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3-Amino-2-hydroxy-propionaldehyde and 3-Amino-1-hydroxy-propan-2-one Derivatives: New Classes of Aminopeptidase Inhibitors

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Abstract—3-Amino-2-hydroxy-propionaldehydes [$H_2NCH(R)CHOHCHO$ with R = H, *i*-Bu, CH_2Ph] were designed as metallo-aminopeptidase inhibitors based on the metal active site chelation concept. These compounds were found to be micromolar inhibitors of aminopeptidase-M (AP-M, EC 3.4.11.2) with potencies similar to bestatin (K_i = 3.5 μM). Notably, compound **5a** (R = H) is a selective inhibitor of AP-M (K_i = 7 μM) with respect to cytosolic leucine aminopeptidase (LAPc, EC 3.4.11.1) (K_i = 385 μM). However, due to their easy oligomerization, these compounds are of low practical value. In contrast, the corresponding isomeric 3-amino-1-hydroxy-propan-2-one derivatives [$H_2NCH(R)COCH_2OH$ with R = H, *i*-Bu, CH_2Ph, *i*-Pr, CH_2Biph] are well defined structures. These hydroxymethylketones also exhibit micromolar affinities on AP-M. Compound **6c** (R = CH_2Ph) was the most potent (K_i = 1 μM). Selectivity studies of **6a** (R = H) and **6b** (R = *i*-Bu) show a preference for AP-M. Compound **6a** is moderately active on AP-M (K_i = 25 μM) and inactive on LAPc. This new class of inhibitors is proposed to bind as bidentates, analogous to hydroxamates. Copyright © 1996 Elsevier Science Ltd

Introduction

Aminopeptidases[1,2] are ubiquitous exopeptidases involved in many biological processes.[3] They specifically cleave N-terminal residues from peptides. For example aminopeptidase M (EC 3.4.11.2) is of prime importance for the catabolism of enkephalins[4] and vasoactive peptides.[5]

Most of the aminopeptidases belong to the metalloproteases family. To date the development of inhibitors for this class of proteolytic enzymes has targeted the zinc ion(s) present in the active site. These inhibitors are usually peptidomimetics which incorporate at their N-terminal residue a hydroxamic acid, a thiol or a carboxylate functionality.[6] Another interesting class of inhibitors includes the natural compounds bestatin[7] and amastatin[8] which have been isolated from *Streptomyces*. Bestatin (Ubenimex) has received considerable interest because of its immunomodulating properties extensively studied by Umezawa and coworkers.[9] Its effectiveness in cancer therapy was the driving force of our search of novel aminopeptidase inhibitors. Bestatin and amastatin are pseudopeptides which contain a β-amino-α-hydroxyamino acid as their N-terminal residue. This moiety provides a strong interaction with zinc ion(s) at the active site. Its binding mode for the di-zinc aminopeptidases, was elucidated from the X-ray structures of bovine lens aminopeptidase complexed with bestatin or amastatin.[10] The amino and hydroxyl groups of the β-amino-α-hydroxyamino acid moiety were shown to be in the coordination sphere of the zinc atoms.

Aldehydes constitute a versatile class of protease inhibitors. α-Aminoaldehydes are potent inhibitors of aminopeptidases[11–13] that are assumed to chelate the catalytic zinc ions via their amine and aldehyde (or their hydrate) functions. By combining the structural features of α-aminoaldehyde and β-amino-α-hydroxy-amide we designed β-amino-α-hydroxypropionaldehydes as a new concept for the development of aminopeptidase inhibitors with potency similar to bestatin. These hydroxyaldehydes were rearranged to corresponding thermodynamically more stable hydroxymethylketones, a new class of aminopeptidase inhibitors.

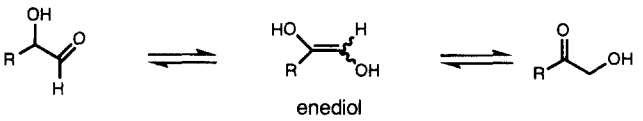
Chemistry

Our first attempted synthesis of 3-amino-2-hydroxy-propionaldehyde was based on a procedure described by Wohl and Schweitzer in 1907.[14] The intermediate 3-BOC-amino-2-hydroxy-propionadehyde diethylacetal (**4ab**) prepared according to Fischer et al.[15] was warmed in aqueous concentrated 37% HCl at 100 °C for 2 min. Rather than the expected hydroxyaldehyde, the isomeric 3-amino-1-hydroxy-2-propanone (**6a**)[16] was obtained, as determined by ^1H and ^{13}C NMR data. Thus isomerization of α-hydroxyaldehydes to hydroxymethylketones had occurred. Deprotonation to an enediol intermediate and subsequent reprotonation as illustrated in Scheme 1 is a likely mechanism. Isomerization of α-hydroxyaldehydes to hydroxymethyl

Abbreviations: BOC, *tert*-butoxycarbonyl; DMF, dimethylformamide; DMSO, dimethylsulfoxide; RT, room temperature; THF, tetrahydrofurane; TMS, tetramethylsilane; *Z*, benzyloxycarbonyl.

ketones had been reported either in basic[17] or acidic media.[18] This process is known in carbohydrate chemistry as the Lobry de Bryn–Alberda van Ehenstein transformation.[19] To eliminate this isomerization, we considered the possibility of cleaving first the acetal under mild acidic conditions and then to generate the free amine by hydrogenolysis of a benzyloxycarbonyl (Z) protecting group.

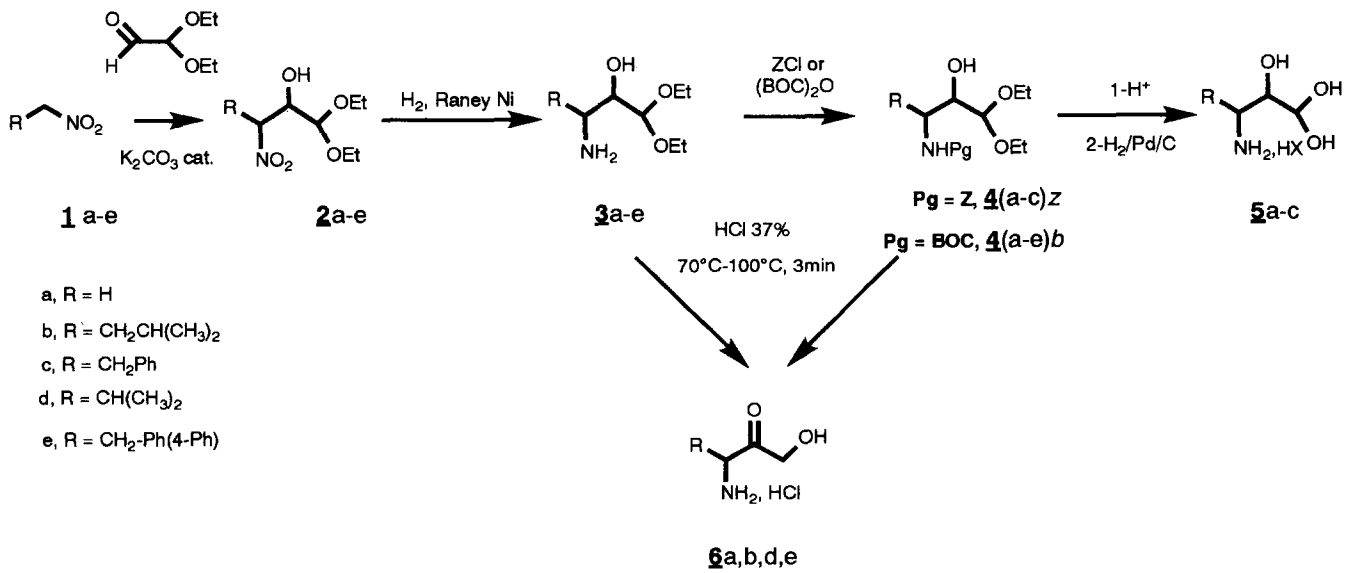
This methodology was extended to 3-substituted analogues of 3-amino-2-hydroxy-propionaldehyde as depicted in Scheme 2. Briefly, the first step involved a Henry reaction. Nitroalkane[20] or nitroaralkanes[21,22] and diethoxyacetaldehyde[23] were condensed under mild basic conditions using potassium carbonate in catalytic amounts. Hydrogenolysis of the resulting 3-nitro-2-hydroxypropionaldehyde acetals (**2**) over Raney nickel generated their corresponding amines. These amines were protected as the benzyloxycarbonyl carbamates (**4z**) by reaction with benzylchloroformate. At this stage diastereoisomers can be separated by flash chromatography on silica gel. The acetal protecting group was then cleaved under mild conditions in a mixture of 1 N aqueous hydrochloric acid and THF (2:1 vol) overnight at room temperature. The resulting 3-Z-amino-2-hydroxy-propionaldehydes were isolated as oligomeric materials which are difficult to analyze by NMR. Nevertheless, the monomeric aldehyde could be detected. For example, 3-Z-amino-2-hydroxy-5-methylhexanal [Z-NHCH(*i*-Bu)CHOHCHO] via formation of the bisulfite adduct and regeneration of the aldehyde with sodium bicarbonate generated a material containing about 60% of the unstable monomeric aldehyde (see Experimental). The existence of dimeric forms of α-hydroxyaldehydes has been reported.[24,25] We were also able to isolate a dimeric form of 3-Z-amino-2-hydroxy-propionaldehyde. Hydrogenolysis of the benzylcarbamate (**4z**) over palladium on charcoal, in the presence of aqueous HCl, generated 3-amino-2-hydroxypropionaldehyde derivatives (**5**) as oligomeric mixtures.



Scheme 1. Lobry de Bryn–Alberda van Ehenstein transformation.

Characterization of the aldehydes **5** was difficult due to their high propensity to oligomerize. The ^1H NMR spectrum of 3-amino-2-hydroxy-propionaldehyde (**5a**) recorded in D_2O shortly after dissolution indicated the presence of numerous oligomers. The existence of these species as monomeric entities has been doubted. Having the α-aminoacetaldehyde (HCl, H_2NCH_2-CHO)[26] in hand we observed a slow conversion of oligomers into the monomeric hydrated form by repeating an NMR experiment on a sample prepared the day before. In an analogous experiment, **5a** hydrated very slowly in D_2O within a week to reach a steady state. The aldehyde hydrate appears more than 90% pure (see Fig. 1). No aldehyde proton was detected. A doublet at δ 5.0 in the ^1H spectrum together with a ^{13}C resonance at δ 92 were assigned to the $CH(OH)_2$ group. In comparison, the depolymerization of α-aminoacetaldehyde is faster and the hydration complete after 8 h. A weak aldehyde proton resonance in this case was also detected by ^1H NMR spectrometry. The monomeric aldehyde accounted for ~5% of the mixture which is in agreement with previous studies.[13] Though less effective, the hydration in D_2O of 3-substituted-3-amino-2-hydroxy-propionaldehydes generated the aldehyde hydrate as the major component of the mixture.

When analyzed by ^1H NMR spectrometry at 360 MHz, **5a** and **5b** were found to be contaminated by minor amounts of isomeric hydroxymethylketones (1–5%). Equilibration of hydroxyaldehydes into the thermo-



Scheme 2. Synthesis of 3-amino-2-hydroxy-propionaldehyde and 3-amino-1-hydroxy-2-propanone derivatives.

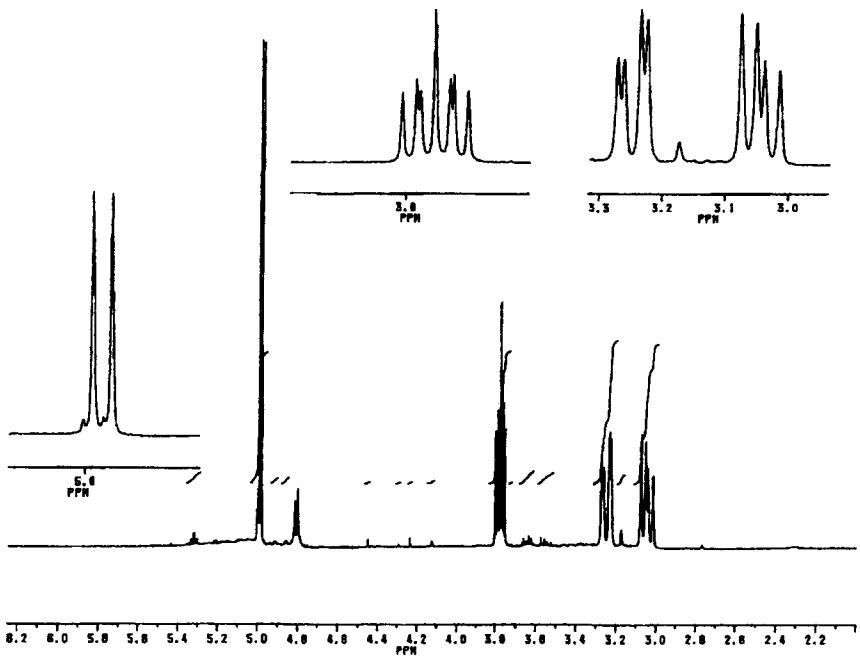


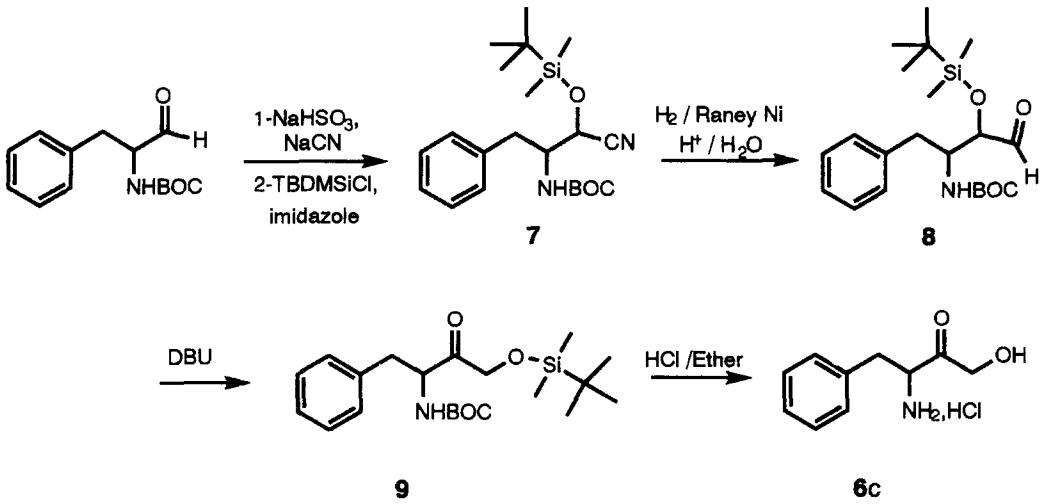
Figure 1. ^{1}H NMR (360 MHz) spectrum of **5a** after 9 days in D_2O.

dynamically more stable hydroxymethylketones at room temperature was excluded since their ratio remained constant with time. We suspected that conversion of acetal to aldehyde with dilute HCl might be a potential source of side reactions. α-Hydroxy ketals have been reported[27] to rearrange into alkoxy ketones under acidic catalysis. Thus, under our conditions, small quantities of ethoxy and hydroxymethylketone could be formed but difficult to detect by NMR spectrometry in the presence of a mixture of oligomeric hydroxyaldehydes.

It is apparent from the previous section that β-amino-α-hydroxy-aldehydes (**5**) are difficult to handle and to characterize since chemically well defined materials could only be obtained in aqueous solution. In contrast, the isomeric hydroxymethylketones do not oligomerize and can be fully characterized. Compounds **6** (except **6a**) have not been previously described. They were prepared either from the BOC-aminohydroxyacetal **4(a,b,d,e)b** or from the aminohydroxyacetal **3(a,b,d,e)** under the Wohl and Schweitzer conditions by heating at 70–100 °C for a few minutes in 37% HCl. We first succeeded in obtaining pure **6b** by warming **4bb** at 70 °C for 3 min. However, when the reaction with **3b** was scaled up to 1 g, due to less efficient thermal exchange, the warming time had to be increased to 7 min, until a substantial amount of hydroxymethylketone appeared in the crude reaction mixture as monitored by ^{1}H NMR spectrometry. On the other hand, longer reaction times and overheating might result in material contaminated by ammonium chloride as evidenced by C, H, N elementary analysis and ^{1}H NMR in DMSO-d_6 on **6b**.[28] In summary, when the isomerization was performed within a narrow range of reaction time and temperature, crystallization of the crude mixture usually generated pure compounds **6**. Yields of isolated hydroxymethylketones from hydroxyacetals were in the range of 30%. These modest yields reflect a rather difficult isomerization process competing with deamination.

In contrast to unsubstituted ketones, the hydroxymethylketones are partially hydrated in aqueous solutions showing that the α-hydroxyl substitution enhances the electrophilic character of the carbonyl group. Compounds **6b** and **6c**, for example, are partially hydrated in D_2O to an extent of 15%. No equilibration into the isomeric hydroxyaldehyde was observed in D_2O or in DMSO-d_6 over several days.

Unexpectedly, when **4cb** (R = CH_2Ph) was stirred in hot 37% HCl, 3-amino-1-chloro-1,2,3,4-tetrahydro-2-naphthalenol hydrochloride precipitated out of the reaction mixture.[29] This compound is formed by a Friedel–Crafts cyclization of the intermediate hydroxyaldehyde followed by SN_1 substitution by chloride ions present in high concentration (12 M). In contrast, 3-BOC-amino-2-hydroxy-4-biphenyl-4-yl-butanal diethyl acetal **4eb** generated the hydroxymethylketone **6e**. The very low solubility of **4eb** is a distinct feature that could influence the course of the reaction. Since the usual approach failed, the revised synthetic pathway which is depicted in Scheme 3 was developed for the preparation of 3-amino-1-hydroxy-4-phenylbutan-2-one (**6c**). The strategy was to isomerize a protected α-hydroxyaldehyde to the hydroxymethylketone under basic conditions. Thus an O-protected cyanhydrin (**7**) derived from *N*-BOC-phenylalaninal was reduced in the O-protected hydroxyaldehyde **8** by hydrogenolysis–hydrolysis over Raney nickel[30] in modest yields (20%). Isomerization into O-protected hydroxymethylketone **9** was achieved under basic conditions by reaction with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The cleavage of the BOC-protecting group was performed in an ethereal solution of hydrochloric acid.



Scheme 3. Synthesis of 3-amino-1-hydroxy-4-phenyl-2-butanone.

Biological Results and Discussion

These compounds were evaluated for their ability to inhibit several aminopeptidases including membrane bound aminopeptidase-M (AP-M, EC 3.4.11.2), cytosolic leucine aminopeptidase (LAPc, EC 3.4.11.1), *Aeromonas proteolytica* aminopeptidase (AP-aero, EC 3.4.11.10) and cytosolic arginyl aminopeptidase (AP-B, EC 3.4.11.6). AP-M contains one zinc ion in its active site while LAPc and AP-aero contain two zinc ions in close proximity. The presence of zinc in AP-B remains controversial. AP-M, LAPc and AP-aero display broad substrate specificity and can accommodate large N-terminal hydrophobic residues in their active site. In contrast, AP-B is specific for N-terminal basic residues.

Inhibition of aminopeptidases by 3-amino-2-hydroxy-propionaldehyde derivatives 5a–c

The α-hydroxy-aldehyde diethylacetals precursors **3a–c** as shown in Table 1, were found to be weak inhibitors of AP-M, LAPc and AP-aero (see Table 1). K_i values of 1.4, 2.8 and 1.9 mM were measured on AP-M for compounds **3a–c** respectively. The corresponding 3-amino-2-hydroxy-propionaldehyde hydrates (**5**) exhibited, as expected, activities in the micromolar range for AP-M, stressing the importance of the aldehyde (or its hydrate) function. Interestingly, 3-amino-2-hydroxy-propionaldehyde hydrate (**5a**) appeared selective for AP-M with a K_i of 7 μM compared to millimolar activities on the other aminopeptidases. These hydroxyaldehydes are less effective on the two zinc ions enzyme LAPc than leucinal ($K_i = 0.06$ μM) and are far less active than bestatin ($K_i = 0.0006$ μM). This could indicate that their binding mode on LAPc differs from that of bestatin.

An important point is whether the free aldehyde or its hydrate binds to the zinc ion in the enzyme active site. Since we have no evidence of the existence of the free aldehyde in solution, we assume that one hydroxyl or the *gem*-diol [$C(OH)_2$] group displaces the catalytic water molecule bound to zinc[32] and that the remaining hydroxyl group(s) further stabilizes the complex by hydrogen-bonding.

Inhibition of aminopeptidases by 3-amino-1-hydroxy-propan-2-one derivatives 6a–c

Similar activities were observed for the related hydroxymethylketones as described in Table 2. Their

Table 1. Inhibitory activity of 3-amino-2-hydroxy-propionaldehyde derivatives on aminopeptidases

Structure	K_i (μM)			
	AP-M	LAPc	AP-aero	AP-B
Bestatin	3.5	0.0006	0.02	6
Leucinal	0.76	0.06	—	—
$H_2NCH_2CH(OH)CH(OEt)_2$ (**3a**)	1400	>5000	>5000	—
$H_2NCH(i\text{-Bu})CH(OH)CH(OEt)_2$ (**3b**)	2800	—	1000	—
$H_2NCH(CH_2Ph)CH(OH)CH(OEt)_2$ (**3c**)	1900	—	>3000	—
HCl, $H_2NCH_2CH(OH)CH(OH)_2$ (**5a**)	7	3850	17 000	5800
HCl, $H_2NCH(i\text{-Bu})CH(OH)CH(OH)_2$ (**5b**)[a]	10	85	10	—
HCl, $H_2NCH(CH_2Ph)CH(OH)CH(OH)_2$ (**5c**)[a]	3	100	30	—

These hydroxyaldehydes were tested as racemic mixtures.
[a]See ref 31.
All these hydroxyaldehydes exhibited a competitive inhibition.
The data for bestatin (except for AP-M and AP-B) and leucinal are reproduced from refs 45 and 13, respectively.

Table 2. Inhibitory activity of 3-amino-1-hydroxy-propan-2-one derivatives on aminopeptidases

Entry	Structure	K_i (μM)			
		AP-M	LAPc	AP-aero	AP-B
1	HCl, $H_2NCH(CH_2Ph)COCH_3$	47	630	2650	2000
2	$H_2NCH(i\text{-Bu})CONHOH$ (L-isomer)	2	47	0.35	—
3	HBr, $H_2NCH(CH_2Ph)COCH_2SH$	1	—	—	—
4	HCl, $H_2NCH_2COCH_2OH$ (**6a**)	25	>3000	210	—
5	HCl, $H_2NCH(i\text{-Bu})COCH_2OH$ (**6b**)	7	160	0.2	900
6	HCl, $H_2NCH(CH_2Ph)COCH_2OH$ (**6c**)	1	—	—	—
7	HCL, $H_2NCH(i\text{-Pr})COCH_2OH$ (**6d**)	500	—	—	—
8	HCl, $H_2NCH(CH_2BiPh)COCH_2OH$ (**6e**)	>100	—	—	—

These hydroxymethylketones were tested as racemic mixtures.
All the compounds are competitive inhibitors. Compound **6d** exhibited a slow binding type inhibition.
The data for the hydroxamate and the thiol derivatives are taken from refs 34 and 35, respectively.

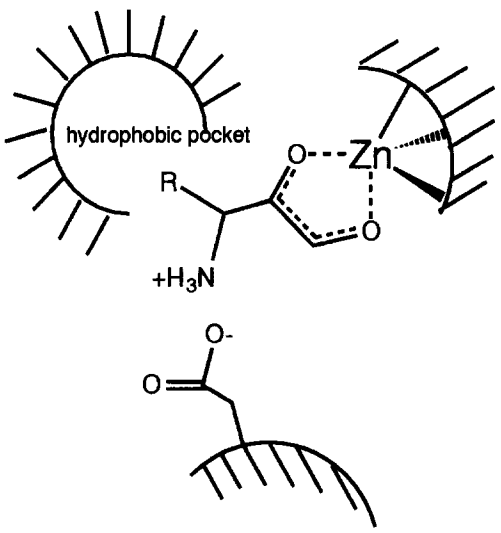
dissociation constant for AP-M is in the micromolar range and the 3-benzyl substituted derivative **6c** is the most potent ($K_i = 1$ μM). Compared to 3-amino-4-phenyl-butan-2-one (phenylalanine methylketone; Table 2, entry 1),[33] **6c** is about 50 times more potent, demonstrating stronger binding due to an additional hydroxyl group. Interestingly, both **6a** (no side-chain) and **6b** (*i*-Bu side-chain) are more potent on AP-M than on LAPc. Selectivity of compound **6a** for AP-M vs LAPc is greater than 120 whereas selectivity of **6b** is only 20. Steric hindrance may explain the poor activity on AP-M of **6d** and **6e**. We can compare the activity of the hydroxymethylketones **6b** (*i*-Bu side-chain) and **6c** (benzyl side-chain) with data found in the literature for related structures. Compound **6b** and the related 2-amino-*N*-hydroxy-4-methyl-pentanoic acid amide (leucine hydroxamate; Table 2, entry 2)[34] are equipotent on AP-M, LAPc and AP-aero. In addition, **6c** and the related 3-amino-1-mercapto-4-phenyl-butan-2-one (Table 2, entry 3)[35] have the same activity on AP-M.

These observations prompt us to suggest an analogous binding mode for these inhibitors (at least on AP-M) and to propose the formation of a bidentate complex[36] with the zinc ion as illustrated in Scheme 4. The catalytic zinc ion would be coordinated by three ligands from the enzyme backbone and by the two hydroxymethylketone oxygen atoms. In analogy with hydroxamate ligands, these may form a distorted trigonal-bipyramidal[37] or a square pyramidal coordination sphere.[38] In the case of one catalytic zinc ion in the active site as for AP-M, the amino group would not interact with the zinc ion but with an acidic residue of the catalytic site.

We propose that most of the aminoketones act as transition state analogues in binding to the catalytic zinc of aminopeptidases in their hydrated form $NH_2—CH(R)—C(OH)_2—R$ where the *gem*-diol group coordinates the zinc ion. Therefore, as alternative mechanism, the binding of the hydroxymethylketone as hydrate $—C(OH)_2—CH_2—OH$ can be considered. But since hydroxymethylketones are structurally related to hydroxamic acids, they are probably better zinc ligands than the hydrate. Therefore, we favor the bidentate interaction described in Scheme 4.

Conclusion

3-Amino-2-hydroxypropionaldehydes oligomerize more readily than usual aldehydes, nevertheless they can be successfully identified by ^1H and ^{13}C NMR spectrometry in aqueous solution as monomeric hydrates. 3-Amino-2-hydroxy-propionaldehyde **5a**, the most interesting inhibitor of this class is a potent and selective inhibitor of AP-M ($K_i = 7$ μM). The hydroxyaldehyde isomerization in hot concentrated hydrochloric acid provides access to the stable and chemically well defined 3-amino-1-hydroxypropan-2-ones. Both inhibitor classes exhibit K_i values in the micromolar range for AP-M. Hydroxymethylketones (**6a**) (no side-chain) and **6b** (*i*-Bu side-chain) are more potent inhibitors of AP-M than of LAPc. Compound **6b** has a potency similar to the related hydroxamate on AP-M, LAPc and AP-aero. Moreover, **6c** (benzyl side-chain) has the same activity on AP-M as the related thiolmethylke-



Scheme 4. Binding mode of hydroxymethylketones.

tone. This leads us to propose a bidentate binding mode for these hydroxymethylketones.

Experimental

Melting points were determined on a Büchi 535 melting point apparatus and have not been corrected. Elementary analyses were obtained on a Carlo Erba model 1106 analyzer. Mass spectra by the chemical ionization (CI) technique were recorded on a Finnigan TSQ 46 or a SSQ 70.

The ^{1}H NMR experiments were performed on a Bruker AM 360 unless otherwise specified. The chemical shifts δ are expressed in ppm relative to TMS as external standard and the coupling constants are expressed in Hz.

Column chromatography and TLC were performed on silica gel 60 (230–400 mesh) and on 0.25 mm plates precoated with silica gel 60 F254 purchased from Merck-Darmstadt, respectively.

Chemistry

3-Amino-2-hydroxy-propionaldehyde, hydrochloride (5a)

2-Hydroxy-3-nitro-propionaldehyde, diethyl acetal 2a (15). A mixture of diethoxy-acetaldehyde (3.9 g, 29 mmol),[23] nitromethane (5.76 g, 94 mmol) and finely divided dried potassium carbonate (0.44 g, 3.2 mmol) was mixed and warmed at 50 °C for 6 h. The resulting orange material was diluted with Et_2O and washed with water and brine. After drying over magnesium sulfate, the solvent was evapd under vacuum. The crude residue was then purified by chromatography on silica gel (50 g, elution with a 15:85 AcOEt:hexane mixture) to yield **2a** (3.84 g, 69%) as an oil, R_f=0.31 (SiO_2, AcOEt:hexane:1:3).

3-Amino-2-hydroxy-propionaldehyde, diethyl acetal (3a). Compound **2a** (3.84 g, 19.9 mmol) dissolved in 2-propanol (120 mL) was hydrogenated at atmospheric pressure over Raney nickel (about 5 g) at rt overnight. The mixture was filtered with caution on celite and evapd under vacuum to yield an oil (3.04 g, 94%) that was used without further purification in the next step, R_f 0.60 (SiO_2, BuOH:AcOH:H_2O, 3:1:1). MS (DCI/CI/NH_3) *m/z* (relative intensity) 164 (MH^+, 100), 181 (MNH_4^+, 10). An analytical sample was obtained by trap to trap distillation at 110 °C under 0.05 mm Hg, Anal. ($C_7H_{17}NO_3$) C, H, N.

3-Benzyloxycarbonylamino-2-hydroxy-propionaldehyde, diethyl acetal (4az). Under nitrogen, benzylchloroformate (0.55 mL, 3.85 mmol) was added dropwise to a soln of **3a** (604 mg, 3.70 mmol) and triethylamine (0.65 mL, 4.7 mmol) in CH_2Cl_2 maintained at −20 °C. The cooling bath was removed and the soln stirred at rt overnight. After following procedure for the synthesis of **2a**, the crude compound was purified on silica gel (50 g SiO_2, elution gradient from 1:4 to 1:3 AcOEt:hexane mixtures) to generate an oil (406 mg, 36%), R_f 0.41 (SiO_2, AcOEt:hexane, 1:1). MS (DCI/CI/NH_3) *m/z* (relative intensity) 252 (100), 298 (MH^+, 15), 315 (MNH_4^+, 60).

3-Amino-2-hydroxy-propionaldehyde, hydrochloride (5a). The acetal **4az** (52 mg, 0.17 mmol) was stirred in aq HCl 1 N at rt for 1.5 h. A precipitant from the reaction mixture was identified as the dimer of 3-Z-amino-2-hydroxy-propionaldehyde. ^{1}HNMR (DMSO-d_6): this material was a mixture of 3 diastereoisomers, the attribution being based on homodecoupling experiments, δ 3.1 (m, 2H), 3.75, 4.15 (m, 1H, CH—O), 4.5, 4.8 (m, 1H, O—CH—O), 5.1 (s, 2H), 6.5, 7.0 (m, 1H, OH), 7.15, 7.3 (m, 1H, NH), 7.45 (m, 5H). Anal ($C_{11}H_{13}NO_4$) C, H ,N; C: calcd 59.19; found 59.85, MS (DCI/CI/NH_3) 241 (MNH_4^+ monomer).

The filtrate was evapd and combined with the dimer. This material (33 mg) dissolved in aq HCl 1 N was hydrogenated over 10% Pd on charcoal (21 mg) at rt and atmospheric pressure for 4 h. Filtration and evapn gave **5a** (15 mg, 70%). Lyophilization and drying under high vacuum over phosphorus pentoxide gave an oil.

^{1}H NMR (D_2O) 9 days after dissolution (see Fig. 1), δ_A 3.04, δ_B 3.25 (ABX spectrum, J_{AB}=13.3, J_{AX}=8.7, J_{BX}=3.6, 2H), 3.78 (ddd, J=3.8, 4.7, 8.6 Hz, 1H), 4.99 (d, J=4.8 Hz, 1H); ^{13}C NMR (D_2O) δ 42.0, 71.4, 91.8.

3-Amino-2-hydroxy-5-methyl-hexanal, hydrochloride (5b)

3-Methyl-1-nitro-butane (1b). This synthesis was adapted from a previously described procedure.[20] Urea (18 g, 0.30 mol) and dropwise 3-methyl-1-bromobutane (30.2 g, 0.19 mol) were added to a soln of sodium nitrite (20.8 g, 0.30 mol) in DMF (400 mL). The mixture was stirred for 4 h at rt, poured into H_2O (1 L) and extracted with Et_2O (4 × 400 mL). The organic layer was washed with H_2O (4 × 100 mL) and dried over magnesium sulfate. Evapn of solvents generated an oily residue (20.5 g) which was distilled under red. press. (31 mm Hg, bp: 72–73 °C) to generate the desired compound (11.6 g, 52%).

2-Hydroxy-3-nitro-5-methyl-hexanal, diethyl acetal (2b). 3-Methyl-1-nitro-butane (8.77 g, 75 mmol) and diethoxy-acetaldehyde (4.40 g, 33 mmol) and potassium carbonate (0.33 g, 2.3 mmol) were reacted as described for **2a**. The crude yellow oil (6.88 g) was purified on silica gel (300 g, elution gradient from 5:95 to 15:85 mixtures of AcOEt:hexane). A yellow oil (5.88 g, 71%) was obtained thereof, R_f 0.25 (SiO_2, AcOEt:hexane, 15:85). MS (DCI/CI/NH_3) *m/z* (relative intensity) 139 (30) 150 (40), 267 (MNH_4^+, 100).

3-Amino-2-hydroxy-5-methyl-hexanal, diethyl acetal (3b). Compound **2b** (5.88 g, 23.6 mmol) was hydrogenated as for **3a** over Raney nickel (5 g) in 2-propanol (120 mL). A yellow oil (4.50 g) was obtained. Trap to

trap distillation at 170 °C under 0.05 mm Hg generated a colorless oil (3.60 g, 70%), Anal. ($C_{11}H_{25}NO_3$) C, H, N. R_f 0.60 (SiO_2, BuOH:AcOH:H_2O, 3:1:1), MS (DCI/CI/NH_3) *m/z* 220 (MH^+).

3-Benzyloxycarbonylamino-2-hydroxy-5-methyl-hexanal, diethyl acetal (4bz). Under nitrogen a 100 mL flask was charged with **3b** (2.66 g, 12.1 mmol), CH_2Cl_2 (40 mL) and triethylamine (1.63 g, 22.2 mmol) and cooled to −20 °C. A soln of benzylchloroformate (2.32 g, 16.2 mmol) in CH_2Cl_2 (41 mL) was added dropwise. The reaction mixture was warmed to 0 °C for 2 h and then to rt overnight. Evapn of solvents yielded a crude material which was eluted on silica gel (400 g, elution gradient from 1:9 to 1:3 mixtures of AcOEt:hexane). Isomer A (1.02 g) and isomer B (1.39 g) were separated, $R_{f_A}=0.22$ and $R_{f_B}=0.095$ (SiO_2, AcOEt:hexane, 1:4) and a mixed fraction (365 mg) was also recovered. The overall yield was 65%. Isomer B crystallized on standing. ^{1}H MNR: isomer A: δ 0.92 (d, $J=6.6$ Hz, $C(CH_3)_2$, 3H), 0.94 (d, $J=6.6$ Hz, $C(CH_3)_2$, 3H), 1.22 (t, $J=7.0$ Hz, OEt, 6H), 1.37 and 1.52 (m, CH_2, 2H), 1.65 (m, $CH(CH_3)_2$, 1H), 2.37 (s, OH, 1H), 3.45 (d, $J=7.4$ Hz, CHOH, 1H), 3.55 and 3.73 (m, OEt, 4H), 3.97 (m, CHN, 1H), 4.26 (d, $J=7.5$ Hz, $CH(OEt)_2$, 1H), 5.04 (d, $J=10$ Hz, NH, 1H), δ_A 5.07 δ_B 5.12 (AB spectrum, $J_{AB}=12.1$ Hz, 2H, OCH_2Ph), 7.35 (m, 5H). Isomer B δ 0.92 (d, $J=5.0$ Hz, $C(CH_3)_2$, 3H), 0.94 (d, $J=4.8$ Hz, $C(CH_3)_2$, 3H), 1.22 (q, $J=6.8$ Hz, OEt, 6H), 1.27 (m, CH_2, 1H), 1.45 (m, CH_2, 1H), 1.65 (m, $CH(CH_3)_2$, 1H), 2.35 (d, $J=3.8$ Hz, OH, 1H), 3.57 and 3.75 (m, OEt, 4H), 3.69 (m, CHOH, 1H), 3.97 (m, CHN, 1H), 4.40 (d, $J=6.2$ Hz, $CH(OEt)_2$, 1H), 4.99 (d, $J=9.4$ Hz, NH, 1H), 5.10 (s, OCH_2Ph, 2H), 7.35 (m, 5H). MS (DCI/CI/NH_3). Isomer A *m/z* (rel. int.) 220 (25), 263 (25), 308 (100), 334 (95), 354 (MH^+, 5); 371 (MNH_4^+, 10); isomer B *m/z* (rel. int.) 308 (100), 334 (20), 354 (MH^+, 30), 371 (MNH_4^+, 60).

3-Amino-2-hydroxy-5-methyl-hexanal, hydrochloride (5b). Compound **4bz** isomer A (236 mg, 0.67 mmol) dissolved in THF (5mL) was mixed with HCl 1 N (5 mL) at rt for 4.5 h. Evapn of this mixture under red. press. gave a polymeric material (181 mg, 97%) as shown by ^{1}H NMR spectrometry. An aliquot (76 mg) was dissolved in methanol (6 mL) and treated with 1 M aq sodium bisulfite (6 mL). Evapn of the mixture and addition of H_2O led to the sepn of the bisulfite adduct as an oil. Regeneration of the aldehyde was performed by stirring an ethereal solution (5 mL) of the bisulfite adduct with an aq soln of 1 M sodium bicarbonate for 2 h. After washing with brine and drying over magnesium sulfate, the etheral solution was evapd to give an oil (30 mg) containing ~60% of free aldehyde based on ^{1}H NMR (90 MHz, acetone-d_6) δ 0.8 (d, 6H), 4.1 (m, 1H), 4.3 (m, 1H), 6.0 (1H), 7.2 (m, 5H), 9.6 (s, 1H). IR ν^{KBr} cm $^{-1}$: 3450, 2950, 1705, 1690, 1530, 1260.

The aldehyde which was purified via the bisulfite adduct (46 mg, 0.16 mmol) was dissolved in a mixture of ethanol (3 mL) and 4 N HCl (1 mL). The mixture was hydrogenated over 10% Pd/C (18 mg) under atmos. press. and rt for 5 h. The mixture was processed as previously established. The resulting oily residue was dissolved in H_2O, treated with charcoal and lyophilized. Additional drying under high vacuum over phosphorus pentoxide for several days produced **5b** as a solid (26 mg, 87%). ^{1}H NMR (D_2O) δ 0.97 (d, $J=6$ Hz, 6H), 1.65 (m, 3H), 3.59 (td, $J=7$, 4 Hz, 1H), 3.72 (t, $J=3$ Hz, 1H), 5.18 (d, $J=4$ Hz, 1H).

3-Amino-2-hydroxy-3-phenyl-butanal, hydrochloride (5c)

(2-Nitro-ethyl)-benzene.[21] A solution of β-nitrostyrene (35.8 g, 0.24 mmol) in dioxane (400 mL) was added dropwise over a 1 h 10 min period to a well-stirred suspension of $NaBH_4$ (20.0 g, 0.53 mmol) in dioxane (400 mL) and absolute ethanol (125 mL). This process was performed under argon gas. The reaction flask was cooled with a cold water bath to maintain the temperature below 30 °C since the reduction reaction is slightly exothermic. Agitation was maintained for an additional 30–45 min and ice-cold water (500 mL) was added to the reaction mixture. The resulting slurry was slowly solubilized by addition of acetic acid:water (1:1, 100 mL). This mixture was then concd under vacuum to about 500 mL and extracted with CH_2Cl_2 (500 mL). The organic layer was washed with brine, dried over magnesium sulfate and evapd. The resulting residue was trap to trap distilled at 165 °C under 15 mm Hg to give the desired compound as an oil (29.5 g, 81%).

2-Hydroxy-3-nitro-4-phenyl-butanal, diethyl acetal (2c). 1-Nitro-2-phenylethane (10 g, 66 mmol), diethoxyacetaldehyde (4.44 g, 34 mmol) and potassium carbonate (0.45 g, 3.3 mmol) were reacted as described for **2a**. The crude product (12.45 g) was purified by chromatography (SiO_2, elution gradient from 1:9 to 1:4 mixtures of AcOEt:hexane). The excess 1-nitro-2-phenylethane (5.77 g) was first recovered R_f 0.50 (SiO_2, AcOEt:cyclohexane, 1:4). Compound **2c** was obtained as a yellow oil (5.90 g, 61%), R_f 0.20 (SiO_2, AcOEt:cyclohexane, 1:4).

3-Amino-2-hydroxy-4-phenyl-butanal, diethyl acetal (3c). Compound **2c** (5.91 g, 20.8 mmol) was reduced over Raney nickel as described for **3a**. A yellow oil (4.89 g, 93%) was obtained. An analytic sample was obtained by trap to trap distillation at 170 °C under 0.1 mBar as a colorless oil, Anal. ($C_{14}H_{23}NO_3$) H, N; C: calcd, 66.37; found, 65.95.

3-Benzyloxycarbonylamino-2-hydroxy-4-phenyl-butanal, diethyl acetal (4cz). Compound **3c** (2.4 g, 9.47 mmol), benzylchloroformate (1.82 g, 10.7 mmol) and triethylamine (1.31 g, 12.9 mmol) were reacted as previously described for **4az**. The crude product (4.34 g) was purified on silica gel (200 g, elution gradient from 1:9 to 1:3, AcOEt:hexane mixtures). Isomer A (1.01 g) and isomer B (0.84 g) were recovered with an overall yield of 50%, $R_{f_A}=0.20$, $R_{f_B}=0.15$ (SiO_2, AcOEt:hexane: 1:3).

3-Amino-2-hydroxy-4-phenyl-butanal (5c). Compound **4cz** isomer A (216 mg, 0.56 mmol) was dissolved in THF (5 mL) and mixed with aq 1 N hydrochloric acid (10 mL). The mixture was stirred overnight at rt. Evapn of solvents gave a residue (160 mg) which was hydrogenated over 10% Pd/C (30 mg) in a mixture of ethanol (6 mL) and aq 4 N hydrochloric acid (4 mL) for 4 h. After following previously described procedure, the product was analyzed by ^{1}H NMR in D_2O, the hydration monitored over two weeks. Lyophilization of the NMR sample yielded **5c** as a yellow solid (29 mg, 23%). ^{1}H NMR (D_2O) δ_A 2.96, δ_B 3.07 (AB spectrum, $J_{AB}=14$, $J_{AX}=8.1$, $J_{BX}=7.3$ Hz, 2H), 3.63 (t, $J=3.0$ Hz, 1H), 3.73 (td, $J=7.8$, <2 Hz, 1H), 5.10 (d, $J=4$ Hz, 1H), 7.35 (m, 5H). ^{13}C NMR (D_2O): δ 137.0, 131.0, 130.7, 129.1, 91.5, 71.7, 54.7, 37.2.

3-Amino-1-hydroxy-propan-2-one, hydrochloride (6a)

3-*tert*-Butoxycarbonylamino-2-hydroxy-propionaldehyde, diethyl acetal (4ab). 3-Amino-2-hydroxy-propionaldehyde, diethyl acetal **3a** (1.41 g, 8.74 mmol) dissolved in methanol (5 mL) was reacted with di-*tert*-butyl-dicarbonate (1.8 g, 8.24 mmol) overnight at rt. Evapn of the solvent gave an oil (2.35 g) which was chromatographed on silica gel (200 g, elution gradient from 1:9 to 1:3 mixtures of AcOEt:hexane) to generate **4ab** as an oil (1.35 g, 59%), R_f 0.21 (SiO_2; 1:3, AcOEt:hexane). MS (DCI:CI:NH_3) *m/z* (rel. int.) 218 (100), 264 (MH^+, 40), 281 (MNH_4^+, 30).

3-Amino-1-hydroxy-propan-2-one, hydrochloride (6a). To the carbamate **4ab** (820 mg, 3.12 mmol) was added cold 37% HCl (5 mL). The mixture was stirred for 5 min at 0 °C and the reaction vessel was immersed in an oil bath at 110 °C for 2 min. The mixture was evapd under vacuum. The crude product was crystallized in a minimal volume of methanol. An oily solid was filtered, washed with methanol and dried over phosphorus pentoxide under high vacuum to give **6a** as a brown solid (78 mg, 20%). This material when recrystallized from methanol gave a melting point of 139 °C. ^{1}H NMR (D_2O): ketone form δ 4.12 (s, 2H), 4.48 (s, 2H); hydrate form: δ 3.16 (s, 2H), 3.64 (s, 2H), ketone:hydrate = 4:1. ^{13}C NMR (D_2O) ketone δ 206.2, 67.2, 46.3, hydrate δ 91.8, 71.4, 42.1. MS (DCI:CI:NH_3) *m/z* (rel. int.) 90 (MH^+, 60), 107 (MNH_4^+, 100). IR ν^{KBr} cm^{-1}: 1730.

3-Amino-1-hydroxy-5-methyl-hexan-2-one, hydrochloride (6b)

To the aminohydroxyacetal **3b** (1.0 g, 4.56 mmol) was added 37% HCl (30 mL). The mixture was stirred at 0 °C for 10 min and warmed in an oil bath at 75 °C for 3 min. The mixture was cooled and evapd. The residue was analyzed by ^{1}H NMR spectrometry. Since hydroxymethylketone was detected only in minor amounts, this mixture was retreated with 37% HCl at 100 °C for 2 min and analyzed. This process was again repeated to improve the conversion rate. The crude product was crystallized in a mixture of methanol and AcOEt and dried under high vacuum to yield a cream colored solid (259 mg, 31%).

Anal. ($C_7H_{15}NO_2$,HCl) C, H, N. ^{1}H NMR (D_2O): ketone δ 0.95 (t, 6H), 1.75 (m, 3H), 4.38 (dd, $J=9.5$, 3.5 Hz, 1H), δ_A 4.47, δ_B 4.58 (AB spectrum, $J_{AB}=19$ Hz, 2H); hydrate: δ 3.40 (dd, 1H), 3.68 (s, 2H). ^{1}H NMR (DMSO-d_6) δ 0.99 (d, $J=6.5$ Hz, 3H), 1.01 (d, 3H), 1.58 (m, 1H), 1.75 (m, 1H), 1.85 (m, 1H), 4.35 (br s or ABX + m, 3H), 5.75 (br s or t, 1H), 8.3 (br s, 3H). ^{13}C NMR (DMSO-d_6): δ 212.0 (C=O), 69.7 (CH_2OH), 57.5 (CHN), 42.0 (CH_2), 27.7 (CH), 26.8 (CH_3), 25.3 (CH_3). IR ν^{KBr} cm^{-1}: 3156, 2961, 1738 (C=O). MS (DCI:CI:NH_3) *m/z* (rel. int.) 146 (MH^+, 100), 163 (MNH_4^+, 60).

3-Amino-1-hydroxy-4-phenyl-butan-2-one, hydrochloride (6c)

3-*tert*-Butoxycarbonylamino-2-hydroxy-4-phenyl-butyronitrile. A mixture of *N*-BOC-L-phenylalaninal (1.12 g, 4.49 mmol) and sodium bisulfite (0.47 g, 4.52 mmol) in H_2O (10 mL) and toluene (10 mL) was stirred at rt overnight. The aq layer was evapd to yield a white solid (1.19 g) which was treated by sodium cyanide (193 mg, 3.94 mmol) in a mixture of AcOEt:water (5 mL:5 mL) at rt for 1.5 h. The mixture was extracted with AcOEt. The resulting mixture was washed with brine, dried over magnesium sulfate and evapd to give the desired compound as a yellow solid (572 mg, 46%). ^{1}H NMR ($CDCl_3$) mixture of diastereoisomers in a 1:1 ratio, δ, 1.4 (s, 9H), 2.92 (ABX spectrum) and δ_A 3.02, δ_B 3.14 (ABX spectrum, $J_{AB}=14$, $J_{AX}=9$, $J_{BX}=6$ Hz) (2H), 3.86 and 4.18 (m, CHN, 1H), 4.49 (d, $J=2.7$ Hz) and 4.57 (br s) ([CH(CN)(OH)], 1H), 4.89 (br d) and 5.00 (d, $J=7.3$ Hz) (NH, 1H), 7.3 (m, 5H).

3-*tert*-Butoxycarbonylamino-2-(*tert*-butyl-dimethyl-silanyloxy)-4-phenyl-butyronitrile (8). Under argon, a soln of 3-*tert*-butoxycarbonylamino-2-hydroxy-4-phenyl-butyronitrile (572 mg, 2.07 mmol) in anhydrous DMF (5 mL) was reacted with *tert*-butyl-dimethylchlorosilane (0.50 g, 3.32 mmol) in the presence of imidazole (0.29 g, 4.26 mmol) at rt overnight. The DMF was evapd under vacuum and the residue was extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over magnesium sulfate and evapd to give a crude product (708 mg) which was purified on silicagel (90 g, AcOEt:cyclohexane, 1:9) to generate **8** as an oil (632 mg, 78%). R_f 0.87 (SiO_2, AcOEt:cyclohexane, 1:9). ^{1}H NMR ($CDCl_3$) mixture of diastereoisomers in a 3:2 ratio, δ 0.12, 0.18 and 0.23 (s, 6H), 0.92 and 0.95 (s, 9H), 1.38 (s, 9H), δ_A 2.75, δ_B 3.15 and δ_A 2.88, δ_B 3.06 (ABX spectra, $J_{AB}=14$, $J_{AX}=9.2$, $J_{BX}=5.5$ Hz, 2H), 4.05 (m, 1H), 4.56 and 4.70 (d, $J=8.3$ Hz, NH, 1H), 4.65 (d, $J=3.9$ Hz, 1H), 7.25 (m, 5H).

3-*tert*-Butoxycarbonylamino-2-(*tert*-butyl-dimethyl-silanyloxy)-4-phenyl-butyraldehyde (9). The nitrile **8** (0.48 g, 1.23 mmol) was reduced over Raney nickel (about 1 g) in a mixture of ethanol:water, 4:1 (80 mL)

and 1 N H_2SO_4 (5 mL) under hydrogen at a pressure of 8.5 Bars for 3 h at rt. The resulting suspension was filtered on Celite and the catalyst was washed with ethanol. Aq sodium bicarbonate was added to the filtrate which was concd under vacuum. The residue was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over magnesium sulfate and evapd to give a residue which was purified by chromatogaphy on silica gel (40 g, elution gradient with AcOEt:cyclohexane mixtures from 1:9 to 1:1) to give **8** as an oil (110 mg, 23%). R_f 0.71 (SiO_2, AcOEt:cyclohexane, 1:9). ^{1}H NMR (200 MHz, $CDCl_3$) 1:1 mixture of isomers δ, 0.08 and 0.10, (s, 6H), 0.95 and 0.97 (s, 9H), 1.40 (s, 9H), 2.9 (m, 2H), 4.07 and 4.25 (br s, 1H), 4.34(q) and 4.6 (m, 1H), 4.75 and 4.85 (d, J=7 Hz, 1H), 7.25 (m, 5H), 9.37 and 9.54 (s, 1H).

3-*tert*-Butoxycarbonylamino-1-(*tert*-butyl-dimethyl-silanyloxy)-4-phenyl-butan-2-one (10). Under argon, a soln of **9** (80 mg, 0.20 mmol) in anhydrous CH_2Cl_2 (5 mL) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 70 μL, 0.47 mmol) at rt for 1.75 h. The mixture was processed as previously described for the synthesis of **8** to give a crude mixture which was eluted on silica gel (20 g, elution gradient with AcOEt:cyclohexane mixtures from 5:95 to 1:9) to give **10** as an oil (48 mg, 61%). $R_{f_{10}}$=0.52, R_{f_9}=0.45 (SiO_2, AcOEt:cyclohexane, 5:95). ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 6H), 0.92 (s, 9H), 1.39 (s, 9H), δ_A 2.95, δ_B 3.16 (ABX spectrum, J_{AB}=14, J_{AX}=6.7, J_{BX}=6.1 Hz, 2H), δ_A 4.17, δ_B 4.32 (AB spectrum, J_{AB}=18 Hz), 4.83 (q, J=7 Hz, 1H), 5.09 (d, J=7.9 Hz, 1H), 7.1 and 7.3 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 208.3 (C=O), 155.0 [O(C=O)N], 136.1, 129.3, 128.6, 126.9, 79.8 [$(CH_3)_3\underline{C}O$], 68.6 (CH_2O), 56.9 (CHN), 37.5 (CH_2Ph), 28.3 [$(\underline{CH_3})_3CO$], 25.8 [$(\underline{CH_3})_2Si$], 18.3 [$(CH_3)_3\underline{C}Si$], −5.5 [$(\underline{CH_3})_3CSi$].

3-Amino-1-hydroxy-4-phenyl-butan-2-one, hydrochloride (6c). A satd soln of HCl in Et_2O was added to protected compound **10** (48 mg, 0.12 mmol) diluted in anhydrous Et_2O. The mixture was left standing for 4 h at rt and the ppt filtered and dried under high vacuum to generate **6c** as a cream coloured powder (12 mg, 54%).

Anal. ($C_{10}H_{13}NO_2$, HCl, 0.25 H_2O) C, H, N. ^{1}H NMR (DMSO-d_6): δ_A 3.01, δ_B 3.18 (ABX spectrum, J_{AB}=14, J_{AX}=7.3, J_{BX}=5.9 Hz, 2H), δ_A 4.08, δ_B 4.23 (AB spectrum, J_{AB}=19 Hz, 2H), 4.48 (br t, J=6.1 Hz, 1H), 5.64 (s, 1H), 7.3 (m, 5H), 8.4 (s, 3H). ^{1}H NMR (200 MHz, D_2O) the mixture contains about 15% hydrate, δ_A 3.05, δ_B 3.50 (ABX spectrum, J_{AB}=13, J_{AX}=12, J_{BX}=4 Hz, 2H), 3.78 (dd, J=12, 4 Hz, 1H), 3.98 (s, 2H). MS (DCI:CI:NH_3) *m/z* (rel. int.) 110 (50), 136 (25), 180 (MH^+, 100), 197 (MNH_4^+, 20).

3-Amino-1-hydroxy-4-methyl-pentan-2-one, hydrochloride (6d)

1-Nitro-2-methyl-propane (1d). This material was prepd from 1-bromo-2-methyl-propane (30.2 g, 0.22 mol) as described for **1b** by reaction with sodium nitrite (20.8 g, 0.30 mol) in DMF in the presence of urea (18 g, 0.30 mol). Distillation of the crude product under 15 mm Hg generated **1d** (8.6 g, 38%).

2-Hydroxy-4-methyl-3-nitro-pentanal, diethyl acetal (2d). This compound was made as previously described, starting with **1d** (9.46 g, 92 mmol), diethoxy-acetaldehyde (2.67 g, 0.20 mmol) and potassium carbonate (276 mg, 2.0 mmol). Purification on silica gel (150 g, elution gradient with AcOEt:cyclohexane mixtures from 1:9 to 1:4) generated **2d** as an oil (2.94 g, 62%) R_f 0.31 (SiO_2, AcOEt:cyclohexane, 1:4).

3-Amino-2-hydroxy-4-methyl-pentanal, diethyl acetal (3d). Compound **2d** (2.94 g, 12.5 mmol) was hydrogenated over Raney nickel as previously described. The crude mixture was distilled trap to trap at 100 °C under 0.1 mBar to give **3d** as a colorless oil (1.86 g, 73%).

3-Amino-1-hydroxy-4-methyl-pentan-2-one, hydrochloride (6d). Compound **3d** (900 mg, 4.39 mmol) was stirred in 37% HCl (30 mL) at 0 °C for 10 min and warmed with an oil bath at 100 °C for 3 min. After cooling at 0 °C, the mixture was evapd under vacuum. The resulting solid was recrystallized in a mixture of MeOH and AcOEt and dried under high vacuum to afford **6d** as a cream coloured powder (211 mg, 29%). Anal. ($C_6H_{13}NO_2$, HCl) C, H, N. ^{1}H NMR (DMSO-d_6): δ 0.95 (d, 3H), 1.15 (d, 3H), 2.40 (m, 1H), 4.25 (br s, 1H), 4.35 (s, 2H), 5.75 (1H), 8.4 (3H). MS (DCI:CI:NH_3) *m/z* (rel. int.) 110 (50), 132 (MH^+, 100), 149 (MNH_4^+, 50). IR ν^{KBr} cm^{-1}: 3439, 3154 , 2973, 1732 (C=0), 1623, 1514.

3-Amino-4-biphenyl-4-yl-1-hydroxy-butan-2-one, hydrochloride (6e)

4-(2-Nitro-vinyl)-biphenyl. This compound was prepared according to ref 22. 4-Biphenylcarboxaldehyde (5.0 g, 27.4 mmol), nitromethane (5 mL, 113 mmol), ammonium acetate (2.0 g, 26 mmol) and acetic acid (20 mL) were refluxed for 2 h. The mixture was poured in ice-water (100 mL). The material that pptd was recrystallized in acetic acid, filtered, washed with EtOH and dried to give the desired compound (4.53 g, 73%).

4-(2-Nitro-ethyl)-biphenyl (1e). 4-(2-Nitro-vinyl)-biphenyl (2.25 g, 10 mmol) was reduced with $NaBH_4$ (0.83 g, 22 mmol) as described for 1-nitro-2-phenyl-ethane. A brown solid (1.97 g, 87%) was obtained which was used without further purification.

4-Biphenyl-4-yl-1,1-diethoxy-3-nitro-butan-2-ol (2e). Compound **1e** (1.91 g, 8.4 mmol) was reacted with diethoxy-acetaldehyde (0.89 g, 6.74 mmol) in the presence of potassium carbonate (0.10 g, 0.7 mmol) as previously described. The crude was purified by chromatography (200 g SiO_2, elution gradient with AcOEt:cyclohexane mixtures from 1:9 to 1:4) to yield the title compound as an oil (2.07 g, 68%).

3-Amino-4-biphenyl-4-yl-1,1-diethoxy-butan-2-ol (3e). Compound **2e** (1.78 g, 4.95 mmol) was reduced by hydrogenolysis over Raney nickel as previously described. A yellow oil (1.65 g, 100%) was obtained.

3-*tert*-Butoxycarbonylamino-4-biphenyl-4-yl-1,1-diethoxy-butan-2-ol (4eb). The amine **3e** (1.46 g, 4.43 mmol) was reacted with di-*tert*-butyl-dicarbonate (1.0 g, 4.58 mmol) in MeOH (50 mL) overnight. The mixture was evaporated and the resulting oil (1.98 g) was purified on silica gel (150 g, elution gradient with AcOEt:cyclohexane mixtures from 1:9 to 1:4). The diastereoisomer that first eluted was a solid (0.46 g, mp 91–92 °C) MS (DCI:CI:NH_3) *m/z* (rel. int.) 328 (30), 384 (100), 430 (MH^+, 30), 447 (MNH_4^+, 5). Anal. ($C_{25}H_{33}NO_5$) C, H, N. The more polar diastereoisomer was obtained as an oil (0.50 g) with an overall yield of 50%.

3-Amino-4-biphenyl-4-yl-1-hydroxy-butan-2-one hydrochloride (6e). A suspension of **4eb** (185 mg, 0.43 mmol) in a mixture of 37% HCl (7 mL) and acetic acid (2 mL) was stirred at 0 °C for 1.75 h and then warmed in an oil bath maintained at 70 °C for 3 min. The reaction flask was cooled at 0 °C and evapd under vacuum to give a solid (55 mg) which was recrystallized in a mixture of MeOH and AcOEt and dried under high vacuum to generate **6e** as a slightly cream coloured powder (28 mg, 22%).

Anal. ($C_{16}H_{17}NO_2$,HCl,0.25H_2O) C, H, N. ^{1}H NMR (DMSO-d_6) δ_A 3.12, δ_B 3.35 (ABX, $J_{AB}=14$, $J_{AX}=7.5$, $J_{BX}=5.5$ Hz, 2H), δ_A 4.30, δ_B 4.40 (ABX, $J_{AX}=J_{BX}=5$ Hz, 2H), 4.63 (br t, $J=6$ Hz, 1H), 5.77 (br t, $J=6$ Hz, 1H), 7.48 (m, 3H), 7.57 (m, 2H), 7.77 (m, 4H), 8.35 (br s, 3H). ^{13}C NMR (DMSO-d_6) δ 211, 143.6, 143.0, 138.0, 134.1, 132.9, 131.4, 130.9, 130.5, 70.3, 59.9, 38.6.

Enzyme inhibitory activity

Enzyme sources. Aminopeptidase-M (AP-M, EC 3.4.11.2) was purified from porcine kidney as a soluble protein.[39] Cytosolic leucine aminopeptidase (LAPc, EC 3.4.11.1) was purchased from Sigma Chemical Co. Cytosolic arginyl aminopeptidase (AP-B, EC 3.4.11.6) was purified from rat liver.[40] *Aeromonas proteolytica* aminopeptidase (AP-aero, EC 3.4.11.10) was purified according to a published procedure.[41]

Determination of enzyme activities. Measurement of inhibitory potency was carried out as described by Schalk et al.[42] Briefly, spectrophotometric assays were performed with L-leucine-*p*-nitroanilide as a substrate for AP-M, LAPc and AP-aero. Arginyl-*p*-nitroanilide was used as a substrate for AP-B. The hydroxyaldehyde inhibitors **5** were kept in aq soln at rt for several days prior to evaluation. The presence of the parent hydrate could be confirmed by ^{13}C NMR spectrometry on the stock solution.

The release of *p*-nitroanilide at 405 nm ($\varepsilon=10\,800$ M^{-1} cm^{-1}) was measured to determine initial velocities.[43] K_i values were determined using a Dixon plot.[44]

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References and Notes

1. Taylor, A. *FASEB J.* **1993**, 290.

2. Sanderink, G.-J.; Artur, Y.; Siest, G. *J. Clin. Chem. Clin. Biochem.* **1988**, *26*, 795.

3. McDonald, J. K.; Schwabe, E. C. *Proteinases in Mammalian Cells and Tissues*; Elsevier/North Holland: Amsterdam, 1977; pp 311–392.

4. Giros, B.; Gros, C.; Solhonne, B.; Schwartz, J. C. *Mol. Pharmacol.* **1986**, *29*, 281.

5. Ward, P. E.; Benter, I. F.; Dick, L.; Wilk, S. *Biochem. Pharmacol.* **1990**, *40*, 1725.

6. Powers, J. C.; Harper, J. W. In *Proteinases Inhibitors* Barrett, A. J.; Salvesen, G. Eds; Elsevier, Amsterdam, 1986; pp 219–298.

7. Nishizawa, R.; Saino, T.; Takita, T.; Suda, H.; Aoyagi, T.; Umezawa, H. *J. Med. Chem.* **1977**, *20*, 510.

8. Tobe, H.; Morishima, H.; Aoyagi, T.; Umezawa, H.; Ishiki, K.; Nakamura, K.; Yoshioka, T.; Schimauchi, Y.; Inui, T. *Agric. Biol. Chem.* **1982**, *46*, 1865.

9. Umezawa, H. In *Small Molecular Immunomodifiers of Microbial Origin, Fundamental and Clinical Studies of Bestatin*; Umezawa, H., Ed.; Japan Scientific Societies and Pergamon: Oxford, 1981.

10. Kim, H.; Lipscomb, W. N. *Biochemistry* **1993**, *32*, 8465.

11. Frick, L.; Wolfenden, R. *Biochem. Biophys. Acta* **1985**, *829*, 311.

12. Andersson, L.; MacNeela, J.; Wolfelden, R. *Biochemistry* **1985**, *24*, 330.

13. Andersson, L.; Isley, T. C.; Wolfenden, R. *Biochemistry* **1982**, *21*, 4177.

14. Wohl, A.; Schweitzer, H. *Chem. Ber.* **1907**, *40*, 92.

15. Fischer, H. O. L.; Baer, E.; Nidecker, H. *Helv. Chim. Acta* **1935**, *18*, 1079.

16. Jackson, A. O.; Marvel, C. S. *J. Biol. Chem.* **1933**, *103*, 191.

17. Waszkuc, W.; Janecki, T.; Bodalski, R. *Synthesis* **1984**, 1025.

18. Kirmann, A.; Druesne, F. *C. R. Acad. Sc. Paris* **1964**, *259*, 3285.

19. Lobry De Bruyn, C. A.; Aberda van Ehenstein, W. *Chem. Ber.* **1895**, *28*, 3078.

20. Kornblum, N.; Larson, H. O.; Blackwood, R. K.; Mooberry, D. D.; Oliveto, E. P.; Graham, G. E. *J. Am. Chem. Soc.* **1956**, *78*, 1497.

21. Varma, R. S.; Kabalka, G. W. *Synth. Commun.* **1985**, *15*, 151.

22. Gairaud, C. B.; Lappin, G. R. *J. Org. Chem.* **1953**, *18*, 1.

23. Sangsari, F. H.; Chastrette, F.; Chastrette, M. *Synth. Commun.* **1988**, *18*, 1343.

24. Stassinopoulou, C. I.; Zioudrou, C. *Tetrahedron* **1972**, *28*, 1257.

25. Kirrmann, A.; Cantacuzène, J.; Vio, L.; Martin, M. *Bull. Soc. Chim. France* **1963**, 1067.

26. Aminoacetaldehyde, diethyl acetal was produced as a side-product during the reduction of **2b** by hydrazine over Raney nickel. Subsequently, BOC-aminoacetaldehyde diethyl acetal was treated with 37% HCl 5 min under reflux to give aminoacetaldehyde hydrochloride.

27. Creary, X.; Rollin, A. J. *J. Org. Chem.* **1977**, *42*, 4231.

28. NH_4Cl displays two pseudo triplets at δ 7.30 and 7.32 with a 51 Hz line separation at 360 MHz.

29. d'Orchymont, H.; Tarnus, C. *US Patent* 1992, 5,106,861.

30. Tinapp, P. *Chem. Ber.* **1971**, *104*, 2266.

31. For **5b** and **5c**, a single pair of enantiomers was tested. It is likely that epimerization of the chiral carbon α to the hydroxyl group occurs rapidly at the test conditions at pH 7–8.

32. For example, the presence of water bound to the catalytic zincs was revealed in the structure of native *Aeromonas proteolytica* aminopeptidase. Chevrier, B.; Schalk, C.; d'Orchymont, H.; Rondeau, J.-M.; Moras, D.; Tarnus, C. *Structure* **1994**, *2*, 283.

33. Fittkau, S.; Jahreis, G.; Peters, K.; Bulaspiri, L. *J. Prakt. Chem.* **1986**, *328*, 529.

34. Wilkes, S. H.; Prescott, J. M. *J. Biol. Chem.* **1983**, *258*, 13517.

35. Chan, W. W.-C.; Dennis, P.; Demmer, W.; Brand, K. *J. Biol. Chem.* **1982**, *257*, 7955.

36. Holmes, M. A.; Matthews, B. W. *Biochemistry* **1981**, *20*, 6912.

37. Borkakoti, N.; Winkler, F. K.; Williams, D. H.; D'Arcy, A.; Broadhurst, M. J.; Brown, P. A.; Johnson, W. H.; Murray, E. J. *Nature Struc. Biol.* **1994**, *1*, 106.

38. Stams, T.; Spurlino, J. C.; Smith, D. L.; Wahl, R. C.; Ho, T. F.; Qoronfleh, M. W.; Banks, T. M.; Rubin, B. *Nature Struc. Biol.* **1994**, *1*, 119.

39. Wacker, H.; Lehky, P.; Fischer, E. H.; Stein, E. A. *Helv. Chim. Acta* **1971**, *54*, 473.

40. Söderling, E. *Prep. Biochem.* **1980**, *10*, 191.

41. Schalk, C.; Remy, J.-M.; Chevrier, B.; Moras, D.; Tarnus, C. *Arch. Biochem. Biophys.* **1992**, *294*, 91.

42. Schalk, C.; d'Orchymont, H.; Jauch, M. F.; Tarnus, C. *Arch. Biochem. Biophys.* **1994**, *311*, 42.

43. Tuppy, H.; Wiesbauer, W.; Wintersberger, E. *Hoppe-Seyler's Z. Physiol.Chem.* **1962**, *329*, 278.

44. Segel, I. H. *Enzyme Kinetics*; John Wiley & Sons, New York, 1975.

45. Wilkes, S. H.; Prescott, J. M. *J. Biol. Chem.* **1985**, *260*, 13154.

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