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An unusual functional group interaction and its potential to reproduce steric and electrostatic features of the transition states of peptidolysis

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Abstract—The donor–acceptor interaction between a tertiary amine and an aldehyde, first observed among a select class of alkaloïds, was deliberately established in a peptidomimetic (**1a–c**) to mimic features of the two principal transition states of peptide hydrolysis. Compounds **1a–c** show preferential adoption in methanol and water of a 'folded' conformation displaying the interaction. Proportions of the folded form in MeOH range from 45% to 70% and can reach 84% in buffer. Significantly, three tendencies for the folded/unfolded equilibrium are observed: increasing solubility and polarity of the medium and decreasing temperature results in a higher extent of folding. In the absence of any parameter set available for this weak bond, no modeling studies were conducted to aid in the design of **1a–c**. The successful straightforward synthesis of **1** and its folding and inhibition results with HIV-1 peptidase using FRET technology encourage studies to further preorganize candidate molecules and to screen the structure space by modeling and parallel combinatorial chemistry.

1. Introduction

The imitation of the features of the transition states of peptide-bond hydrolysis has long been applied to the design of potent inhibitors of aspartic peptidases as part of a fundamental teaching of enzymology.[1] This strategy evolved from the discovery that certain naturally occurring peptides, containing a non-proteinogenic amino acid called statine (pepstatine, Fig. 1), represent a general inhibitory principle for the majority of aspartic peptidases.[2]

With the advent of the age of structure-based design of potent HIV-1 peptidase inhibitors at the end of the 1980s, researchers have chosen mainly the hydroxyethylene moiety (Fig. 1) contained in statine as the point of departure to elaborate.[3] It is now well-established knowledge that the hydroxyl group therein interacts with the catalytic machinery of the active site, that is, with the two aspartic acid residues.[4] This interaction results in the extrusion of the localized water molecule to be found in crystal structures of ligand-free peptidases between the two aspartic residues.[5] This water molecule is generally held to be the second substrate molecule of peptide hydrolysis, itself being a bimolecular reaction. This has led to the view that hydroxyethylene-based

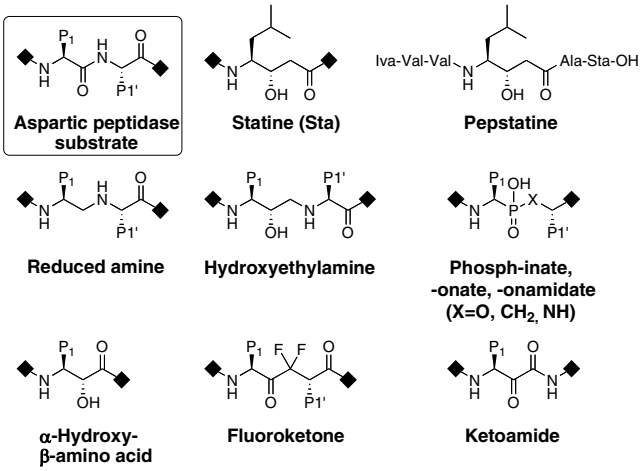

Figure 1. A selection of transition-state isosteres of peptide hydrolysis.

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inhibitors act as 'collected-substrate' or 'multi-substrate' inhibitors,[6] thus benefiting from a considerable entropic advantage for increased affinity. These highly potent inhibitors have met with remarkable success in the struggle against related diseases.

It is also generally maintained that the tetrahedral nature of the secondary alcohol function in the hydroxyethylene moiety reproduces the geometry of the 'tetrahedral intermediate,' or 'transition state,' as is sometimes used in its place, which led to the introduction of the term 'transition-state isostere.' Many other such isosteres have been explored in the wake of the statin-based approach, such as phosphonic acid- and phosphinic acid-based moieties,[7] α-perfluorinated ketones,[8] α-ketocarboxamides,[9] or sulfoxide and sulfone-containing units (Fig. 1). This listing does not account for the other penultimate constituent of the transient species on the reaction coordinate of peptide hydrolysis, namely the amine leaving group. In fact, many of the above isosteres have been combined with substituents on the C-terminal side representing this part, which, if still containing a nitrogen atom, often necessitate their spatial separation from the CHOH moiety by at least one carbon atom (see 'hydroxyethylamine,' Fig. 1), in effect rendering the nitrogen a very basic center with the pK_a of a tertiary amine. A more close resemblance with species on the reaction coordinate can be found in phosphonates and phosphonamidates;[10] which led to the design of numerous potent hydrolase inhibitors. However, their electrostatic properties have been calculated to be different from those of transition states associated with alkaline ester hydrolysis.[11] They also suffer from the practical inconvenience of hydrolytic instability in aqueous media which somewhat limits their pharmaceutical applications. Sulfonamidates appear to have had little success in inhibitor design.[12]

In fact, as many theoretical studies have shown, the reaction coordinate of carboxamide hydrolysis features two main transition states (TS1, TS2, Fig. 2) separated by the tetrahedral intermediate (INT).[13,14] Some of these workers find a third barrier leading to product formation, following a second, fully zwitterionic, intermediate. According to the main pillar of enzymology, enzymes have evolved catalytic power by *differentially binding* to transition state(s) (here: TS1, TS2) over ground states (ES, EP).[15,16]

In the context of enzyme inhibition, as well as that of catalytic-antibody induction, it has thus been found regrettable that current transition-state isosteres are often far from faithfully reproducing steric *and* electrostatic elements of the respective transition state(s) (Fig. 2),[17,18,11] in this case a partial charge on the carbonyl oxygen with an associated pK_a value distinctly different from that of either hydroxyl groups or phosphinates, -onates, and -onamidates, a C–O bond order around 1.5, a pseudo-tetrahedral nature of the carbon, and, for the 2nd transition state, an additional partial charge on the nitrogen and an unusually long C–N single bond (Table 1).

2. Results and discussion

We wish to explore an infrequently observed functional group for its capacity to mimic at the same time some essential geometric *and* also electrostatic features of the transition state(s), both being different from those of the tetrahedral intermediate.[19] This molecular moiety results from the weak interaction of a tertiary amine with a ketone (or aldehyde) function and has been observed initially in a particular class of alkaloids[20,21] in the form of trans-annular functional group interactions, subsequently studied in further detail in nature-inspired compounds[22] and eventually helped in the establishment of the Bürgi–Dunitz trajectory as a fundamental element of Physical Organic Chemistry[23] (Fig. 3).

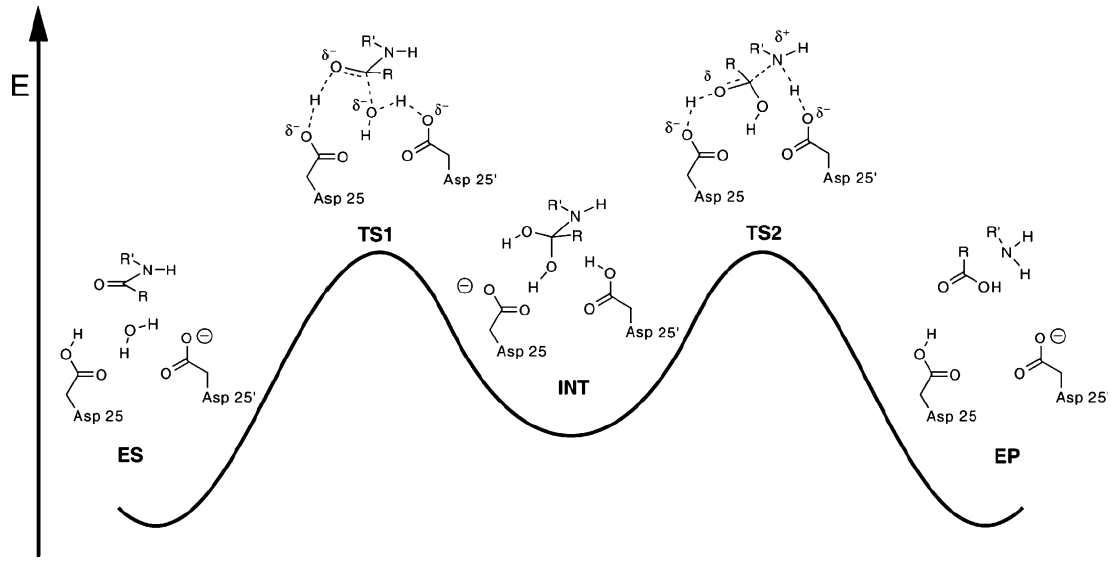


Figure 2. A schematic reaction coordinate of the mechanism of peptide hydrolysis within the active site of aspartic peptidases.

Table 1. Comparison of bond lengths

Bond nature	Bond length [Å]	Ref.
Ground state		
C–N ($MeNH_2$)	1.47	25,26
C–NR_3^+	1.49	25
C–O (MeOH)	1.41	25
C=O (MeCHO)	1.19	25
Ground-state peptide bond		
C=O	1.23	25
C–N	1.33	25
Transition state peptide bond		
C–N calculated in TS 1, TS 2	1.52–2.21	27,28
C–O calculated in TS 1	<1.296	12
GS $^{\delta+}N \rightarrow C{=}O^{\delta-}$ (X-ray)		
C–N	1.62–2.91	29,35
C=O	1.21–1.31	29,35
GS R_3N^+–CHOH (X-ray)		
C–N	1.58	30,31
C–O	1.37	30

GS, ground state.

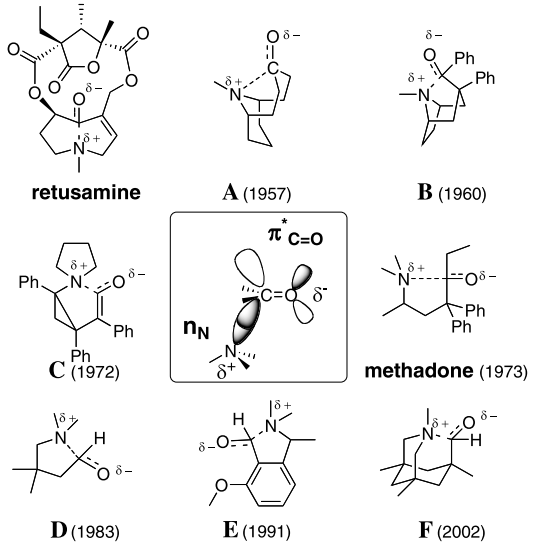


Figure 3. Examples of compounds displaying the tertiary amine-carbonyl interaction.

The $^{\delta+}N \rightarrow C{=}O^{\delta-}$ interaction has been described as a through-space homoconjugation of the *n* and π molecular orbitals of the nitrogen and C=O double bond, respectively, where electron density is shifted from the *n* orbital to the π* orbital, giving rise to an enhanced dipole moment and a hypsochromic shift of the carbonyl UV absorption (see below).[24]

Its attractive features in the context of this project are: (a) an oxygen-bearing carbon with a transient configuration between sp^2 and sp^3, (b) an oxygen carrying a partial negative charge, (c) a carbon–oxygen bond of intermediate length, (d) an unusually long nitrogen–carbon bond distance, and (e) a partial positive charge on the nitrogen (see Table 1). In effect, the nucleophilic approach of a tertiary nitrogen on a carbonyl group as a point of reference for the description of generic nucleophile approach on carbonyl-containing substrates (being the subject of the study of Bürgi and Dunitz) finds its inverse parallel in the final step (TS2) of carboxamide hydrolysis.

By contrast, a forced interaction between a tertiary amine and a carboxylic acid on a preorganizing matrix, even though an attractive solution on initial consideration, cannot be envisaged for inhibitor design: while Dunitz et al. observed a C—N distance of only 2.49 Å (the van der Waals distance is $r_C + r_N = 3.20$ Å) in the crystal structure of a naphthalene-based system containing a tertiary amine group and a *carboxylic acid* group,[32] in aqueous solution at around pH 7 only the classic zwitterionic amino acid form would exist.

Some more recent sporadic observations of the weak $^{\delta+}N \rightarrow C{=}O^{\delta-}$ interaction (**C**,[33] **D**,[34] **E**,[30] and **F**[35]) have helped us in assessing the feasibility of introducing it into a *peptide-derived peptidomimetic*.[5] Importantly, detection of the $^{\delta+}N \rightarrow C{=}O^{\delta-}$ interaction is not limited to a trans-annular context, nor does it require the grafting of the interaction partners onto a rigid framework. Equally important is the fact that the weak interaction is favored in protic, polar media over aprotic nonpolar solvents. In logic extension of such findings made in the 1950s and 1960s, rather recent reports confirm this effect to be even more pronounced in water.[30,34,35] This may help in designing molecules that adopt in free aqueous solution the (cyclic) conformation meant to be complementary to the active site. What has emerged from these past observations is thus a novel functional group with rather unique stereoelectronic properties that may be displayed as the core feature of a peptidomimetic, provided that its backbone is sufficiently preorganized.

Importantly, the design of such a first example of inhibitor candidate in this paper was to be conducted without knowing the actual structure of the inhibitor (aside from the configuration of the two asymmetric carbons) nor having carried out any modeling attempts outside or within the active site.[36]

2.1. Design and synthetic strategy

The need for incorporation of a tertiary amine function into the final target has led us to choose as an initial point of departure a dipeptide mimic (Fig. 4) of the substrate sequence preferentially cleaved by HIV-1 peptidase (HIV1-PR), that is, Phe-Pro. Here, one only needs to introduce a molecular scaffold (bridge) linking the α-carbon of Phe with the nitrogen of Pro in order to

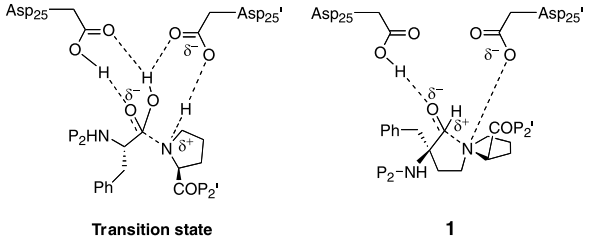


Figure 4. A dipeptidic transition-state mimic (**1**).

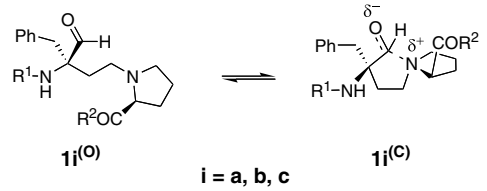
obtain a molecule (**1**) with increased chances of showing the desired interaction in aqueous media. The design of **1** was inspired by the observed ring-open/ring-closed equilibrium of compound **D**[34] in different media, a behavior largely identical to those of compounds **E** and **F** (Fig. 3).

Compound **1** has been chosen as a prototype on which to study the conformational equilibrium between the open and the closed form in different media, its stereochemical preferences, and its affinity to the model enzyme, namely HIV-1 PR. Three separate derivatives of **1** were targeted, **1a**–**c** (Fig. 5). They differ in their periphery around **1**, either by carrying, on the C terminus, a methyl ester, a *t*-butylated carboxamide terminus, or a *t*-butylated valine residue, and, on the N terminus, either an Alloc-protecting group stemming from synthetic requirements or a Cbz-protected valine residue.

2.2. Synthesis

Compound **6** is the common synthetic intermediate to the three targets. The quaternary carbon center in **3** was obtained using the method of 'self-regeneration of stereocenters' developed by Seebach.[37] *cis*-Oxazolidinone **2**, synthesized from L-phenylalanine by known procedures,[38] was converted to the corresponding enolate by treatment with potassium hexamethyldisilazide in THF at −78 °C. The diastereoselective alkylation of the resulting chiral anion with 2-bromoethyl triflate afforded **3** in 85% yield as a single diastereomer with retention of configuration at the starting stereocenter. The stereochemistry of **3** was confirmed by its NOESY spectrum, which revealed NOE contacts between the *t*-Bu group and the benzyl methylene group and between the methine and the methylene group of the introduced moiety. The bromide **3** was then reacted with commercially available H-Pro-O-*t*-Bu in the presence of Et_3N to give **4** in 82% yield.

Careful reduction of **4** with lithium aluminum hydride at −78 °C afforded oxazolidinol **5** in 50% yield. To our knowledge, this reaction constitutes the first example of the reductive transformation of a sterically hindered 2,5-disubstituted oxazolidinone into an oxazolidinol, achieved by the use of LAH, without exhaustive reduction into a ring-opened diol as has been observed for 2-substituted oxazolidinols.[39] In parallel to what is frequently seen in carbohydrate chemistry, the oxazolidinol **5** was then directly converted into the dithioketal **6** in 83% yield by a $BF_3{\cdot}OEt_2$-catalyzed exchange reaction with 1,2-ethanedithiol. Methylation of **6** with trimethylsilyldiazomethane in benzene/methanol afforded the ester **7** in 64% yield. Finally, deprotection of **7** with $HgO/BF_3{\cdot}OEt_2$ produced the aldehyde **1a** in 47% yield. Target compound **1a** was thus prepared via an efficient six-step synthesis with 8.7% overall yield (Scheme 1).

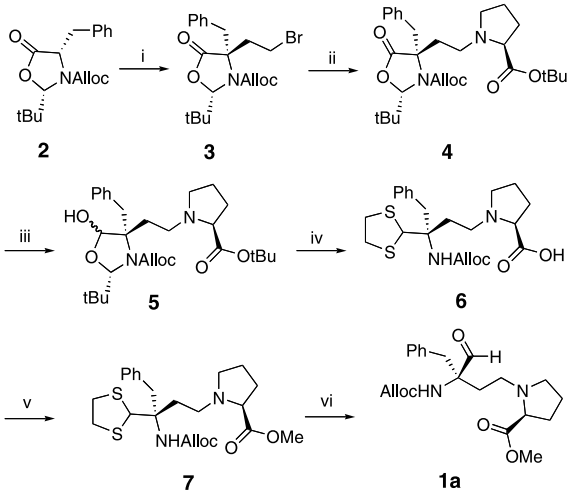


1a R^1= Alloc, R^2= OMe; **1b** R^1= CbzVal, R^2= NHtBu;
1c R^1= CbzVal, R^2= ValNHtBu

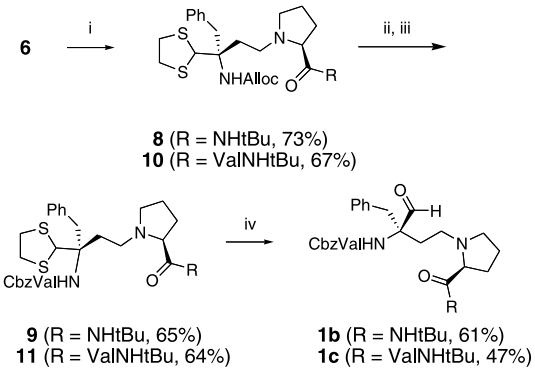
Figure 5. The folding equilibrium of the target peptidomimetics **1a**–**c**.

The synthesis of compounds **1b** and **1c** started with the coupling of **6** with *tert*-butylamine and H-Val-NH-*t*-Bu, respectively, using the peptide-coupling cocktail DCC/HOBt; compounds **8** and **10** were thus obtained in 73% and 67%, respectively (Scheme 2). The Alloc-protecting group was then selectively removed via $Pd(Ph_3)_4$-catalyzed allyl transfer in the presence of dimedone.[40] Peptide coupling under classic coupling conditions with the thus liberated amine function adjacent to a quaternary center failed.[41] We successfully coupled the sterically hindered free amine with N-Cbz-L-valine using the reagent cocktail CIP/HOAt to afford **9** and **11** in 65% and 64% yield, respectively.[42] The synthesis was finished with their deprotection by use of $HgO/BF_3{\cdot}OEt_2$ and aldehydes **1b** and **1c** were obtained in 61% and 47% yield, respectively.

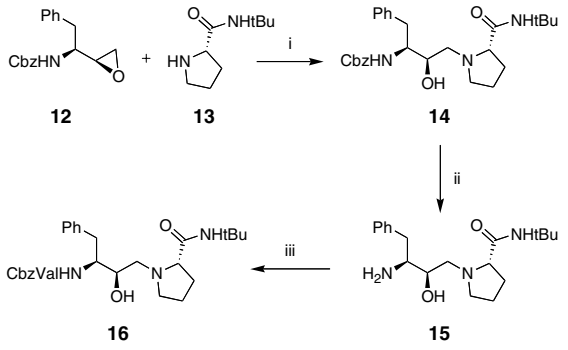
For the inhibition studies we were in need for a control inhibitor containing a transition-state isostere of proven potency while mimicking the Phe-Pro dipeptide targeted in this study. We therefore synthesized hydroxyethylamine **16** (Scheme 3). The synthetic intermediate **14** was synthesized by regioselective nucleophilic attack of aminoamide **13**[43] on chiral α-amino epoxide **12**[44] in 74% yield. Removal of the Cbz-protecting group from **14** by Pd/C-catalyzed hydrogenolysis afforded **15** in quantitative yield. Hydroxyethylamine **16** was then obtained in 65% yield by coupling of the amine **15** with N-Cbz-L-valine using CIP/HOAt.



Scheme 1. Synthesis of **1a**. Reagents and conditions: (i) (1) KHMDS (1 equiv), THF, −78 °C, 30 min; (2) $Br(CH_2)_2OTf$, −78 °C to rt, overnight, 85%; (ii) H-Pro-O-*t*-Bu, Et_3N Et_3N, DMF, 60 °C, 3 days, 82%; (iii) LAH (2 equiv), THF, −78 °C, 48 h, 50%; (iv) $HS(CH_2)_2SH$ (4 equiv), $BF_3{\cdot}OEt_2$ (3 equiv), CH_2Cl_2, 24 h, 83%; (v) $TMSCHN_2$, C_6H_6/MeOH 7:2, 1.5 h, 64%; (vi) $HgO/BF_3{\cdot}OEt_2$, THF/H_2O 85:15, 2 h, 47%.



Scheme 2. Synthesis of compounds **1b** and **1c**. Reagents and conditions: (i) RNH_2, DCC, HOBt, THF, overnight; (ii) $Pd(Ph_3)_4$, dimedone, THF, overnight; (iii) CbzValOH, CIP, HOAt, CH_2Cl_2, 2 days; (iv) $HgO/BF_3{\cdot}OEt_2$, THF/H_2O 85:15, 2 h.



Scheme 3. Synthesis of **16**. Reagents and conditions: (i) MeOH, reflux overnight, 74%; (ii) H_2, Pd/C, ethanol, 2 h, quantitative; (iii) CbzValOH, CIP, HOAt, CH_2Cl_2, 0 °C, 2 h, 65%.

2.3. Conformational analysis in solution

Before testing **1a**–**c** for their ability to inhibit peptidolytic activity, it was of fundamental importance to determine their actual conformational and configurational preferences.

When ^{13}C NMR spectra of **1a** were recorded in $CDCl_3$ and MeOH-d_4, respectively, it was found that the methanol spectrum displayed a splitting for each signal essentially giving rise to two independent sets. The only significant difference was found for the signal corresponding to the aldehyde carbon that had been shifted upfield for more than 100 ppm, residing now at 97.8 ppm, right in the middle of the region found for common organic compounds between the olefinic/aromatic and the aliphatic region. It may be deduced that the carbon corresponding to this signal adopts a hybridization state between those of sp^2 and sp^3 as is desired as part of the fundamental aims of this project. This behavior found its parallel in the ^1H NMR spectra (Fig. 6) where only one set of signals was observed in chloroform, but two sets for the MeOH-d_4 solution. The set displaying higher intensity did not contain a signal in the aldehydic region (9.35 ppm), but instead a singlet was found at 3.87 ppm. A weak change in shift was observed for the methyl singlet corresponding to the methyl ester moiety, as is likely the case for all multiplets as

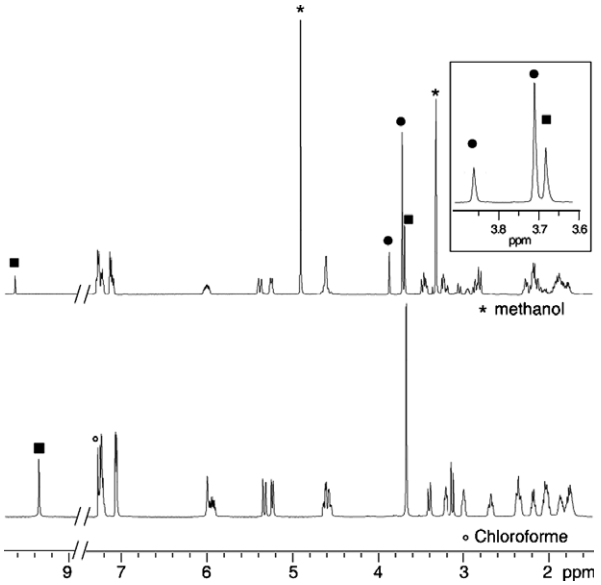


Figure 6. ^1H NMR spectra (500 MHz) of **1a** in $CDCl_3$ (■ signal of the open-form aldehydic proton) and MeOH-d_4 (■ signals of the open-form aldehydic proton and its corresponding methyl ester group; ● signals of the folded-form methine proton and its corresponding methyl ester group).

well. Thus, the two sets of signals may be attributed to a slow exchange compared to the NMR time scale of two forms of **1a**. The correspondence of the signal at 3.87 ppm and that at 97.8 ppm in the ^{13}C NMR spectrum was proven by a two-dimensional HMQC experiment (^1H–^{13}C). A correlation signal due to $^1J_{H,C}$ heteronuclear coupling was found for the postulated methine group in **1a**. Integration of the signals at δ_H 9.63 ppm and at δ_H 3.87 ppm allows us to estimate the conformational equilibrium constant between the closed and the open form: at 20 °C in MeOH-d_4 a 70/30 ratio is found, corresponding to a $K^{obs} = 2.33$.[45]

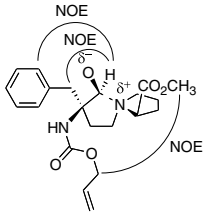
In order to potentially obtain thermodynamic data on the open–close equilibrium of **1a**, we conducted a temperature-dependent ^1H NMR experiment (DNMR) at high temperatures (300–350 K, at 200 MHz), only to realize that even at elevated temperatures the establishment of the equilibrium remains slow on the NMR time scale.[45] This may not be too surprising in view of the necessary change of numerous dihedral angles and the movement of large substituents compared to the process of simple rotamer exchange where coalescence points are frequently determined. By integration of the proton signals of the aldehyde and the methine group, respectively, it was found that the proportion of the cyclic form decreases with rising temperature. Over a range of 50 °C the proportion of the cyclic form diminishes by 38%.[47] This behavior is in agreement with those of other reports.[35]

In view of the above results, a DNMR experiment at low temperatures (213–293 K, 500 MHz) was also carried out so as to potentially freeze out the cyclic form.[45] As was expected from the high-temperature experiment, the proportion of the cyclic form increased with falling temperature to attain a value of 90% at 213 K. The slow appearance of an extra singlet at 3.73 ppm, tentatively

assigned to the methyl ester group of the cyclic form because of its integration value, may be explained with the emergence of two distinct conformers of the spiro-type structure of **1a**.

A *conformational* analysis of **1a** alone does not suffice when taking into account the possibility of observing four distinct configurational isomers arising from the formation of two pseudo-tetrahedral asymmetric centers. In our case, the NMR spectra appear to indicate the existence of but one diastereomer of the cyclic form. On this assumption, we conducted a NOESY experiment at 500 MHz. In spite of the difficulty of interpreting the 2D spectrum due to the presence of two molecular species, three NOE contacts were identified.

One of them is attributed to an interaction between the methine group and one of the benzyl methylene protons, another to the one between the methine group and aromatic protons, and yet another to the one between the methyl ester protons and the methylene of the Alloc-protective group. While one has to be cautious in attributing the latter due to the closeness of the methyl signals of the closed *and* open forms, it is assumed that the open form will likely exist in an extended conformation and thus not give an NOE contact. On the grounds of the NOESY data, we tentatively assign the following configuration (figure) to the cyclic form of **1a** in MeOH at room temperature.



The conformational equilibria of compounds **1b** and **1c** were also investigated by NMR experiments. As expected from the above observations with **1a**, in chloroform both compounds exist only in the ring-open form as evidenced by the existence of only an aldehydic signal. By contrast, the data obtained from the experiment in methanol are slightly more complicated to interpret than for **1a**. Aside from the typical aldehydic signal, **1b** shows several new signals in the region where the methine proton of **1a** was observed. Compound **1c** in methanol also displays several signals in that same region around 4 ppm being superposed with those stemming from the α protons of the valine residues. These findings suggest that compounds **1b** and **1c** exist at the same time in an extended conformation and *several* ring-closed forms in slow equilibrium on the NMR time scale. As was pointed out above, the latter may be explained by the adoption of different configurations of the $^{\delta+}N \rightarrow C{=}O^{\delta-}$ functional group or by different conformations of the five-membered rings of the spiro form or again by a mixture of both phenomena. An estimation of the proportion of the extended form can nevertheless be furnished on the basis of the integration of the corresponding aldehydic signal: **1b** and **1c** adopt to 45% and 55% the open form, respectively. A higher proportion of the cyclic form may be observed in water and can no doubt be obtained by introducing additional elements of preorganization.

UV spectroscopy presents another independent means of estimating the open-chain/cyclic ratio of a given candidate compound.[46] UV spectra of **1a**–**c** were thus determined and found to be similar in appearance (Fig. 7): in chloroform, an intense band at 245 nm is observed that may be attributed to the $\pi \rightarrow \pi^*$ absorption; in methanol, a decrease of absorption intensity of this band is accompanied by a concomitant emergence of a new, very intense band at around 220 nm that stems from the newly formed $^{\delta+}N \rightarrow C{=}O^{\delta-}$ moiety in accord with results from previous reports.[46]

Aqueous solutions of **1a**–**c** buffered at pH 4.7 (10% DMSO, acetate buffer), while not being sufficiently concentrated for NMR spectroscopy, were subjected to the same UV analysis. The results[47] support the existence of an equilibrium of two protonated forms, namely the ring-open (**1i**$^{(o)}$-NH^+) and the cyclic conformation

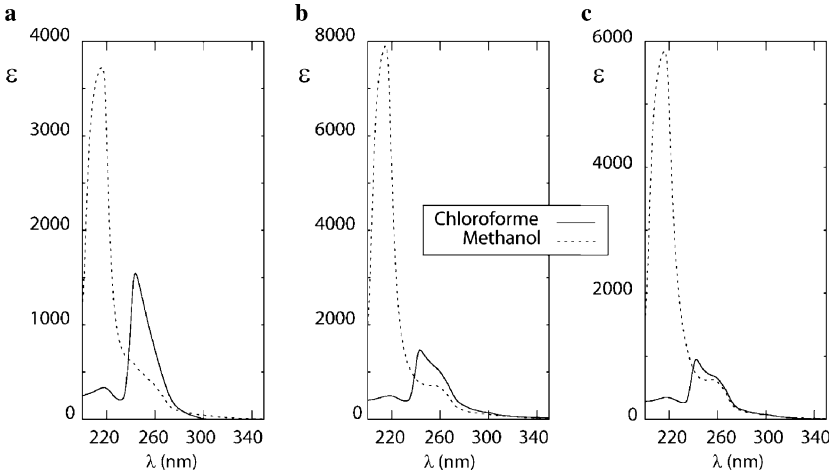


Figure 7. UV spectra in chloroform (continuous line) and in methanol (dotted line) of (a) **1a**, (b) **1b**, and (c) **1c**.

(**1i**$^{(c)}$-OH^{+}) (Fig. 5), giving rise to a regular ammonium ion and a quaternary hemiaminal. It is interesting to note that the decrease in the folded form in passing from **1a** to **1c** is matched by a concomitant decrease in solubility by these compounds in the applied buffer system.

2.4. Inhibition studies

The inhibitory potency of candidate compounds **1a**–**c** was tested with HIV-1 peptidase (HIV-1 PR). A well-established continuous assay based on the FRET technology (fluorescence resonance energy transfer) was used[48] in order to benefit from minimal substrate, enzyme and inhibitor consumption while rigorously determining kinetic data. We employed a commercially available dodecapeptide substrate specific for HIV-1 PR, ArgGlu(EDANS)SerGlnAsn**Tyr**+**Pro**IleValGln-Lys(DABCYL)Arg, that is a derivative of a previously introduced version,[49] and that displays excellent spectroscopic properties for analysis by a microplate spectrofluorimeter.

First, the kinetic parameters for the FRET substrate were determined in order to evaluate our assay conditions in regard to literature protocols. A mean value of $K_M = 27 \pm 1$ μM and $V_{max} = 267 \pm 10$ nM/min was obtained via different methods. These numbers are comparable to those published for the original FRET substrate ($K_M = 103$ μM; $V_{max} = 164$ nM/min at 30 °C).[49] The potency of **1a**–**c** was first estimated by determining their IC_{50} values.[50] Initial rates were measured for several inhibitor concentrations and IC_{50} values deduced by a nonlinear fit to $v/v_0 = 1/(1 + [I]/IC_{50})$ (Table 2). The final reaction mixtures contained 10% DMSO as cosolvent in order to assure complete solubilization of **1b** and **1c** (with $[\mathbf{1b}]_{max} = 1$ mM; $[\mathbf{1c}]_{max} = 200$ μM). As controls we used compound **16** and pepstatine, the latter being the benchmark for aspartic peptidase inhibitors. Table 2 illustrates to what extent **1b** and **1c** can compete with the hydroxyethylene inhibitors.

Candidate **1a**, being the smallest of the candidate inhibitors, showed an IC_{50} value of greater than 1 mM, and was not analyzed further. Candidate **1b** displaying the same number and the same nature of side chains as the conformationally flexible hydroxyethylamine **16** gave $IC_{50} = 545$ μM, thus being roughly 1000 times less potent than the latter. Pepstatine, being of molecular weight comparable to that of **16**, surpasses the potency of **16** by one order of magnitude.

The exact inhibition constants were derived from the same set of experiments by using several substrate concentrations (Table 2).[52] The choice of our methodology has been validated by the results obtained for pepstatine. In fact, its IC_{50} and K_i literature values determined for HIV-1 PR inhibition vary between 0.4 and 250 nM according to experimental conditions ($K_i = 17$ nM when using a FRET substrate similar to ours).[49] The present inhibition constants (Table 2) illustrate the additional importance of interactions between the periphery on the central moiety and the active site. By progressively attaching amino acid residues (and/or groups that are known to also satisfy to a certain degree the recognition subsites, such as Cbz and *t*-Bu) to the N-terminal and C-terminal sides of **1a**, a gradual increase in affinity of an approximate total of two orders of magnitude is observed.

3. Conclusion

It has been pointed out by use of numerous examples to what great extent the optimization of electrostatic interactions can be productive for achieving high-affinity ligands.[51] The $^{\delta+}N \rightarrow C{=}O^{\delta-}$ interaction as part of a transition-state analogue is proposed here for this particular reason. An efficient synthesis of a first prototype has been developed, and its conformational analysis in polar media demonstrated that the moiety resulting from this interaction can be deliberately incorporated into a peptidomimetic, or, for that matter, any molecular framework allowing for a minimal degree of preorganization. Three tendencies for the folded/unfolded equilibrium of compounds **1a**–**c** are observed: increasing solubility and polarity of the medium and decreasing temperature results in a higher extent of folding.

The conformational constraints imposed by this interaction render a considerable part of the inhibitor rigid which may have a favorable impact not only on bioavailability of future inhibitors displaying this moiety,[53] but also on affinity, provided a structure truly complementary to the active site is adopted. It remains to be seen to what extent candidate molecules displaying the $^{\delta+}N \rightarrow C{=}O^{\delta-}$ interaction are able to reproduce dihedral angles Cα–C–N–Cα′ of the scissile carboxamide bond found on the reaction coordinate.[54,12]

The aim of observing an $^{\delta+}N \rightarrow C{=}O^{\delta-}$ interaction as part of a peptide backbone can only be reached when a bridging moiety is incorporated, here an ethylene

Table 2. IC_{50} values obtained by data fitted to $v/v_0 = 1/(1 + [I]/IC_{50})$, K_i values obtained by use of three different methods, and molecular weights for each compound

Compound	K_i (μM)			IC_{50} (μM)	MW (g/mol)
	Lineweaver–Burk[51]	Hanes[51]	Dixon[51]		
1a	—	—	—	>1000	388
1b	574	618	577	545	578
1c	99	96	96	204	677
16	0.463	0.497	0.758	0.469	566
Pepstatine	0.036	0.036	0.036	0.036	686

bridge, that links both fragments displaying the tertiary amine and the aldehyde, respectively. Through the discovery of high-affinity inhibitors diverging significantly from the steric consumption of natural peptidic substrates, aspartic peptidases have been shown to tolerate extra steric bulk remarkably well, in part due to the flexibility of the domains ('flaps') covering the active site. Bioavailability also critically depends on the molecular weight of a given inhibitor as has been observed in the quest for a bioavailable peptide-derived renin inhibitor.[5] These points illustrate the need for finding new transition-state isosteres with superior affinity to their target site, allowing for reduction of overall inhibitor size.

The central transition state-analogue moiety **1** alone does not show any appreciable inhibitory activity toward HIV-1 PR. When increasing the resemblance to a natural substrate sequence by attachment of extra amino-acid residues, a successive increase in affinity is observed, reaching a K_i of 97 μM for the best candidate. This value is still three to four orders of magnitude higher than that found in classic hydroxyethylene-based inhibitors. Structural incongruency with the active site may have been caused by a counterproductive conformation of the newly formed heterocycle and/or the incapacity of **1a** to satisfy the recognition subsites due to a bad spatial separation of the benzyl group and the pyrrolidine moiety. It is also known that subtle differences in configuration of just one stereocenter can have a huge impact on affinity, in an extreme case approaching a K_i difference of seven orders of magnitude.[1d,55] Computational exploration outside and within the active site will be necessary of molecular matrices promoting $^{\delta+}N \rightarrow C{=}O^{\delta-}$ interactions for their tendencies to adopt a particular conformation and configuration of the newly formed stereogenic centers.[36]

4. Experimental

4.1. General

All reactions were carried out in anhydrous solvents under argon atmosphere in dried glassware. CH_2Cl_2 and DMF were distilled under argon on CaH_2, THF on Na/benzophenone. Compounds **2**, **12**, and **13** were prepared according to literature protocols. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 200 spectrometer (200 MHz) and a Varian Unity 500 spectrometer (500 MHz). Coupling patterns in the ^1H NMR spectra are designated as s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Mass spectra were recorded by the Centre de Spectrométrie de Masse, Université de Lyon, France. Elemental analyses were carried out by the Service Central d'Analyses, CNRS. Specific rotations were measured on a Jasco-P1010 polarimeter. UV spectra were recorded on a JASCO V550 UV–vis spectrophotometer.

4.2. Enzyme assays

4.2.1. Materials. rec. HIV-1 PR was purchased from Bachem and HIV protease substrate 1 (Arg-Glu(EDANS)SerGlnAsnTyrProIleVal-GlnLys-(DABCYL)Arg) was from Sigma–Aldrich.

4.2.2. Methods. Enzymatic activity and inhibition were determined by a fluorogenic assay using a microplate spectrofluorimeter (Spectramax Gemini XS, Molecular Devices) and black 384-well plates (NUNCLONE, Nunc Inc.). Hydrolysis of the fluorogenic substrate was investigated at pH 4.7, 37 °C, in 0.1 M acetate, 1 M NaCl, 1 mM EDTA, 1 mM dithiothreitol, and 1 mg/mL bovine serum albumin. The entire time course was recorded by monitoring the fluorescence at 490 nm with an excitation wavelength set to 340 nm. Each initial rate was the average value of five independently performed reactions.

K_M determination was performed using a range of fluorogenic substrate concentrations from 1 to 10 μM and an HIV-1 PR concentration of 4 nM. Final solutions contained 5% DMSO.

For *determination of IC_{50} and K_i values*, inhibitor was preincubated with HIV-1 PR for 30 min. IC_{50} determinations were performed using 10% DMSO solutions containing a final 5 nM HIV-1 PR and 4 μM fluorogenic substrate. Initial rates were determined for at least five inhibitor concentrations. K_i determinations were performed using 10% DMSO solutions containing a final 5 nM HIV-1 PR. Initial rates were determined for at least five inhibitor concentrations and four fluorogenic substrate concentrations (2, 3, 4, and 5 μM).

4.3. Bromide 3

To a stirred solution of **2** (11.61 g, 36.5 mmol) in dry THF (500 mL) at −78 °C was added dropwise a 0.5 M solution of KHMDS in toluene (77 mL, 38.4 mmol) under argon atmosphere. The solution was stirred for 1 h at −78 °C and then 2-bromoethyltriflate (10.30 g, 40.15 mmol) was added dropwise. The mixture was maintained at −78 °C for 3 h and then allowed to warm to room temperature overnight. The reaction was quenched by adding saturated NH_4Cl solution and then extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate and the solvents were removed by vacuo. The compound **3** was obtained as a yellow oil after flash column chromatography, using cyclohexane/ethyl acetate 95:5 as eluant (13.15 g, 85%, single diastereomer). $[\alpha]_D^{21}$ +42 (*c* 0.75 in $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 0.61 (s, 9H), 2.28–2.43 (m, 1H), 2.85–3.37 (m, 5H), 4.65 (d, $J = 6$ Hz, 2H), 5.27–5.43 (m, 3H), 5.83–6.00 (m, 1H), 7.22 (br s, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 24.36, 24.94, 37.71, 39.81 (br), 42.22, 66.95, 67.79, 95.22, 120.27, 127.38, 128.30, 130.96, 131.23, 134.83, 154.57, 172.39; HRMS: *m/z*: calcd for $C_{20}H_{27}BrNO_4$: 424.1124; found: 424.1126 [M^++H]; elemental analysis calcd (%) for $C_{20}H_{26}BrNO_4$: C, 56.61; H, 6.18; N, 3.30; found: C, 57.06; H, 6.36; N 3.25.

4.4. Tertiary amine 4

A stirred solution of **3** (13 g, 30.6 mmol), H-Pro-O-*t*-Bu (6.8 g, 39.8 mmol), and Et_3N (5.6 mL, 39.8 mmol) in

DMF (40 mL) was heated at 60 °C for 3 days under argon atmosphere. The reaction mixture was cooled and then diluted with water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried with sodium sulfate and the solvents were removed by vacuo. The compound **4** was obtained as a yellow oil after flash column chromatography, using cyclohexane/ethyl acetate 9:1 then 8:2 as eluant (12.91 g, 82%). $[\alpha]_D^{21}$ −16 (*c* 2.7 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ 0.54 (s, 9H), 1.37 (s, 9H,), 1.66 (m, 1H), 1.76 (m, 2H), 1.92 (m, 2H), 2.26 (m, 2H), 2.45–2.58 (m, 2H), 2.98 (m, 2H), 3.21 (m, 2H), 4.49 (dd, J = 12 Hz, J = 6 Hz, 1H), 4.60 (dd, J = 12 Hz, J = 6 Hz, 1H), 5.21–5.34 (m, 2H), 5.34 (s, 1H), 5.84 (m, 1H), 7.12–7.20 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 22.43, 24.70, 27.61, 28.42, 37.22, 42.45, 47.91, 52.81, 65.22, 66.29, 66.48, 80.29, 94.70, 119.08, 126.70, 127.78, 130.64, 131.21, 135.21, 154.75 (br), 172.51, 172.88; HRMS: *m*/*z*: calcd for $C_{29}H_{43}N_2O_6$: 515.3121; found: 515.3119 [M^++H]; elemental analysis calcd (%) for $C_{29}H_{42}N_2O_6$: C, 67.68; H, 8.23; N, 5.44; found: C, 67.23; H, 8.55; N 5.38.

4.5. Lactol 5

To a stirred solution of **4** (12.71 g, 24.7 mmol) in dry THF (120 mL) at −78 °C was added carefully per portion LAH (1.87 g, 49.4 mmol) under argon atmosphere. The solution was stirred for 48 h at −78 °C. The reaction was quenched very carefully at −78 °C by adding water (1.9 mL), 15% NaOH (1.9 mL), and water (5.7 mL). The mixture was allowed to warm to room temperature and stirred for 15 min. After filtration of white aluminum salts, the organic layer was dried over magnesium sulfate and the solvent was removed by vacuo. The compound **5** was obtained as a colorless oil after flash column chromatography, using pentane/ether 7:3 then 5:5 as eluant (6.42 g, 50%). ^1H NMR ($CDCl_3$, 200 MHz): δ 0.57 (s, 9H), 1.43 (s, 9H), 1.75–2.05 (m, 5H), 2.30–2.69 (m, 4H), 2.94–3.02 (m, 2H), 3.27–3.39 (m, 2H), 4.61–4.70 (m, 2H), 5.06 (s, 1H), 5.19–5.41 (m, 3H), 5.84–6.04 (m, 1H), 7.12–7.24 (m, 5H), 8.90 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 22.79, 26.05, 28.06, 29.41, 38.61, 42.51, 47.27, 52.76, 64.54, 66.10, 69.08, 81.23, 93.71, 101.01, 118.72, 126.66, 128.11, 131.09, 132.37, 136.77, 155.15 (br), 171.95; HRMS (LSIMS): *m*/*z*: calcd for $C_{29}H_{45}N_2O_6$: 517.3278; found: 517.3273 [M^++H]; elemental analysis calcd (%) for $C_{29}H_{44}N_2O_6$: C, 67.41; H, 8.58; N, 5.42; found: C, 67.18; H, 8.67; N 5.30.

4.6. Dithiolane 6

To a solution of **5** (6.25 g, 12.1 mmol) in dry CH_2Cl_2 (100 mL) at 0 °C were added 1,2-ethanedithiol (4.07 mL, 48.4 mmol) and $BF_3{\cdot}OEt_2$ (4.6 mL, 36.3 mmol) under argon atmosphere. After 3 h at 0 °C, the mixture was warmed to room temperature and stirred for a night. After adding saturated NH_4Cl, the mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over sodium sulfate and the solvent was removed by vacuo. The compound **6** was obtained as a colorless oil after flash column chromatography, using ethyl acetate/methanol 6:4 as eluant (4.54 g, 83%). $[\alpha]_D^{22}$ −14 (*c* 0.74 in $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 1.83–1.90 (m, 2H), 2.11–2.22 (m, 2H), 2.34–2.45 (m, 2H), 2.73–3.37 (m, 9H), 3.59 (t, J = 7 Hz, 1H), 3.71–3.77 (m, 1H), 4.49 (d, J = 5.6 Hz, 2H), 4.93 (s, 1H), 5.15–5.31 (m, 2H), 5.61 (br s, 1H), 5.78–5.94 (m, 1H), 7.17–7.29 (m, 5H), 7.90 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 23.47, 29.10, 31.15, 38.92, 39.23, 42.81, 51.28, 53.99, 61.29, 61.53, 65.44, 69.09, 117.83, 127.16, 128.52, 130.59, 132.70, 135.46, 155.02, 170.82; HRMS (LSIMS): *m*/*z*: calcd for $C_{22}H_{31}N_2O_4S_2$: 451.1725; found: 451.1724 [M^++H].

4.7. Methyl ester 7

To a stirred solution of **6** (1 g, 2.22 mmol) in a benzene/methanol 3.5:1 mixture (20.5 mL) was added a 2 N solution of trimethylsilyldiazomethane in hexane (1.44 mL, 2.89 mmol). The reaction mixture was stirred for 1.5 h at room temperature and then the solvent was removed by vacuo. The compound **7** was obtained as a colorless oil after flash column chromatography, using pentane/ethyl acetate 8:2 as eluant (656 mg, 64%). $[\alpha]_D^{22}$ −75.4 (*c* 1.08 in $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ = 1.70–2.24 (m, 9H), 2.84–3.28 (m, 7H), 3.23 (s, 3H), 3.23–3.66 (m, 1H), 4.56 (d, J = 5 Hz, 2H), 5.14–5.37 (m, 2H), 5.80 (s, 1H), 5.84–5.96 (m, 1H), 7.12–7.25 (m, 5H), 8.21 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 22.99, 29.08, 29.29, 38.50, 39.68, 42.90, 50.17, 51.69, 52.25, 62.34, 62.41, 64.63, 65.72, 116.43, 126.52, 128.04, 130.50, 133.52, 137.32, 155.36, 173.63; HRMS (LSIMS): *m*/*z*: calcd for $C_{23}H_{33}N_2O_4S_2$: 465.1882; found: 465.1886 [M^++H]; elemental analysis calcd (%) for $C_{23}H_{32}N_2O_4S_2$: C, 59.45; H, 6.94; N, 6.03; found: C, 59.36; H, 6.98; N 5.87.

4.8. Target compound 1a

To a stirred solution of HgO (570 mg) in a THF/water 85:15 mixture (10 mL) was added $BF_3{\cdot}Et_2O$ (3.56 mL) under argon atmosphere. After discoloration of the orange solution, a solution of **7** (530 mg, 1.14 mmol) in a THF/water 85:15 mixture (25 mL) was added dropwise. After the mixture was stirred for 2 h, saturated sodium bicarbonate solution was added. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate and the solvent was removed by vacuo. The compound **1a** was obtained after flash column chromatography as a colorless oil, using cyclohexane/ethyl acetate 1:1 as eluant (207 mg, 47%). $[\alpha]_D^{21}$ −5.8 (*c* 0.63 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ 1.75–1.91 (m, 3H), 2.00–2.17 (m, 2H), 2.19–2.22 (m, 1H), 2.34–2.39 (m, 2H), 2.66–2.71 (m, 1H), 2.99–3.03 (m, 1H), 3.14 (d, J = 14 Hz, 1H), 3.21–3.24 (m, 1H), 3.41 (d, J = 14 Hz, 1H), 3.69 (s, 3H), 4.56–4.65 (m, 2H), 5.24 (d, J = 10.5 Hz, 1H), 5.33 (d, J = 17.5 Hz, 1H), 5.91–5.98 (m, 1H), 6.00 (s, 1H), 7.06–7.07 (m, 2H), 7.22–7.28 (m, 3H), 9.35 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 23.14, 29.14, 33.98, 39.56, 48.87, 51.73, 52.34, 65.14, 65.37, 65.76, 117.37, 126.71, 128.11, 130.37, 132.90, 135.59, 155.06, 174.16, 197.76; HRMS (LSIMS): *m*/*z*: calcd for $C_{21}H_{29}N_2O_5$: 389.2076; found: 389.2079 [M^++H]; elemental analysis

calcd (%) for $C_{21}H_{28}N_2O_5$: C, 64.93; H, 7.27; N, 7.21; found: C, 65.07; H, 7.33; N 7.08.

4.9. Allyl carbamate 8

To a stirred solution of **6** (425 mg, 0.94 mmol) in dry THF (5 mL) at 0 °C were added HOBt (254 mg, 1.88 mmol) then DCC (213 mg, 1.03 mmol) under argon atmosphere. After the mixture was stirred for 10 min, was added *t*-BuNH$_2$ (300 μL, 2.82 mmol). The mixture was maintained for 1 h at 0 °C and then allowed to warm to room temperature and stirred for a night. After filtration of DCU, the solution was concentrated in vacuo and ethyl acetate was added. The organic layer was washed with 3 N citric acid solution, saturated sodium bicarbonate solution and brine, dried over sulfate sodium and then the solvent was removed by vacuo. The compound **8** was obtained as a colorless oil after flash column chromatography, using pentane/ethyl acetate 6:4 as eluant (347 mg, 73%). $[\alpha]_D^{21}$ −47.1 (*c* 0.30 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.29 (s, 9H), 1.61–1.68 (m, 3H), 1.99–2.37 (m, 5H), 2.67–2.80 (m, 2H), 3.06–3.29 (m, 7H), 4.47 (d, J = 4.7 Hz, 2H), 5.14–5.30 (m, 3H), 5.70 (s, 1H), 5.80–5.94 (m, 1H), 6.96 (s, 1H), 7.22 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 23.65, 28.62, 30.27, 33.98, 38.69, 39.13, 42.18, 50.12, 51.06, 53.50, 61.42, 62.06, 65.08, 68.89, 117.48, 126.92, 128.32, 130.55, 132.80, 136.00, 154.61, 173.62; HRMS (LSIMS): *m*/*z*: calcd for $C_{26}H_{40}N_3O_3S_2$: 506.2511; found: 506.2510 [M$^+$+H].

4.10. Benzyl carbamate 9

To a degassed mixture of **8** (230 mg, 0.45 mmol) and dimedone (318 mg, 2.27 mmol) in dry THF (3 mL) was added Pd(Ph$_3$)$_4$ (26 mg, 0.02 mmol) under argon atmosphere. The reaction mixture was stirred for 24 h at room temperature. After the solution was concentrated in vacuo, ethyl acetate was added and the organic layer was extracted with 1 N HCl. The aqueous layer was neutralized by adding carefully potassium carbonate and then extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate and the solvent was removed by vacuo. The deprotected amine was obtained as a yellow oil used without further purification (190 mg). To a solution of Cbz-Val-OH (90 mg, 0.36 mmol) and HOAT (49 mg, 0.36 mmol) in dry CH_2Cl_2 (3 mL) at 0 °C were added Et_3N (150 μL, 1.08 mmol), CIP (100 mg, 0.36 mmol) and then the deprotected amine (150 mg, 0.36 mmol) under argon atmosphere. The mixture was maintained for 1 h at 0 °C and then allowed to warm to room temperature and stirred for 2 days. The reaction mixture was then diluted with CH_2Cl_2. The organic layer was washed with 3 N citric acid solution, saturated sodium bicarbonate solution and brine, dried over sulfate sodium and then the solvent was removed by vacuo. The compound **9** was obtained as a colorless oil after flash column chromatography, using pentane/ethyl acetate 1:1 as eluant (153 mg, 65% on 2 steps). $[\alpha]_D^{21}$ −51.3 (*c* 0.25 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.89 (d, J = 6.7 Hz, 3H), 0.98 (d, J = 6.7 Hz, 3H), 1.29 (s, 9H), 1.60–1.74 (m, 3H), 1.98–2.37 (m, 6H), 2.58 (m, 1H), 2.74–2.79 (m, 1H), 3.01–3.19 (m, 6H), 3.38 (d, J = 13.7 Hz, 1H), 3.89 (dd, J = 9 Hz, J = 6.3 Hz, 1H), 5.02–5.13 (m, 2H), 5.29 (br s, 1H), 5.50 (m, 1H), 6.72 (br s, 1H), 6.83 (br s, 1H), 7.15–7.36 (m, 10H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 17.74, 19.46, 23.73, 28.75, 30.38, 30.57, 34.99, 38.87, 38.96, 42.42, 50.27, 51.09, 53.68, 61.55, 61.72, 62.15, 66.93, 68.27, 126. 98, 128.03, 128.35, 128.45, 130.64, 135.98, 136.35, 156.28, 170.83, 173.50; HRMS (LSIMS): *m*/*z*: calcd for $C_{35}H_{51}N_4O_4S_2$: 655.3352; found: 655.3351 [M$^+$+H].

4.11. Target compound 1b

To a stirred solution of HgO (110 mg) in a THF/water 85:15 mixture (6 mL) was added $BF_3{\cdot}Et_2O$ (690 μL) under argon atmosphere. After discoloration of the orange solution, a solution of **9** (144 mg, 0.22 mmol) in a THF/water 85:15 mixture (6 mL) was added dropwise. After the mixture was stirred for 2 h, saturated sodium bicarbonate solution was added. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate and the solvent was removed by vacuo. The compound **1b** was obtained after flash column chromatography as a colorless oil, using cyclohexane/ethyl acetate 1:1 as eluant (78 mg, 61%). $[\alpha]_D^{21}$ −1.2 (*c* 0.25 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 0.84 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H), 1.33 (s, 9H), 1.58–1.73 (m, 5H), 1.94–2.42 (m, 6H), 2.63–2.80 (m, 2H), 3.50 (d, J = 13.9 Hz, 1H), 3.92–3.98 (m, 1H), 5.09 (s, 2H), 5.31 (d, J = 8.9, 1H), 6.52 (s, 1H), 6.62 (s, 1H), 6.93–6.96 (m, 2H), 7.13–7.16 (m, 3H), 7.34 (m, 5H), 9.37 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 17.43, 19.53, 23.66, 28.79, 30.62, 30.98, 32.18, 39.31, 50.59, 50.77, 54.07, 60.55, 66.83, 67.03, 69.49, 127.18, 128.01, 128.16, 128.44, 128.53, 129.93, 134.38, 136.29, 156.22, 171.15, 173.75, 198.94; HRMS (LSIMS): *m*/*z*: calcd for $C_{33}H_{47}N_4O_5$: 579.3546; found: 579.3548 [M$^+$+H].

4.12. Allyl carbamate 10

To a stirred solution of **6** (1.5 g, 3.33 mmol) in dry THF (7.5 mL) at 0 °C were added HOBt (900 mg, 6.66 mmol) and then DCC (756 mg, 3.66 mmol) under argon atmosphere. After the mixture was stirred for 10 min was added dropwise using a cannula a THF solution of H-Val-NH-*t*-Bu prepared by adding Et_3N (585 μL, 4.16 mmol) to a solution of HCl·H-Val-NH-*t*-Bu (624 mg, 4.00 mmol) in dry THF (7.5 mL). The mixture was maintained for 30 min at 0 °C and then allowed to warm to room temperature and stirred for a night. After filtration of precipitates, the solution was concentrated in vacuo and ethyl acetate was added. The organic layer was washed with 3 N citric acid solution, saturated sodium bicarbonate solution and brine, dried over sulfate sodium and then the solvent was removed by vacuo. The compound **10** was obtained as a colorless oil after flash column chromatography, using pentane/ethyl acetate 1:1 as eluant (1.34 g, 67%). $[\alpha]_D^{21}$ −52.7 (*c* 0.76 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.90 (t, J = 6.2 Hz 6H), 1.28 (s, 9H), 1.59–1.86 (m, 4H), 2.01–2.16 (m, 4H), 2.26–2.31 (m, 1H), 2.46–2.52 (m, 1H), 2.75–2.77 (m, 1H), 3.00–3.24 (m, 8H), 3.94 (dd,

J = 8.9 Hz, J = 7.2 Hz, 1H), 4.50 (d, J = 5.5 Hz, 2H), 5.13 (s, 1H), 5.13–5.32 (m, 2H), 5.70 (s, 1H), 5.75 (s, 1H), 5.80–5.99 (m, 1H), 7.12–7.13 (m, 5H), 7.73 (d, J = 9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 18.24, 19.40, 24.16, 28.69, 30.66, 30.80, 34.02, 38.79, 39.12, 41.91, 50.86, 51.30, 53.39, 58.88, 61.51, 62.06, 65.14, 67.46, 117.43, 126.83, 128.28, 130.76, 133.16, 136.39, 154.98, 169.96, 174.97; HRMS (LSIMS): *m/z*: calcd for $C_{31}H_{49}N_4O_4S_2$: 605.3195; found: 605.3196 [M^++H]; elemental analysis calcd (%) for $C_{31}H_{48}N_4O_4S_2$: C, 61.56; H, 8.00; N, 9.26; found: C, 61.23; H, 8.08; N, 9.04.

4.13. Benzyl carbamate 11

To a degassed mixture of **10** (604 mg, 1 mmol) and dimedone (701 mg, 5 mmol) in dry THF (9 mL) was added $Pd(Ph_3)_4$ (58 mg, 0.05 mmol) under argon atmosphere. The reaction mixture was stirred for 24 h at room temperature. After the solution was concentrated in vacuo, ethyl acetate was added and the organic layer was extracted with 1 N HCl. The aqueous layer was neutralized by adding carefully potassium carbonate and then extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate and the solvent was removed by vacuo. The deprotected amine was obtained as a yellow oil used without further purification (520 mg).

To a solution of Cbz-Val-OH (251 mg, 1 mmol), and HOAT (136 mg, 1 mmol) in dry CH_2Cl_2 (8 mL) at 0 °C were added Et_3N (420 μL, 3 mmol), CIP (278 mg, 1 mmol) then the deprotected amine (520 mg, 1 mmol) under argon atmosphere. The mixture was maintained 1 h at 0 °C and then allowed to warm to room temperature and stirred for 3 days. The reaction mixture was then diluted with CH_2Cl_2. The organic layer was washed with 3 N citric acid solution, saturated sodium bicarbonate solution and brine, dried over sulfate sodium and then the solvent was removed by vacuo. The compound **11** was obtained as a colorless oil after flash column chromatography, using pentane/ethyl acetate 2:8 as eluant (485 mg, 64% over two steps). $[\alpha]_D^{22}$ −75.4 (*c* 0.57 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.86–1.00 (m, 12H), 1.26 (s, 9H), 1.63–2.55 (m, 12H), 2.91–3.16 (m, 6H), 3.35 (d,J = 13.8 Hz, 1H), 3.81–3.92 (m, 2H), 5.05 (d,J = 12.3 Hz, 1H), 5.13 (d,J = 12.3 Hz, 1H), 5.38 (br s, 1H), 5.70 (br s, 1H), 5.87 (d, J = 8.7 Hz, 1H), 6.92 (br s, 1H), 7.18–7.36 (m, 10H), 7.46 (d, J = 9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 17.30, 17.93, 19.31, 19.56, 23.87, 28.65, 30.38, 30.45, 30.66, 33.83, 38.88, 39.10, 42.25, 50.85, 51.33, 53.39, 59.23, 61.62, 62.59, 63.36, 66.80, 67.45, 126.87, 128.03, 128.08, 128.31, 128.48, 130.77, 136.20, 136.54, 156.39, 170.05, 171.07, 174.5; HRMS (LSIMS): *m/z*: calcd for $C_{40}H_{60}N_5O_5S_2$: 754.4036; found: 754.4039 [M^++H].

4.14. Target compound 1c

To a stirred solution of HgO (270 mg) in a THF/water 85:15 mixture (15 mL) was added $BF_3{\cdot}Et_2O$ (1.7 mL) under argon atmosphere. After discoloration of the orange solution, a solution of **11** (405 mg, 0.54 mmol) in a THF/water 85:15 mixture (15 mL) was added dropwise. After the mixture was stirred for 2 h, saturated sodium bicarbonate solution was added. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate and the solvent was removed by vacuo. The compound **1c** was obtained after flash column chromatography as a colorless oil, using cyclohexane/ethyl acetate 1:1 as eluant (170 mg, 47%). $[\alpha]_D^{24}$ −8.6 (*c* 0.42 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.83–0.96 (m, 12H), 1.31 (s, 9H), 1.61–1.80 (m, 5H), 2.01–2.45 (m, 8H), 2.86–3.09 (m, 3H), 3.35 (d, J = 13.9 Hz, 1H), 4.05–4.17 (m, 2H), 5.06 (s, 2H), 5.51 (d, J = 9.1 Hz, 1H), 5.77 (m, 1H), 6.98–7.01 (m, 2H), 7.13–7.17 (m, 3H), 7.32 (m, 5H), 7.65 (d. J = 9.4 Hz, 1H), 9.38 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 17.44, 18.48, 19.36, 23.90, 28.68, 30.65, 30.98, 31.39, 32.04, 39.65, 51.03, 51.60, 53.65, 58.37, 59.86, 66.17, 66.70, 68.97, 127.00, 127.97, 128.26, 128.40, 130.10, 134.32, 136.45, 156.17, 171.06, 171.55, 174.45, 200.03; HRMS (LSIMS): *m/z*: calcd for $C_{38}H_{56}N_5O_6$: 678.4231; found: 678.4230 [M^++H].

4.15. Hydroxyethylamine 14

A stirred mixture of H-Pro-NH-*t*-Bu **13** (149 mg, 0.87 mmol) and *N*-Cbz-phenylalanine epoxyde **12** (260 mg, 0.87 mmol) in dry methanol (5 mL) was refluxed for a night under argon atmosphere. The reaction mixture was cooled to room temperature, and the solvent was removed by vacuo. The compound **14** was obtained as a colorless oil after flash column chromatography, using Et_2O/methanol 9:1 as eluant (302 mg, 74%). $[\alpha]_D^{24}$ −47.8 (*c* 0.30 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.29 (s, 9H), 1.61–1.90 (m, 3H), 2.00–2.20 (m, 1H), 2.38–2.51 (m, 1H), 2.65 (d, J = 6.1 Hz, 1H), 2.81–2.96 (m, 3H), 3.18 (m, 1H), 3.60–3.68 (m, 1H), 3.80–3.91 (m, 1H), 4.93 (s, 1H), 4.99 (s, 2H), 6.96 (s, 1H), 7.12–7.31 (m, 10H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 24.32, 28.71, 30.94, 35.57, 50.50, 55.58, 56.28, 59.81, 66.78, 68.94, 72.11, 126.60, 127.90, 128.08, 128.47, 128.57, 129.27, 136.31, 137.44, 156.55, 174.26; HRMS (LSIMS): *m/z*: calcd for $C_{27}H_{38}N_3O_4$: 468.2862; found: 468.2858 [M^++H].

4.16. Compound 15

A mixture of **14** (246 mg, 0.53 mmol) and 10% Pd/C (28 mg, 0.026 mmol) in ethanol (3 mL) was stirred under nitrogen atmosphere (1 atm) for 1 h. After filtration on Celite, the solvent was removed by vacuo. The compound **15** was obtained as a white solid used without further purification (176 mg, quantitative). Mp 144 °C; $[\alpha]_D^{24}$ −53.9 (*c* = 0.55 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.31 (s, 9 H), 1.62–1.85 (m, 3H), 2.06–2.25 (m, 1H), 2.54–2.96 (m, 8H), 3.07–3.12 (m, 1H), 3.24–3.28 (m, 1H), 3.59–3.65 (m, 1H), 7.14–7.31 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 24.27, 28.62, 30.88, 37.37, 50.42, 55.94, 56.15, 58.99, 68.64, 71.93, 126.56, 128.68, 129.17, 138.48, 174.42; HRMS (LSIMS): *m/z*: calcd for $C_{19}H_{32}N_3O_2$: 334.2495; found: 334.2496 [M^++H].

4.17. Target compound 16

To a solution of Cbz-Val-OH (68 mg, 0.27 mmol), and HOAT (37 mg, 0.27 mmol) in dry CH_2Cl_2 (1.5 mL) at

0 °C were added Et_3N (115 μL, 0.81 mmol), CIP (75 mg, 0.27 mmol) then **15** (90 mg, 0.27 mmol) under argon atmosphere. The mixture was maintained 2 h at 0 °C. The reaction mixture was then diluted with CH_2Cl_2. The organic layer was washed with 3 N citric acid solution, saturated sodium bicarbonate solution and brine, dried over sulfate sodium and then the solvent was removed by vacuo. The compound **16** was obtained as a colorless oil after flash column chromatography, using cyclohexane/ethyl acetate 3:7 as eluant (100 mg, 65%). $[\alpha]_D^{24}$ −59 (*c* 0.25 in $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (d, $J = 6.8$ Hz, 3H), 0.83 (d, $J = 6.8$ Hz, 3H), 1.29 (s, 9H), 1.60–1.85 (m, 4H), 1.95–2.16 (m, 2H), 2.41–2.50 (m, 1H), 2.62–2.97 (m, 5H), 3.22 (m, 1H), 3.54–3.68 (m, 2H), 3.83 (dd, $J = 5.8$ Hz, $J = 8.1$ Hz, 1H), 4.10–4.19 (m, 1H), 5.00 (s, 1H), 5.06 (s, 2H), 6.24 (d, $J = 7.4$ Hz, 1H), 6.99 (s, 1H), 7.10–7.24 (m, 5H), 7.33 ppm (s, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 17.28, 19.26, 24.30, 28.61, 30.34, 30.96, 34.93, 50.47, 54.19, 56.18, 59.56, 60.77, 67.16, 68.92, 72.01, 126.58, 128.09, 128.29, 128.56, 129.06, 136.06, 137.50, 156.32, 171.57, 174.39; HRMS (LSIMS): *m/z*: calcd for $C_{32}H_{47}N_4O_5$: 567.3546; found: 567.3548 $[M^+ + H]$.

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Supplementary data

NMR spectra, UV data, and kinetic plots (PDF). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.01.031.

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