α-Keto Amide Inhibitors of Aminopeptidases[1]

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The design and synthesis of 3-amino-2-oxo-4-phenylbutanoic acid amides (α-keto amides), a new class of aminopeptidase inhibitor, are described. These compounds, illustrated by the Phe-Leu analogue **2**, are effective inhibitors of arginyl aminopeptidase (K_i = 1.5 μM), cytosol aminopeptidase (K_i = 1.0 μM), and microsomal aminopeptidase (K_i = 2.5 μM). The ketone carbonyl of the α-keto amide was found to hydrate readily in an aqueous DMSO solution, due to the electron-withdrawing effect of the neighboring amide group. A mechanism of inhibition is proposed for the α-keto amides that is similar to that proposed for the structurally related aminopeptidase inhibitor bestatin and its analogues, wherein the inhibitor may interact with the S_1'–S_2' subsite of the enzyme rather than the S_1–S_1' subsite. Like bestatin, the α-keto amides are slow-binding inhibitors of all three enzymes.

α-Keto esters have been successfully incorporated into peptidic molecules by Powers and co-workers[2] to generate potent inhibitors of the serine proteinase elastase. More recently, α-keto esters and α-diketones have been shown to be very effective inhibitors of α-chymotrypsin and the cysteine proteinase calpain.[3,4] In the course of our study on the mechanism of inhibition of aminopeptidases (APases) by bestatin[5] (**1**) and its analogues, we prepared low molecular weight ketone analogues of bestatin (e.g. **2**), which are quite similar electronically to the reported α-keto esters. Enzyme inhibitors that contain electron-deficient carbonyl groups can be hydrated in aqueous solution and are expected to be excellent protease inhibitors since the transition state for hydrolysis of an amide bond is more closely mimicked by the sp^3-hybridized hydrate (*gem*-diol) than by the sp^2-hybridized ketone. Herein we report the synthesis of several ketone analogues of bestatin; these α-keto amides are effective aminopeptidase inhibitors.

1 2

Results

Chemistry. The synthesis of the requisite Boc-protected α-hydroxy amide starting materials has been previously described.[6,7] The Boc ketones **5–7** (Table I) were synthesized in moderate yields (35–55%) by using pyridinium dichromate (PDC) in acetic acid. Low yields of ketone were generally caused by incomplete oxidation, and thus starting material could be recycled. It should be noted that oxidations employing activated DMSO (e.g. SO_3-py, DMSO, NEt_3) gave very poor yields of desired product (<25%). The methyl ketone (**10**) derived from Boc-leucine, was obtained by addition of methyllithium to the free acid of Boc-leucine. The Boc group was removed from the α-keto amides by using 4 N HCl–dioxane, and the HCl salts **2–4** were isolated by filtration after trituration with ether (Scheme I).

Inhibition constants for each analogue were determined as previously described.[7] Initially we attempted to determine the importance of chirality at the side-chain bearing carbon (C-3) on inhibitor potency by oxidizing the 3*R* and 3*S* alcohol precursors separately. However, epimerization adjacent to the ketone occurred rapidly so that the resulting ketones were obtained as variable mixtures of the two enantiomers at C-3. When the pure 3*R* and 3*S* alcohol precursors were oxidized, the resulting ketones were

Table I. Physical Constants of Ketone Analogues

compd	A	R1	% yield	formula	anal.
2	HCl·H	Ph	97	$C_{15}H_{23}N_2O_2Cl$	CHN
3[b]	HCl·H	$CH(CH_3)_2$	67	$C_{12}H_{25}N_2O_2Cl\cdot{}^3/_5H_2O$	CHN
4[c]	HCl·H	$CH(CH_3)_2$	67	$C_{12}H_{25}N_2O_2Cl$	CHN
5	Boc	Ph	52	$C_{20}H_{30}N_2O_4$	CHN
6[b]	Boc	$CH(CH_3)_2$	35	$C_{17}H_{32}N_2O_4$	a
7[c]	Boc	$CH(CH_3)_2$	55	$C_{17}H_{32}N_2O_4$	CHN

[a] Calcd: C, 62.17; H, 9.82; N, 8.53. Found: C, 61.75; H, 9.95; N, 8.37. [b] Derived from the 3*S* amine. [b] Derived from the 3*R* amine.

Scheme I

PDC / HOAc → 5

4N HCl / dioxane → 2

(1) (a) Abbreviations used: Boc, *tert*-Butyloxycarbonyl; AHBPA, 3-amino-2-hydroxy-4-phenylbutanoic acid; EtOAc, ethyl acetate; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; Ac, acetyl; Bn, benzyl; Iva, isovaleryl; Iaa, isoamylamide; Z, benzyloxycarbonyl; AP-B, aminopeptidase B or arginyl aminopeptidase; AP-M, aminopeptidase M or microsomal aminopeptidase; LAP, leucine aminopeptidase or cytosol aminopeptidase. S_1, S_1', P_1, P_1', etc. follow the conventions of Schecter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162. (b) Abstracted in part from the Ph.D. Thesis of T. D. Ocain, submitted to the Graduate School of the University of Wisconsin-Madison, April 1986.

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Table II. Kinetic Constants for Inhibitors

compd	K_i (10^{-6} M) cytosolic AP	microsomal AP	argininyl AP
2	1.0	2.5	1.5
3	1.9	10.5	3.2
4	>15[a]	18.6	6.5
8[b]	5.4	24	>300
9[b]	0.51	20	39
10	>600	430	>1000
11[c]	1100	K_{is} 660[d] K_{ii} 940	130
12[c]	57	K_{is} 79[d] K_{ii} 260	0.17

* "S" derived. ** "R" derived. [a] Solubility limit. [b] Reference 7. [c] Reference 9. [d] Noncompetitive, slow-binding.

enriched in the corresponding precursor.

NMR and Hydration Results. Boc keto amides 5–7 showed two peaks in the proton NMR spectrum for the Boc resonance (~1.45 ppm), which was subsequently shown to be due to the presence of multiple conformers in solution (vide infra). An extra Boc peak is also seen in the ^{13}C NMR spectra for 5 and 7 but was not observed for 6. (This is probably simply a detection problem since the extra Boc peak in the proton spectrum of 6 was very small.) Except for the extra signal in the spectrum of 7, the ^{13}C NMR spectra for 6 and 7 were *identical*. The effect of solvent on conformation was determined by obtaining the proton NMR spectrum of 5 in both hydrogen bonding and non-hydrogen bonding solvents. In the non-hydrogen bonding solvent, $CDCl_3$, the phenyl protons appeared as multiple signals in contrast to the singlets normally observed for the starting alcohol and related compounds (data not shown). When the solvent was switched to the hydrogen bonding solvent, DMSO-d_6, the multiplicity collapsed and the phenyl protons appeared as a singlet. These data establish that the multiplicities observed in $CDCl_3$ arise from conformational differences induced by intramolecular hydrogen bonding.

It was not possible to determine the extent of hydration of the ketone group in a completely aqueous system, because of the low solubility of the ketones in water. However, when D_2O was added to a DMSO-d_6 solution of ketone 2 (17% v/v solubility limit) a new set of resonances was detected in the ^{13}C NMR spectrum. New peaks at 94.21 and 172.2 ppm are consistent[8] with a hydrated ketone

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(*gem*-diol) α to an amide and the adjacent amide carbonyl carbon; a smaller peak at 161 ppm is due to residual amide carbonyl from the nonhydrated α-keto amide. About 50% of the ketone group is hydrated in 17% D_2O/DMSO-d_6 (v/v).

Addition of methanol to the ketone carbonyl group also occurred in both the Boc-protected and free amino ketones (**5** and **2**); the hemiacetals were assigned from their resonances at 100.11 and 97.3 ppm, respectively.

Biological Results. The keto amides reported here are potent competitive inhibitors of three aminopeptidases (Table II). In general, they are better aminopeptidase inhibitors than the recently reported[9] ketomethylene inhibitors, with the exception of the inhibition of arginyl aminopeptidase by compound **12**. The ketomethylene results cited here serve as a reference for the inhibition produced by ketone analogues that do not readily hydrate in solution. For the α-keto series with the isobutyl side chain at C-3, the 3*S*-derived compound **3** is slightly more potent than the 3*R*-derived compound **4**. Although this center readily undergoes epimerization, it appears that the 3*S*-derived ketone ($[\alpha]^{23}_D$ +24.3° (c = 0.12, $CHCl_3$)) binds more favorably to the aminopeptidases than the 3*R*-derived ketone ($[\alpha]^{23}_D$ −18.2° (c = 0.11, $CHCl_3$)). In most cases, the ketones are more potent inhibitors of aminopeptidases than the 2*S* hydroxy analogues. The 2*R* analogues of bestatin have been shown previously to be much less active inhibitors of APases.[6] For example, compound **2** has a K_i of 1.0 μM for cytosol aminopeptidase and 1.5 μM for arginyl aminopeptidase, whereas alcohol **9** has a K_i of 20 and 29 μM for cytosol aminopeptidase and arginyl aminopeptidase, respectively. Only in the case of microsomal aminopeptidase is the hydroxy analogue slightly more effective than the ketone (0.51 vs 2.5 μM). In general, the ketomethylene analogues and the α-hydroxy compounds did not display slow binding kinetics. In contrast, the α-keto amides showed dramatic time-dependent, slow-binding inhibition, similar to that noted with other potent inhibitors of aminopeptidases, e.g. bestatin.[6] Finally, the methyl ketone of *L*-leucine **10** is a poor inhibitor of aminopeptidases that does not show time-dependent inhibition.

Discussion

Potent inhibitors of all classes of peptidases have been obtained by replacing the scissile amide bond of a peptidase substrate with a carbonyl functionality. This design principle was first suggested by the discoveries of antipain (**13**), a good inhibitor of cysteine proteinases,[10] and leupeptin (**14**), an excellent inhibitor of cysteine and serine proteases.[11] Aldehydic inhibitors of aspartyl proteases (e.g. renin[12]) and metalloproteases (e.g. aminopeptidases[13]) are also known. In 1980, Almquist[14] reported a substrate-derived ketone inhibitor **15** which strongly inhibited the metalloprotease angiotensin converting enzyme (ACE). Gordon and co-workers have described novel amino ketone inhibitors (e.g. **16**) of ACE as well.[15] It rapidly became apparent that ketone-containing compounds would serve as inhibitors for a broad range of enzymes, although often inhibiting by different mechanisms. Szelke discovered that replacement of the scissile amide bond with a ketomethylene isostere gave very good inhibitors of renin.[16] Rich et al.[17] showed that ketone analogues of potent statine-containing compounds ("statones" e.g. **17**) are good aspartyl protease inhibitors. The ketone group in these inhibitors and in ketomethylene compounds[18] was shown by ^{13}C NMR to exist as a *gem*-diol when bound to the enzyme (porcine pepsin), which is formed via an enzyme-catalyzed addition of water.[8] However, the ketone inhibitors are less potent than their hydroxy counterparts, due to the sacrifice of binding energy needed to drive the hydration process. Abeles and co-workers incorporated fluorine into statone-like inhibitors to render the carbonyl more electron-deficient. Difluorostatone derivatives (e.g. **18**), which are very potent inhibitors of procine pepsin,[19]

H2N NH HN (CH2)3 HO2C N H O NH O (CH2)3 N H H O H2N NH HN H2N NH HN (CH2)3 Ac-Leu-Leu-NH H O
13 14

O C6H5 NH-Bn N O CO2H C6H5 Bn-NH O N H N O CO2H
15 16

Iva-Val-Val-NH O O Ala-Iaa Iva-Val-Val-NH F F O O Ala-Iaa
17 18

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form a stable *gem*-diol hydrate in aqueous solution thereby eliminating the need for enzyme-catalyzed hydration of the ketone. The concept of di- or trifluoro ketones has been extended to inhibitors of potentially therapeutically useful enzymes including renin[20] and elastase.[21] Several groups have extended this concept by placing a nonhalogen electron-withdrawing group adjacent to the carbonyl, for example an ester group or another ketone. Powers and co-workers[2] synthesized α-keto esters (e.g. **19**) as effective inhibitors of elastase. Bey and co-workers[3,4] have synthesized α-keto esters (e.g. **20**) and α-diketones (e.g. **21**) as inhibitors of serine and cysteine proteinases. Hu and Abeles have also described α-keto esters, amides, and acids and diketones as inhibitors of the cysteine proteinases Cathepsin B and papain.[22] These "activated" ketones, like the fluoro ketones, easily hydrate in aqueous solution.

Z-Ala-Ala-NH ... OEt **19**; Z-NH ... OEt **20**; Z-NH ... CH_3 **21**

The aminopeptidases, a diverse group of metalloproteases that cleave the N-terminal amino acid from peptides and proteins, also are inhibited by carbonyl compounds. In 1982 Wolfenden and co-workers[13] demonstrated that leucinal (**22**) and related compounds are very potent ($K_i = 10^{-7}$–10^{-8} M) inhibitors of microsomal aminopeptidase and cytosol aminopeptidase. Deuterium

H_2N ... H **22**

isotope effect experiments[23] suggested that leucinal binds to the enzyme as an oxygenated adduct, either as the *gem*-diol hydrate (Figure 1B) or a hemiacetal derived from addition of an enzyme-derived amino acid side chain hydroxy group; in this regard, the *gem*-diol is expected to mimic the tetrahedral intermediate for amide bond hydrolysis (Figure 1A). Umezawa reported that the arphamenines (**23** and **24**), natural products that contain the ketomethylene moiety, are effective inhibitors of arginyl aminopeptidase.[24] Unlike the aldehydic inhibitors, hydration does not occur to an appreciable extent in solution. Synthetic ketomethylene inhibitors that incorporate substrate specificity for aminopeptidases have been shown to

A. Hydrolysis:

-HN ... NH ... NH- —Enz / H_2O→ [-HN ... HO OH ... NH ... NH-] (R_1, R_2)

B. Hydrated Leucinal

H_2N ... H ⇌ (H_2O) H_2N ... HO OH H

C. Activated ketone inhibitor:

-HN ... X (R_1) ⇌ (H_2O) -HN ... HO OH X

X = $\bar{e}$-withdrawing group

Figure 1. (A) Transition state of hydrolysis. (B) Structure of leucinal as a hydrate. (C) Activated ketone inhibitor.

be good inhibitors of other aminopeptidases[9] (e.g. **25**) but not of leucine aminopeptidase. Finally, Delaney et al.[25] have reported amino ketones that are very potent inhibitors of rat brain aminopeptidase (e.g. **26**).

H_2N, NH, HN, $(CH_2)_3$, H_2N ... OH, R — **23** R=H, **24** R=OH; H_2N ... Val-Iaa, CH_3 **25**; H_2N ... N H **26**; H_3N ... P ... NH ... O^- **27**

As an indication of the susceptibility of the ketones to hydration, our NMR results indicate that the ketone group of the α-keto amides is readily hydrated in aqueous DMSO to form the *gem*-diol. Thus, the α-keto amides resemble difluoro ketones, α-keto esters, and α-diketones in that a neighboring electron-withdrawing group enables the ketone to readily undergo hydration in aqueous solution (Figure 1C). The α-keto amides are less potent inhibitors of aminopeptidases than the simple amino acid aldehydes, even

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Figure 2. Proposed modes of binding of aminopeptidase inhibitors.

though the ketones contain potential P′ substituents that could interact with the enzyme S′ subsites. The α-keto amides **2–4** are slightly better inhibitors than the ketomethylene compounds, e.g. **11–12** (with the exception of **12** on arginyl aminopeptidase). The α-keto amides are also slightly more potent than the structurally related carbamoyl phosphinic acid analogue **27** reported by Giannousis and Bartlett[26] ($K_i = 56 \times 10^{-6}$ M; cytosol aminopeptidase). The α-keto amides **2–4** are slightly better than the corresponding precursor hydroxy analogues **8–9**.

At the time this project was initiated, it was anticipated that bestatin and related analogues might bind to the enzyme as analogues of the transition state for amide bond hydrolysis. In that mode, the critical hydroxyl group of bestatin was expected to mimic the *gem*-diol group of the tetrahedral intermediate (Figure 1A) and to bind to the enzyme in a fashion analoguous to substrate, leucinal, or the ketomethylene-containing arphamenines (see Figure 2, parts A–C), as shown in Figure 2D. Subsequently, Harbeson and Rich[9] established by kinetic methods and structure–activity comparisons that bestatin was most likely binding to arginyl aminopeptidase at the S_1'–S_2' sites (Figure 2E), rather than at the S_1–S_1' sites. Further, they suggested that, if arginyl aminopeptidase is related to the other aminopeptidases, then bestatin binds to these APase at the S_1'–S_2' sites. Recently, Burley et al. reported the X-ray crystal structure of bestatin bound to bovine lens leucine aminopeptidase at 2.3-Å resolution.[28] In that structure, the critical 2(*S*)-hydroxyl group and the N-terminal amino groups are complexed to the loosely bound zinc ion. Burley et al. suggested that the benzyl and isobutyl side chains of the AHPBA and Leu residues, respectively, were binding in the S_1–S_1' subsites (Figure 2G) and not in the S_1'–S_2' subsites as proposed for arginyl aminopeptidase.[28]

The data reported herein are not easily reconciled with this reassessment of the mechanism of action of bestatin. The α-keto amides confer only a small advantage over the ketomethylene inhibitors and are actually less effective on arginyl aminopeptidase than the ketomethylene compound **12**. If the leucine-derived side chain of the α-keto amides were interacting at S_1, then like leucinal, the dioxygenated adduct could be expected to impart much greater inhibition either due to an increased resemblance to the transition state (much like the difluoroketones on aspartyl proteinases) or increased interaction with the active-site zinc atoms. Instead, the α-keto amides are only slightly more potent than their hydroxy counterparts, as would be expected for bestatin analogues acting at the S_1'–S_2' site (Figure 2F). Alternatively, it is possible that the precise geometrical binding requirements for binding bestatin at S_1–S_1' are not being met by the α-keto amides; diol binding to the two active site zinc atoms may be disrupting the important proposed hydrogen bonds between the amide and Lys-262 and Leu-360. The lack of conformational flexibility of the amides versus leucinal may result in unfavorable interactions with the enzyme.

The X-ray structure of bestatin complexed to bovine lens leucine aminopeptidase reported by Burley et al.[28] was interpreted to be an analogue of the tetrahedral intermediate for the APase-catalyzed hydrolysis of substrate, but bestatin instead might mimic a collected product complex in which the chelating amino and hydroxyl groups mimic amine and carboxyl portions of the departing products (Figure 2F). Many angiotensin-converting enzyme (ACE) inhibitors are thought to bind to ACE as analogues of collected products, rather than as analogues of the tetrahedral intermediate.[29] Such a binding mode would be consistent with the reported structure–activity relationships and kinetic data; however, this binding mode is not consistent with the apparent absence of an additional unoccupied S_1 subsite in the bestatin–LAP complex, which would be expected for a collected-product complex.[28] Alternatively, the structure described by Burley et al.[28] approaches the geometry attained in the transition-state complex (Figure 2G), but the transition state is sterically demanding and cannot easily accommodate the phosphonate **27** and *gem*-diol (Figure 2G) transition-state analogues (or the thiolbestatin analogue[7]) in the active site of LAP without some energy penalty due to their slightly larger size.

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Experimental Section

A. Materials and Methods. Melting points were determined using a Fischer-Johns melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 polarimeter in a 1.000-dm cell. Proton nuclear resonance spectra were recorded on a Varian EM390 (90 MHz), a JEOL FX-90Q FT (90 MHz), or a Bruker W-270 (270 MHz) FT spectrometer. Chemical shifts are reported in ppm from an internal TMS standard. Carbon-13 spectra were recorded on the JEOL FX-90Q (22.5 MHz) spectrometer, and chemical shifts are reported in ppm downfield from internal TMS. Low-resolution mass spectra were obtained on a Finnegan 1015 GC-mass spectrometer interfaced to a Finnegan M6000 data system. Elemental analyses were determined by Galbraith Laboratories.

TLC was performed on 0.25-mm thickness silica gel plates (Merck, silica gel F-24). TLC solvent systems used were (v/v) (A) 5% methanol in chloroform; (B) 20% EtOAc/CH_2Cl_2; (C) 1-butanol/acetic acid/water, 4:1:1. Compounds were visualized on the plates by reaction with: (a) ultraviolet light; (b) ninhydrin; (c) chlorox-*o*-tolidine; (d) 5% phosphomolybdic acid; (e) iodine; (f) water.

Kinetic methods and analysis have been previously described.[7]

B. General Synthetic Procedures. General Procedure A: Removal of the Boc Group Using HCl/Dioxane. The *N*-Boc amino acid or peptide was dissolved in 4 N HCl in *p*-dioxane (10 equiv) and stirred for half an hour at room temperature under anhydrous conditions. The reaction mixture was concentrated in vacuo, and the residue was reevaporated from anhydrous diethy ether (2×) and then dried in a vacuum dessicator. The hydrochloride salt was triturated with ether and collected on a sintered-glass funnel.

General Procedure B: Oxidations of Alcohols with Pyridinium Dichromate. The alcohol was dissolved in glacial acetic acid (5 mL/mmol), solid pyridinium dichromate (3 equiv) was added, and the reaction mixture was stirred for 2 h at room temperature. Longer reaction times (3–24 h) or warming (50–60°) did not result in complete conversion of starting material, but rather led to an increase in 0 R_f byproducts. The reaction mixture was diluted with EtOAc and washed repeatedly (3–5×) until the aqueous layer was no longer colored. The organic layer was dried with anhydrous $MgSO_4$, concentrated in vacuo, and chromatographed over silica gel to give pure product.

C. Specific Synthetic Procedures. 3-[(*tert*-Butyloxycarbonyl)amino]-2-oxo-4-phenylbutanoic Acid *N*-Isoamylamide (5). The title compound was prepared from Boc-AHPBA-Iaa[7] according to general procedure B: R_f (A) 0.66; MS *m/e* 363 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.95 (d, 6 H, *J* = 6.5 Hz), 1.25–1.72 (m, 12 H), 3.04–3.44 (m, 4 H), 5.09 (br, 1 H), 5.34 (q, 1 H, *J* = 6 Hz), 6.86 (br, 1 H), 7.08–7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 22.37, 25.94, 28.01, 28.27, 37.76, 38.08, 42.64, 56.99, 80.07, 127.04, 128.61, 129.48, 136.03, 155.05, 159.27, 196.22.

3-Amino-2-oxo-4-phenylbutanoic Acid *N*-Isoamylamide Hydrochloride (2). The title compound was prepared from **5** according to general procedure A: R_f (C) 0.63 (with streaking); mp >80 °C dec; ^{1}H NMR (270 MHz, DMSO-d_6) δ 0.89 (d, 6 H, *J* = 6 Hz), 1.34 (q, 2 H, *J* = 7.5 Hz), 1.45–1.60 (m, 1 H), 3.14 (q, 2 H, *J* = 7.5 Hz), 3.19–3.23 (dd, 2 H, *J* = 5, 5 Hz), 5.01 (br, 1 H), 7.18–7.37 (m, 5 H), 8.55 (br, 3 H), 9.0 (t, 1 H, *J* = 6 Hz); ^{13}C NMR (DMSO-d_6) δ 22.16, 25.04, 35.17, 36.95, 37.22, 54.89, 127.10, 127.86, 129.38, 134.25, 159.12, 193.46.

3-[(*tert*-Butyloxycarbonyl)amino]-5-methyl-2-oxohexanoic Acid *N*-Isoamylamide (6). The title compound was prepared from the 3*S* alcohol[7] according to general procedure B: R_f (A) 0.65; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.93, 1.04 (2 d, 12 H, *J* = 6, 3 Hz), 1.21–1.83 (m, 15 H, includes 1.43, 1.49, 2 s, 9 H), 3.29 (q, 2 H, *J* = 6 Hz), 4.96 (br, 2 H), 6.86 (br, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.56, 22.37, 23.24, 25.25, 25.90, 28.06, 28.33, 37.71, 38.08, 40.96, 54.72, 80.02, 155.37, 159.44, 197.52.

3-Amino-5-methyl-2-oxohexanoic Acid *N*-Isoamylamide Hydrochloride (3). The title compound was prepared from **6** according to general procedure A: R_f (C) 0.73 (with streaking); mp >100 °C dec; ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.82 (d, 12 H, *J* = 6 Hz), 1.0–1.70 (m, 6 H), 2.68–3.50 (m, includes H_2O peak), 4.70 (br, 1 H), 7.78 (t, 2 H, *J* = 50 Hz), 8.33 (br, 1 H), 8.96 (t, 1 H, *J* = 5 Hz); ^{13}C NMR (DMSO-d_6) δ 21.30, 22.17, 22.65, 23.68, 25.09, 36.95, 37.27, 37.60, 52.53, 159.11, 194.11.

3-[(*tert*-Butyloxycarbonyl)amino]-5-methyl-2-oxohexanoic Acid *N*-Isoamylamide (7). The title compound was prepared from the 3*R* alcohol[7] according to general procedure B: R_f (A) 0.65; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.93 (d, 6 H, *J* = 6 Hz), 1.03 (d, 6 H, *J* = 6 Hz), 1.30–88 (m, 15 H, includes 1.43, 1.50, 2 s, 9 H), 3.32 (q, 2 H, *J* = 6.5 Hz), 5.05 (br, 2 H), 6.86 (br, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.56, 22.37, 23.24, 25.25, 25.90, 28.33, 37.71, 38.08, 40.96, 54.72, 80.02, 155.37, 159.44, 197.52.

3-Amino-5-methyl-2-oxohexanoic Acid *N*-Isoamylamide Hydrochloride (4). The title compound was prepared from **7** according to general procedure A: R_f (C) 0.73 (with streaking); mp >100 °C dec; ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.87 (d, 12 H, *J* = 6 Hz), 1.10–1.77 (m, 6 H), 3.14 (br, 2 H), 4.67 (br, 1 H), 7.34 (t, 2 H, *J* = 50 Hz), 8.49 (br, 1 H), 9.00 (t, 1 H, *J* = 5 Hz); ^{13}C NMR ($CDCl_3$) δ 21.34, 22.37, 23.19, 24.87, 26.00, 37.81, 38.08, 38.84, 54.93, 158.84, 194.54.

3(*S*)-Amino-5-methyl-2-hexanone Hydrochloride (10). The title compound was prepared from the methyl ketone of Boc-leucine[27] according to general procedure A: R_f (C) 0.42; mp 148–150 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.92 (d, 3 H, *J* = 7.5 Hz), 0.97 (d, 3 H, *J* = 7.5 Hz), 1.49–1.94 (m, 3 H), 2.29 (s, 3 H), 4.09 (br, 1 H), 8.39 (br, 3 H).

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Registry No. **2**·HCl, 138153-71-6; **2** (free base), 138153-77-2; **3**·HCl, 138153-72-7; **3** (free base), 138153-78-3; **4**·HCl, 138153-73-8; **4** (free base), 138153-79-4; **5**, 138153-74-9; **6**, 138153-75-0; **7**, 138153-76-1; **10**·HCl, 123768-64-9; **10** (free base), 114416-27-2; Boc-AHPBA-Iaa, 138153-80-7; Boc-Leu-Me, 85613-63-4; BocNHCH(*i*-Bu)CH(OH)CONHCH$_2$CH$_2$CHMe$_2$, 105166-94-7; cytosolic AP, 9001-61-0; microsomal AP, 9054-63-1; argininyl AP, 9073-92-1.