8.0$ Hz, 1H, d2), 3.69 (s, 2H, d1), 2.17 (m, 1H), 1.33 (d, $J = 6.8$ Hz, 3H, d2), 1.32 (d, $J = 6.8$ Hz, 3H, d1), 0.97 (m, 6H). IR: 3274, 3255, 3000−3100, 2800−3000, 1739, 1658, 1637,1538, 1582, 859, 838, 800, 772, 722, 1329 cm^{-1}. ESMS: 569.2 (M + H^+). Isolated yield: 5.1 mg, 37%.

(*S*)- *N*-Boc-3-amino-1-*N*-(3-(2-pyridyl)-phenyl acetyl)-amino-butan-2-ol. (*S*)-*N*-Boc-3-amino-2-hydroxy-butylamine (6.4 g, 31.37 mmol) was dissolved in DMF (60 mL). Then, *N*-methyl morpholine (3.5 mL, 31.7 mmol), 3-(2-pyridyl)-phenyl acetic acid (6.7 g, 31.37 mmol), and HBTU (12 g, 31.7 mmol) were added, and the reaction mixture was stirred overnight. Then the reaction mixture was concentrated in vacuo and chromatographed on silica gel to yield the title compound as a white solid (5.7 g, 45%): ^{1}H NMR (400 MHz, CDCl3): δ 8.65 (d, 1H), 7.90 (s, 1H), 7.85−7.70 (m, 3H), 7.46−7.40 (m, 1H), 7.38−7.30 (m, 1H), 7.25 (t, 1H), 6.50 (br s, 1H), 4.85 (d, 1H), 3.70−3.60, 3.50 (s, 2H), 3.40−3.30 (m, 1H), 3.20−3.10 (m, 1H), 1.40 (s, 9H), 1.15 (d, 3H). MS (ES+) 400.2 (M + H^+).

(*S*)-3-Amino-1*N*-(3-(2-pyridyl)-phenyl acetyl)-amino-butan-2-ol. (*S*)- *N*-Boc-3-amino-1-*N*-(3-(2-pyridyl)-phenyl acetyl)-amino-butan-2-ol (2.6 g, 65 mmol) was dissolved in 4 M HCl in dioxane (80 mL) and was stirred at room temperature overnight. Toluene (200 mL) was added, the reaction mixture was concentrated in vacuo, and the resulting title compound was used in the following reaction without further purification. (*S*)-3-Amino-1*N*-(3-(2-pyridyl)-phenyl-acetyl)-amino-butan-2-ol (2.4 g, 6.5 mmol) was dissolved in DMF (25 mL). Then *N*-methyl morpholine (3.5 mL, 31.7 mmol), Boc-leucine (2.0 g, 8 mmol), and HBTU (3.0 g, 8 mmol) were added, and the reaction mixture was stirred overnight. Then the reaction mixture was concentrated in vacuo, the crude product was triturated in ether, and the white solid was used in the next reaction without further purification (2.65 g, 80%), ^{1}H NMR (400 MHz, $CDCl_3$): 8.52 (d, 1H), 7.37−7.75 (m, 3H), 7.50−7.30 (m, 4H, 5.70 (d, 1H), 3.90−3.85 (m, 1H), 3.68 (s, 2H), 3.60−3.50 (m, 2H), 3.05−2.95 (m, 1H), 1.70−1.60 (m, 1H), 1.45 (s, 9H), 1.15 (d, 3H), 0.95 (t, 6H). MS (ES+) 513.4 (M + H^+).

(*S*)-3*N*-(*N*-(Benzothiophene-2-carbonyl)-L-leucinyl)-amino-1-*N*-(3-{2-pyridyl}phenylacetyl)-amino-butan-2-ol. (*S*)-3-*N*-(*N*-Boc-L-leucinyl)-amino-1-*N*-(3-(2-pyridyl)-phenylacetyl)-amino-butan-2-ol (1.4 g, 2.73 mmol) was dissolved in 4M HCl in dioxane (60 mL) and CH_2Cl_2 (100 mL) and was stirred at room temperature overnight. The

reaction mixture was concentrated in vacuo toluene was added (20 mL), the solution was concentrated in vacuo, and the crude product was used in the next reaction without further purification. MS (ES): 413.3 ($M + H^+$). (*S*)-3-*N*-(L-leucinyl)-amino-1-*N*-(3-(2-pyridyl)-phenyl acetyl)-amino-butan-2-ol·2HCl (0.92 g, 1.9 mmol) was dissolved in DMF (15 mL) and *N*-methyl morpholine (0.82 mL, 7.5 mmol), thianaphthenyl-2-carboxylic acid (0.338 g, 1.9 mmol), and HBTU (0.72 g, 1.9 mmol) were added, and the reaction mixture was stirred for 4 h. Then, the reaction mixture was poured into water (10 mL), and the aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organics were dried with MgSO4, filtered, and concentrated in vacuo, the crude product was triturated in ether, and the white solid was used in the next reaction without further purification (0.65 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$/MeOD): 8.61δ (d, 1H), 7.92 (s, 1H), 7.78−7.70 (m, 6H), 7.50−7.28 (m, 5H), 4.70−4.60 (m, 1H), 4.0−3.85 (m, 1H), 3.65 (s, 2H), 3.60−3.52 (m, 1H), 3.30−3.20 (m, 1H), 3.15−3.05 (m, 1H), 1.75−1.60 (m, 3H), 1.18 (d, $J = 6.78$ Hz, 3H), 1.0−0.9 (m, 6H). IR (KBr): 3328, 1642, 1622, 1535 cm $^{-1}$. HRMS: 572.2453.

(*S*)-3*N*-(*N*-(Benzothiophene-2-carbonyl)-L-leucinyl)-amino-1*N*-(3-{2-pyridyl}phenylacetyl)-amino-butan-2-one. (*S*)-3*N*-(*N*-(benzothiophene-2-carbonyl)-L-leucinyl)-amino-1-*N*-(3-{2-pyridyl}phenylacetyl)-amino-butan-2-ol (0.155 g, 0.27 mmol) was heated in CH_2Cl_2 (70 mL) until it fully dissolved. It was then cooled to room temperature, Dess−Martin periodinane (0.172 g, 0.40 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. Aqueous 10% $Na_2S_2O_3$ (10 mL) and aqueous 10% $NaHCO_3$ (10 mL) were added, and the reaction mixture was stirred for 10 min. The organic layer was dried ($MgSO_4$), filtered, concentrated in vacuo, and chromatographed on silica gel (3% MeOH, CH_2Cl_2) to yield the title compound as a white solid (0.13 g, 84%). ^{1}H NMR (360 MHz, $CDCl_3$): 8.69 (d, $J = 2.1$ Hz, 1H), 7.94 (s, 1H), 7.85-7.75 (m, 6H), 7.50−7.35 (m, 4H), 7.30−7.26 (m, 1H), 7.10 (d, $J = 6.49$ Hz, 1H), 6.84 (d, $J = 8.08$ Hz, 1H), 6.40 (t, $J = 4.91$ Hz, 1H), 4.66-4.60 (m, 1H), 4.50-4.40 (m, 1H), 4.20 (ABq, $\delta\Delta = 4.02$ Hz, 2H), 3.70 (s, 2H), 1.80-1.60 (m, 3H), 1.33 (d, $J = 7.16$ Hz, 3H), 0.94 (2D, $J = 6.1$ Hz, $J = 6.02$ Hz, 6H). IR (KBr): 3383, 1732, 1649, 1626, 1537 cm^{-1}. HRMS: 570.2295.

Inhibition Assay Conditions. Inhibitors were evaluated for inhibition against purified recombinant cathepsin K as described in ref 8b. Inhibitors were assayed against human liver Cathepsin L (Calbiochem) and human liver Cathepsin B (Calbiochem) with the following substrates: Cbz-Phe-Arg-AMC at 5 μM ($K_m = 3$ μM) and Cbz-Phe-Arg-AMC at 50 μM ($K_m = 140$ μM), respectively, in 100 mM acetate, 20 mM cysteine, 5 mM EDTA, pH 5.5 buffer, with a final DMSO concentration of 10%.

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