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Synthesis, biological evaluation, and modeling studies of inhibitors aimed at the malarial proteases plasmepsins I and II

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Abstract—The increasing resistance of the malarial parasite to antimalarial drugs is a major contributor to the reemergence of the disease and increases the need for new drug targets. The two aspartic proteases, plasmepsins I and II, from *Plasmodium falciparum* have recently emerged as potential targets. In an effort to inhibit these hemoglobinases, a series of inhibitors encompassing a basic hydroxyethylamine transition state isostere as a central fragment were prepared. The synthesized compounds were varied in the P1′ position and exhibited biological activities in the range of 31 to >2000 nM. To try to rationalize the results, molecular docking and 3D-QSAR analysis were used.

1. Introduction

Malaria is considered to be the most serious tropical parasitic disease in the world, killing more people each year than any other contagious disease except tuberculosis. It is spread by the bite of a female *Anopheles* mosquito and is caused by protozoan parasites of the genus *Plasmodium*. It is estimated that there are at least 400 million clinical cases every year, with at least 1 million fatalities.[1] Mortality is highest among young children and greatly exceeds the number of deaths from AIDS. The species responsible for almost all fatal cases is *Plasmodium falciparum*. Some *P. falciparum* strains have now been identified that are resistant to all known antimalarial drugs except artemisinin derivatives.[2] This problem of resistance has increased the need for the discovery of new macromolecular targets for malaria. The *P. falciparum* genome hosts several proteases involved in the degradation of hemoglobin,[3] among these are the cysteine proteases, metalloproteases, and the aspartic proteases constituting plasmepsin I (Plm I), plasmepsin II (Plm II), plasmepsin IV (Plm IV), and histoaspartic protease (HAP).[4–9] Plm I and Plm II, which are present in the digestive food vacuole of the parasite, cleave hemoglobin and have generated interest as antimalarial drug targets.[9] These plasmepsins exhibit 73% sequence identity, suggesting that a single compound might be able to efficiently inhibit both of them. In this context, it is important to emphasize that it was recently demonstrated that all four plasmepsins have overlapping functions and that the parasite can survive even when only one of the plasmepsins is inhibited.[10] Plm I and Plm II also show a rather high structural similarity (>50%) to the human protease cathepsin D (Cat D), suggesting potential problems with selectivity.[11–13] In 1996, Silva et al. reported the crystal structure of Plm II in complex with pepstatin A, which is a potent inhibitor with a $K_i = 0.006$ nM.[14] With pepstatin A as a lead, a series of phenylalanine–statin analogues were synthesized and among these the picoline derivative **1**, exerting an impressive Plm II activity ($K_i = 0.56$ nM) and a 38-fold selectivity over Cat D, was identified (Fig. 1).[14] More recently, Ellman's group used several iterative focused libraries to generate potent and selective Plm II inhibitors, for example, **2**.[15] Importantly, they also revealed that large P1′ substituents seemed to be easily accommodated in the flexible S1′ site of Plm II.

Since it is well-established that basic antimalarials like chloroquine accumulate in the acidic food vacuole of the parasite,[16] we decided to make Plm I and Plm II inhibitors containing a basic nitrogen. Hence,

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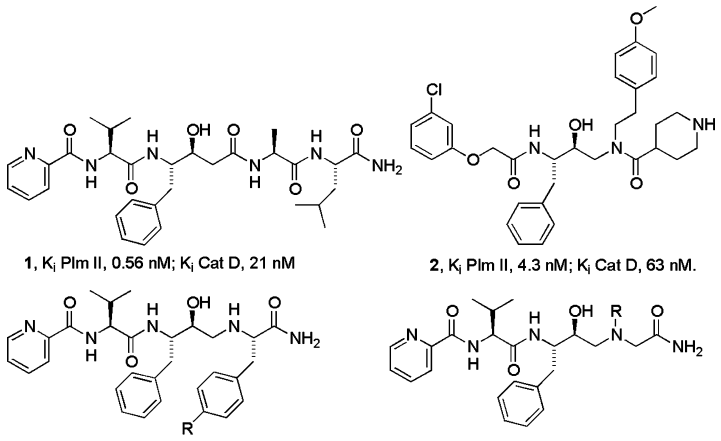


Figure 1. Two potent Plm II inhibitors (**1** and **2**) shown together with two earlier synthesized compounds with different inhibitory potencies, indicating the importance of a large P1′ side chain (**3** and **4**), and the generic structure of the inhibitors presented in this work (**5**).

compounds with the following characteristics were synthesized: (a) a basic hydroxyethylamine transition state mimicking scaffold that previously had been used successfully, for example, renin and HIV-protease inhibitors,[17–19] (b) alkylphenyl groups or alkylbiphenyl groups as P1′ substituents, and (c) a non-prime side and a C-terminal primary amide group derived from the potent lead compound **1**.[14] We previously demonstrated that some of the compounds with these characteristic features, exemplified by **3**, not only showed submicromolar affinity toward Plm I and Plm II but also exerted good inhibitory effect on the growth of the parasite in infected red blood cells.[20] In agreement with Ellman's previous findings, we confirmed that large P1′ substituents were required for activity also in this series of inhibitors as shown by the replacement of the 4-phenyl benzyl group of inhibitor **3** for a benzyl group, that delivered compound **4** devoid of plasmepsin inhibitory activity.

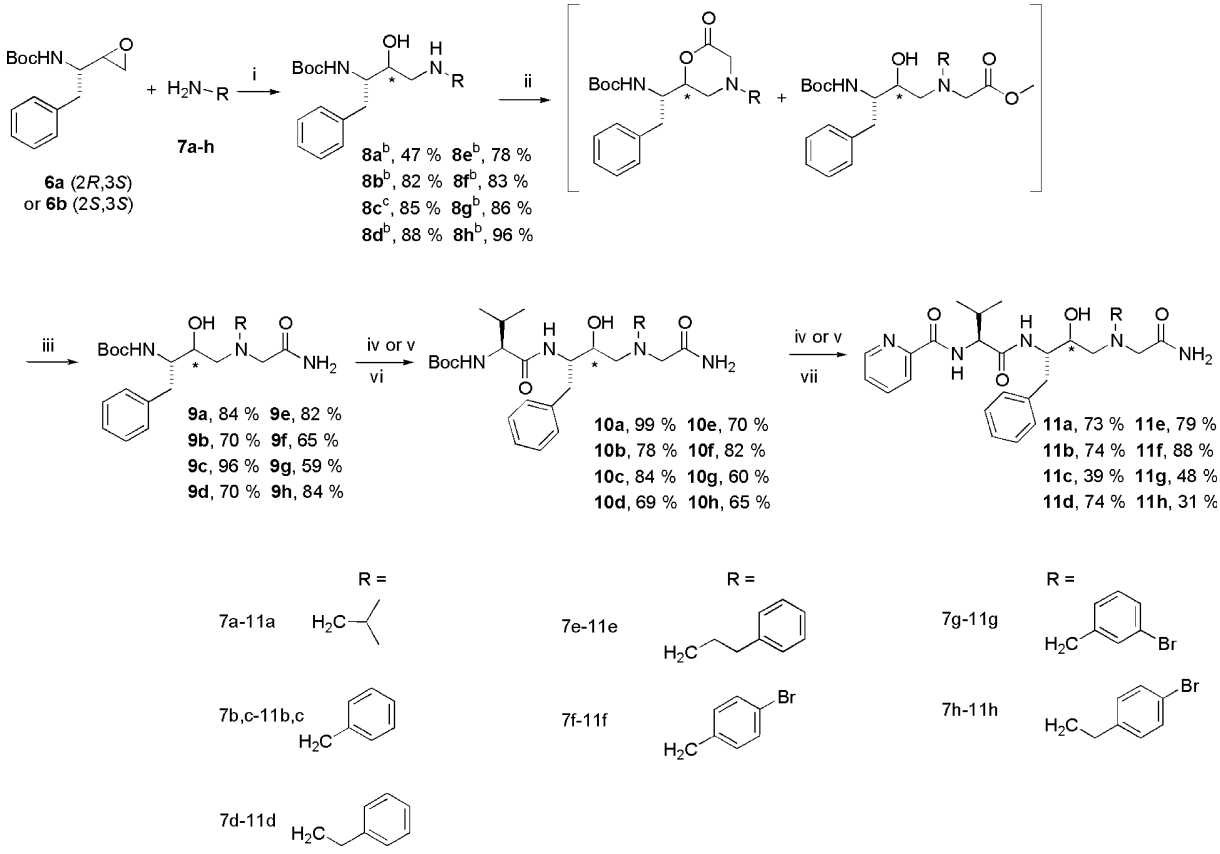
We speculated that the transfer, for example, the large biphenyl P1′ substituent from C-α of **3** to a potentially protonated sp^3-hybridized nitrogen would preserve the interactions of P1′ with the enzyme which could result in inhibitors with similar or better activities. To probe this hypothesis, a series of compounds with the generic structure **5** have now been synthesized (these will be referred to as N-substituted and those with the P1′ substituents at C-α as C-substituted). We herein report that, to our surprise, the transfer of the 4-phenylbenzyl group of **3** and other similar large substituents to the adjacent nitrogen delivered inactive compounds, while migration of a small benzyl group afforded a Plm II inhibitor (**11b**; K_i = 46 nM), which was even more potent than **3** (K_i = 117 nM). In an attempt to rationalize these unexpected findings, we docked the compounds into the active site of Plm II and evaluated their docking poses. Furthermore, to obtain a quantitative understanding of the SAR of these inhibitors a 3D-QSAR analysis was performed that included both the N-substituted analogues of **5** and the previously reported C-substituted analogues related to **3** and **4**[20,21] using the docked poses as alignment.

2. Results and discussion

2.1. Chemistry

The first step in the synthesis used the epoxide **6a** (prepared according to the method of Romeo and Rich)[22] and in the case of the synthesis of the epimer **8c**, epoxide **6b** (prepared according to the method of Brånalt et al).[19] These epoxides stirred with four different primary amines (**7a**–**7e**, where **7b** = **7c**) in isopropanol at 50 °C overnight afforded **8a**–**8e** (Scheme 1). These secondary amines were alkylated with methyl bromoacetate which yielded a mixture of the methyl ester and the lactone, the latter formed after a subsequent ring closure. The mixture was directly allowed to react with ammonia-saturated methanol to deliver the primary amides **9a**–**e**. The Boc groups were removed using an acidic solution and the resulting primary amines were coupled with Boc-protected valine using TBTU as coupling agent to give compounds **10a**–**10e**. After Boc deprotection, the resulting amines were reacted with picolinic acid, again with TBTU as the coupling agent, to deliver the target compounds **11a**–**11e**.

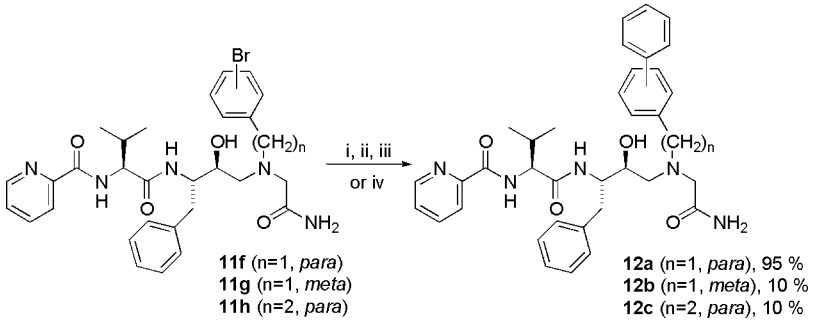
Since we had previously observed that extensions and enlargements in the P1′ site of the related inhibitors (e.g., **3**) significantly improved the inhibition of Plm I and II,[20,21] we prepared compounds **11f**–**11h** utilizing the procedure in Scheme 1. These compounds differ from **8b** and **8d** in that the phenyl ring on the P1′ side chain is substituted with bromine serving as a handle for the substitutions in the *para* (**11f** and **11h**) and *meta* (**11g**) positions. From these bromo compounds, the respective phenyl-substituted compounds **12a**–**12c** were synthesized applying a Suzuki protocol ($Pd(PPh_3)_2Cl_2$,



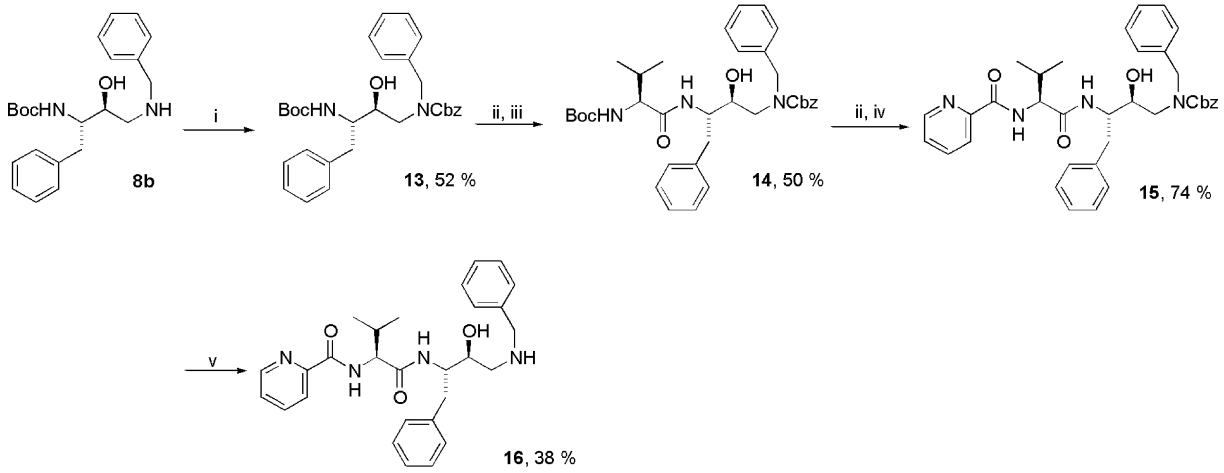
Scheme 1. Reagents and conditions: (i) isopropanol, overnight, 50 °C; (ii) K_2CO_3, methyl bromoacetate, DMF, overnight, rt; (iii) NH_3 (satd), MeOH, overnight, rt; (iv) HCl, EtOAc, 1 h, rt; (v) TFA, CH_2Cl_2, rt, 15 min; (vi) BocValOH, TBTU, DIEA, DMF, 2 h, rt; (vii) picolinic acid, TBTU, DIEA, DMF, 2 h, rt. [b]After reaction with **6a**. [c]After reaction with **6b**.

$PhB(OH)_2$, and Cs_2CO_3) under microwave irradiation (Scheme 2).[23,24] Under these conditions, the Suzuki coupling was accompanied with hydrolysis of the primary amide bond as deduced from LC–MS. The formation of carboxylic acid was probably attributed to an intramolecular attack of the hydroxyl group at the amide carbonyl group to form a lactone that was subsequently hydrolyzed by traces of water. Therefore, the crude reaction mixture, after filtering and evaporating, was subjected to acidic methanol to obtain the methyl ester. The crude product was thereafter stirred in an ammonia-saturated methanol solution to provide product **12a** in an excellent overall yield. For the synthesis of **12b** and **12c** a slightly modified condition was employed wherein Cs_2CO_3 was replaced by $NaHCO_3$. This modification suppressed the amide hydrolysis completely, although the yield for these reactions dropped considerably from 95% for **12a** to 10% for both **12b** and **12c**.

To assess the importance of the terminal amide functionality for binding, we prepared compound **16** (Scheme 3). Thus, compound **8b** was stirred with Cbz-Cl and K_2CO_3 in a mixture of water and CH_2Cl_2 to give the protected amine **13** in 52% yield. The Boc group of compound **13** was removed using a mixture of TFA and CH_2Cl_2, and the resulting primary amine was coupled with BocValOH utilizing TBTU as a coupling agent to afford the amide **14** in 50% yield. The Boc



Scheme 2. Reagents and conditions: (i) $PhB(OH)_2$, Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, DME, EtOH, 20 min, 130 °C; (ii) HCl, MeOH, rt, overnight; (iii) NH_3 (satd), MeOH, rt, overnight; (iv) $PhB(OH)_2$, $NaHCO_3$, $Pd(PPh_3)_2Cl_2$, DME, EtOH, 20 min, 140 °C.



Scheme 3. Reagents and conditions: (i) Cbz-Cl, K_2CO_3, H_2O, CH_2Cl_2, rt, 2 h; (ii) TFA, CH_2Cl_2, rt, 15 min; (iii) BocValOH, TBTU, DIEA, DMF, 2 h, rt; (iv) picolinic acid, TBTU, DIEA, DMF, 2 h, rt; (v) TfOH, anisol, CH_2Cl_2, rt, 15 min.

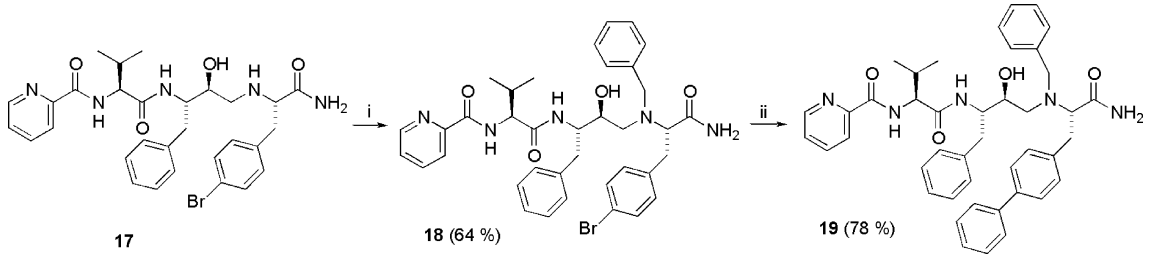
group of compound **14** was removed, the resulting amine was coupled to picolinic acid, and the amide **15** was isolated in 74% yield. The Cbz group was removed using triflic acid in CH_2Cl_2 with anisol as a scavenger to finally deliver the secondary amine **16** in 38% yield.

For preparation of **19**, the following procedure was employed: compound **17** (the 4-bromo benzyl equivalent of **3**) was treated with Cs_2CO_3 and benzyl bromide in DMF to give the tertiary amine **18** (Scheme 4). The aryl bromide **18** was subjected to the Suzuki protocol with phenylboronic acid (vide supra) to provide compound **19**, which is a hybrid of the dual Plm I and Plm II inhibitors **3** and **11b**.

2.2. Biological evaluation

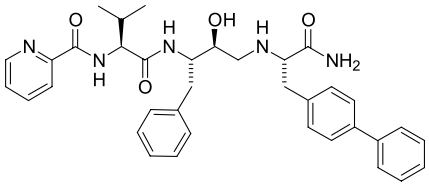
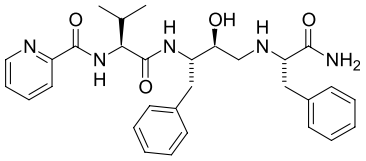
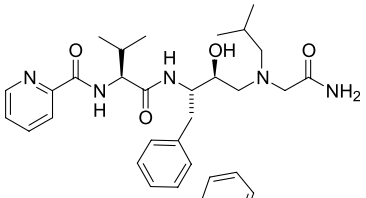
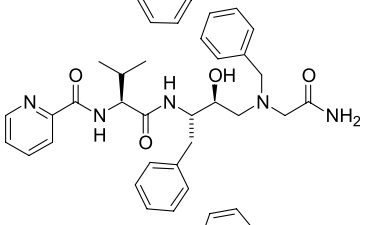
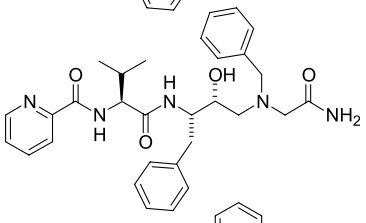
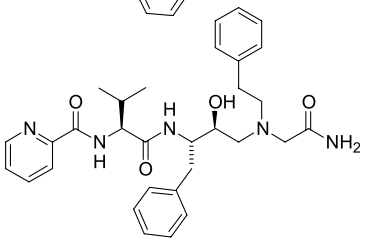
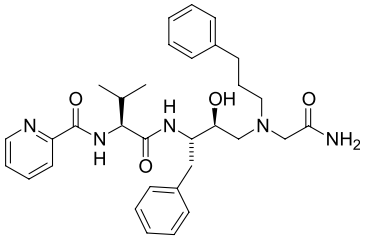
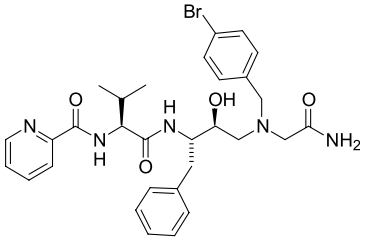
The results from the enzyme inhibition studies of the compounds in the Plm I, Plm II, and Cat D assays are presented in Table 1. A comparison between the biological activity of **3** and the analogues **12a**–**12c** revealed that the SAR differs in the two series (C- and N-substituted). While **3** requires the biphenyl in P1′ to achieve higher affinity, this biphenyl on **12a**–**12c** renders these molecules inactive against Plm I and Plm II ($K_i > 1000$ nM), except for **12b** with a Plm I $K_i = 350$ nM. The bromo-substituted compounds (**11f**–**11h**), which are intermediates in the synthesis of the phenyl-substituted analogues (**12a**–**12c**), were also inactive in the plasmepsin assays. This again points out the difference in the SAR between the two series, since the C-substituted analogue **24** has a K_i of 98 and 540 nM against Plm I and Plm II, respectively. We further observed that the phenylethyl derivative **11d**, which has an ethylene bridge, exhibited lower activity in Plm I and Plm II as compared to the methylene-bridged **11b**. Further elongation of the side chain to phenylpropyl, **11e**, resulted in complete loss of activity. Compound **11b** is also more potent than **11a**, which comprises an isobutyl group in the place of the benzyl. To find out whether the stereochemistry at the hydroxyl carbon was critical, compound **11c** was synthesized. This epimer of **11b** with inverted stereochemistry at the hydroxyl-bearing carbon was inactive, suggesting that the *S*-configuration is the preferred configuration in this position. The inhibitor **11b** exhibited the highest activity of all N-substituted compounds toward the plasmepsins, however, it showed no selectivity for the plasmepsins over Cat D, whereas **11a** showed a 30-fold selectivity for the plasmepsins over Cat D.

The terminal amide group was also found to be critical for activity, since the secondary amine **16**, which lacks this functionality, was completely devoid of any plasmepsin activity ($K_i > 2000$ nM). On the contrary **15**, an intermediate in the synthesis of **16**, possessing an ester functionality showed some activity with K_i of 800 and 380 nM toward Plm I and Plm II, respectively. Combining the P1′ of the most active N-substituted inhibitor **11b** with the P1′ of **3** and **24** gave **19** and **18**, respectively. Both these inhibitors had reduced activities (**18**, K_i Plm I = 573 nM and K_i Plm II = 833 nM and **19**, K_i Plm



Scheme 4. Reagents and conditions: (i) Cs_2CO_3, BnBr, DMF, 50 °C, overnight; (ii) $PhB(OH)_2$, Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, DME, EtOH, 20 min, 130 °C.

Table 1. Biological activities of the compounds in this study

Structure	Compound	K_i (nM)		
		Plm I	Plm II	Cat D
	**3**[a]	68	117	>2000
	**4**[a]	~2100	~3200	>2800
	**11a**	170	220	>6000
	**11b**[b]	31	46	38
	**11c**	650	>2000	>5900
	**11d**	~1900	~900	480
	**11e**	>2000	>2000	>2000
	**11f**	1600	>2000	1012

(*continued on next page*)

Table 1 (*continued*)

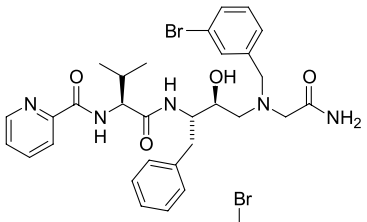
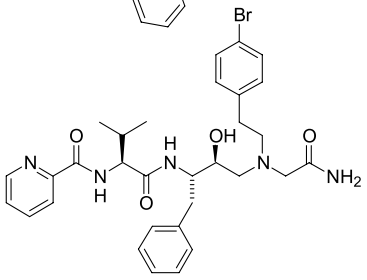
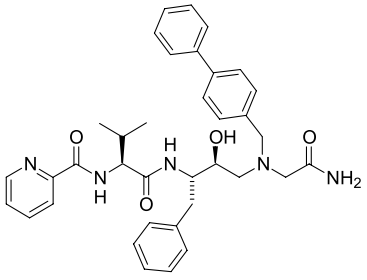
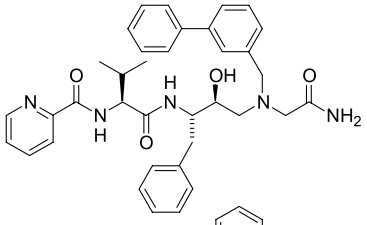
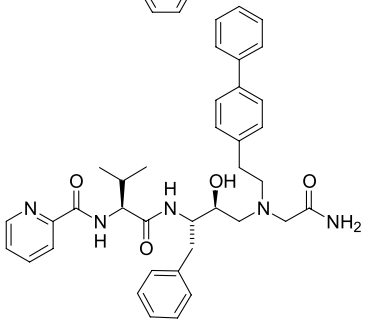
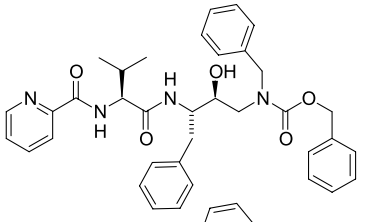
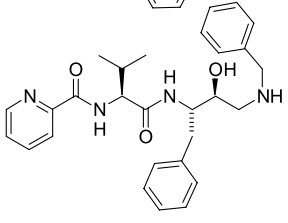
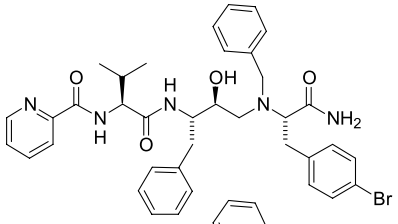
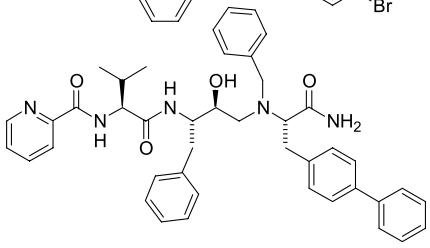
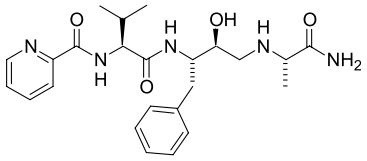
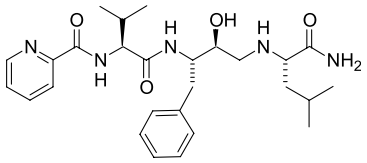
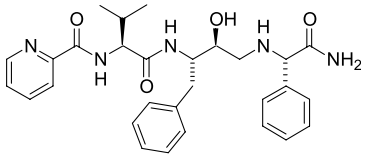
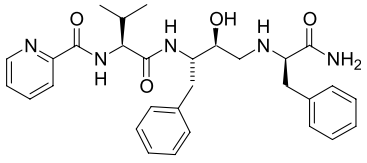
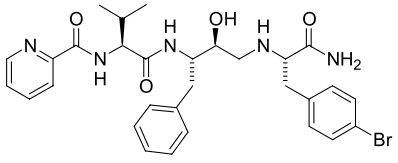
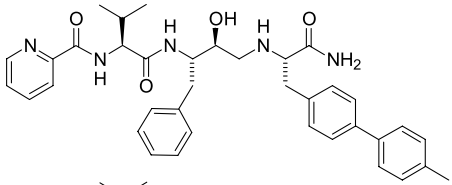
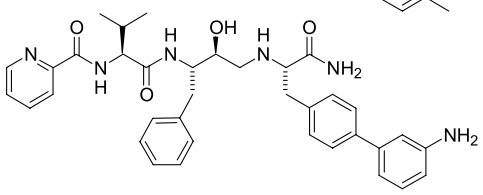
Structure	Compound	K_i (nM)		
		Plm I	Plm II	Cat D
	**11g**	1200	>2000	>2000
	**11h**	>2000	>2000	>2000
	**12a**	>2000	>2000	>2000
	**12b**	350	1300	>2000
	**12c**	>2000	1600	>2000
	**15**	~800	~380	>2000
	**16**	>2000	>2000	>2000

Table 1 (*continued*)

Structure	Compound	K_i (nM)		
		Plm I	Plm II	Cat D
	**18**	573	833	>2900
	**19**	220	650	>2900
	**20**[a]	>2000	>2000	>2000
	**21**[a]	~2900	~3300	>2800
	**22**[a]	>2000	>2000	>2000
	**23**[a]	~8000	~3500	>2000
	**24**[a]	98	540	>2000
	**25**[a]	115	121	~1700
	**26**[a]	63	150	~1000

(*continued on next page*)

Table 1 (*continued*)

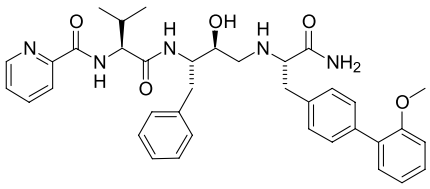
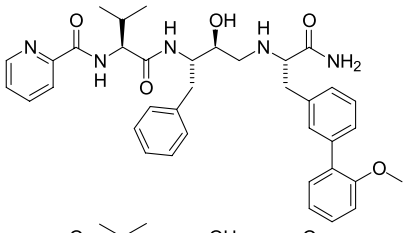
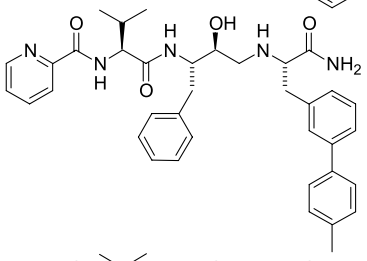
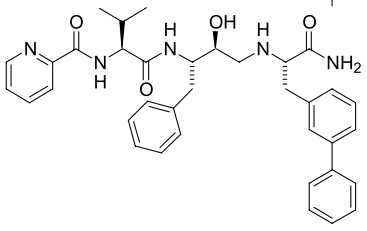
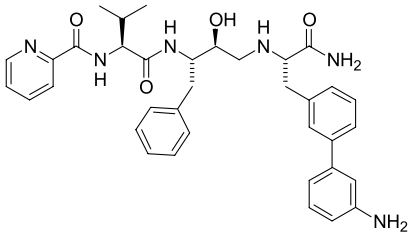
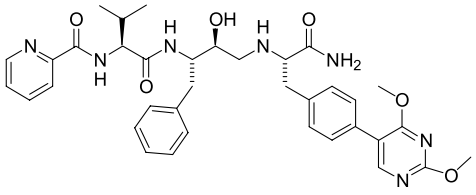
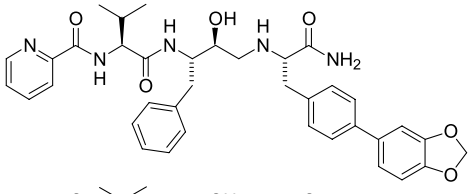
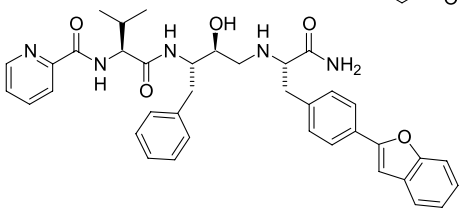
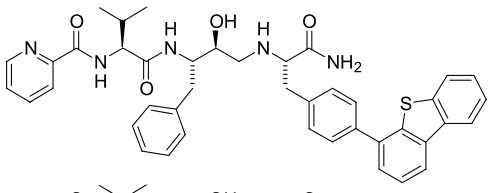
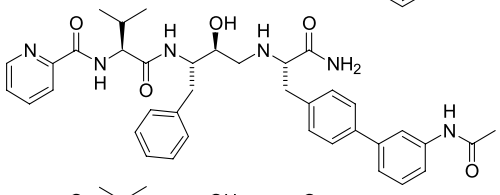
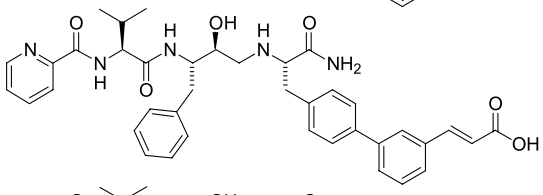
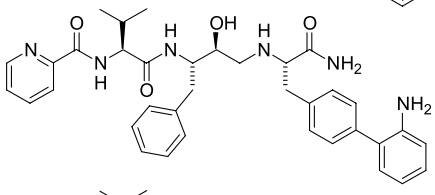
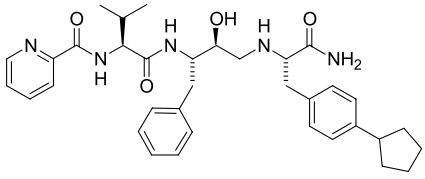
Structure	Compound	K_i (nM)		
		Plm I	Plm II	Cat D
	**27**[a]	437	379	~1700
	**28**[a]	1300	1430	>2000
	**29**[a]	1100	930	1900
	**30**[a]	1000	579	1600
	**31**[a]	530	129	>2000
	**32**[c]	170	360	>2900
	**33**[c]	23	52	1933
	**34**[c]	13	30	1400

Table 1 (*continued*)

Structure	Compound	K_i (nM)		
		Plm I	Plm II	Cat D
	**35**[c]	220	490	>2900
	**36**[c]	99	92	>2900
	**37**[c]	42	110	300
	**38**[c]	610	600	>5900
	**39**[c]	130	1300	>2900

[a] Compounds from Nöteberg et al.[20]
[b] Permeability on Caco 2-cells determined; P_{app} value = 4.5×10^{-6} cm/s; fair permeability (20–75%).
[c] Compounds from Nöteberg et al.[21]

I = 220 nM and K_i Plm II = 650 nM) compared to their parent compounds (**24**, K_i Plm I = 98 nM and K_i Plm II = 540 nM), indicating that the disubstitution had no synergistic effect. The potent Plm I and II inhibitor **11b** was evaluated in the Caco-2 cell assay,[25] and although this compound contains three amide bonds and one hydroxyl group it still exhibited fair (20–75%) cell penetration properties.

2.3. Docking

Docking studies were performed only on Plm II. The large and solvent exposed active site of this enzyme makes docking a challenging task. The problem becomes even more difficult due to the large number of rotatable bonds (~15) present in the inhibitors and the conformational changes that are known to occur in the protein upon ligand binding.[14] To take the protein flexibility into account, we chose to use the docking software FLO by McMartin and Bohacek,[26] which allows user-defined constraints to be assigned to the residues of choice. The residues Tyr77-Ser79, Gly216-Ser218, and Ile290-Val296 were allowed to move under constraints during docking.

The resulting poses were similar in their backbone orientation and showed good overlap with the cocrystallized inhibitors EH58 and rs370.[14,27,28] The H-bonding pattern seen for the docked inhibitors, exemplified by **11b** in Figure 2, was also similar to the pattern reported for the known cocrystallized ligands. The binding modes of the ligands can be generalized as follows: The transition state mimicking hydroxyl group of the hydroxyethylamine was found between the catalytic aspartic acids, Asp34 and Asp214, forming an H-bond with the carboxylate oxygens of the aspartates at a distance of around 2.2 Å. The P1 benzyl side chain showed π-interactions with the aromatic rings of Phe111, Phe120, and Tyr77 and was stacked with the side chain of Ile123. The P1 amide hydrogen was found at about 2.0 Å from the backbone carbonyl oxygen of Gly216. The carbonyl oxygen of P2 valine showed an H-bond (2.7–2.9 Å) with

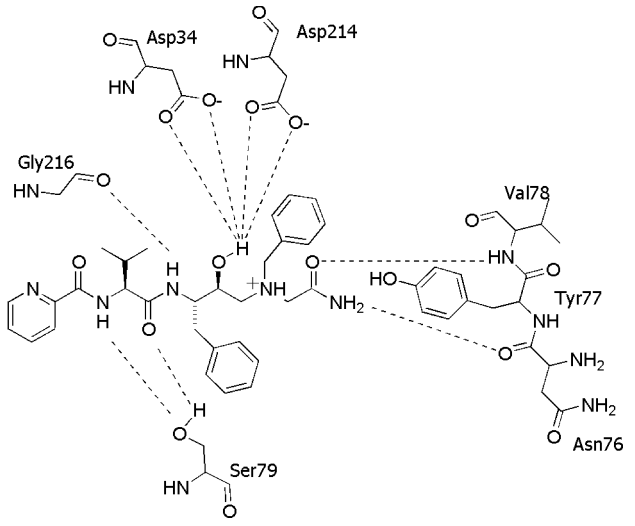


Figure 2. Schematic representation of H-bonding network for **11b**.

the hydroxyl group of Ser79. Hydrophobic interactions were observed between the P2 side chain and residues Thr217, Val78, and Ile290. The 2-pyridinecarboxamide (P3 substituent) resides in the S3 pocket close to residues Ser79 and Thr114, while the amide nitrogen makes an H-bond to the γ-O of Ser79. We also observed π-stacking between the aromatic P1 and P3 rings, which is in accordance with the X-ray structure of 1W6H.[29]

Large P1′ substituents were shown to accommodate well in the S1′ pocket of Plm II[15] as has also been pointed out by our prior investigations.[20] This was illustrated by the increase in the activity of compound **3**, having a $K_i = 117$ nM, compared to **4**, with a $K_i \sim 3200$ nM. The docking studies showed that the P1′ groups of the C-substituted inhibitors **3**, **4**, and **20**–**39**[20,21] were located in the S1′ pocket in van der Waals contact with Leu292 and Pro295. These poses were in agreement with the observed binding of the P1′ of EH58 in Plm II.[28] It was seen that large P1′ substituents could easily fit in the open S1′ site, once again demonstrating that large P1′ substituents are well tolerated in this pocket. The docking modes of the N-substituted compounds were similar to the C-substituted ones, but these inhibitors exhibited a reverse trend in the activities, that is, the smaller P1′ had higher activity than the larger P1′. This could in part be due to the observation that the smaller P1′ substituents (**11a** and **11b**) occupied a different region in the S1′ pocket, where they were surrounded by Ile212, Phe294, and Ile300. The binding mode of the P1′ substituents is in accordance with the one seen for inhibitor rs370 in 1LF2 bearing a small P1′ substituent.[27] Furthermore, a closer inspection of the poses revealed a possible π-interaction of the P1′ group of **11b** with Phe294 which could explain its higher activity compared to **11a** which lacks this interaction. We also observed that the large substituents on some of the N-substituted compounds (**12a**, **12c**) occupy the same space in the S1′ site, as do the corresponding potent large C-substituted compounds. Therefore, no simple relationship between the effect of the various P1′ substituents and the experimentally deduced K_i values could be found.

The interactions in the S2′ pocket are weak and primarily electrostatic in nature. Residues Val78 and Asn76 show H-bonds with the terminal amide. In case of compound **11b**, the carbonyl of the terminal amide is at an H-bonding distance of 1.7 Å from backbone amide of Val78, while the amide hydrogen forms an H-bond with backbone carbonyl of Asn76. The latter interaction is not seen in the case of **15** ($K_i = \sim 380$ nM), while **16** ($K_i > 2000$ nM) lacks both of the above-mentioned interactions due to the absence of this terminal amide group, which could explain their reduced activities.

Compounds **18** and **19** were prepared to probe whether the different requirements for the P1′ side chain in these series may be due to the side chains binding to different sites in the enzyme. The docked pose of **18** shows that the *N*-benzyl group fits into the S1′ site, while the *p*-bromobenzyl lies in the S2′ pocket close to Tyr192 and Leu131. On the contrary, **19** has the biphenyl group reaching into the S1′ site in accordance with observed docked pose of compound **3**, while the *N*-benzyl occupies the S2′ site. It was seen that the accommodation of these substituents in the S2′ pocket forced the terminal amide out of the pocket resulting in a loss of interaction with Asn76, while maintaining the H-bond with Val78. This could provide an explanation for the drop in their activity (**18**, $K_i = 833$ nM and **19**, $K_i = 650$ nM), once again demonstrating the importance of the terminal amide interactions.

On the basis of these results, we were able to generalize the various binding modes of the inhibitors, leading to a reasonable qualitative interpretation of the activity values. However, due to the limitations of the scoring functions, it is often difficult to establish a quantitative correlation between the calculated and the experimentally derived activity values. This poses a problem for establishing a structure–activity relationship. Due to this reason, we decided to derive a quantitative structure–activity relationship employing CoMFA using the docked conformations as an alignment.

2.4. 3-D QSAR model

Comparative molecular field analysis (CoMFA) is a technique that relates the biological activity to the chemical structure through multivariate statistics. It requires that the molecules to be investigated be aligned in their bioactive conformations. Here, we aligned the C- and N-substituted compounds using the docked poses (Fig. 3). LOO cross-validated PLS analysis was used to determine the optimal number of components in the final CoMFA. This analysis yielded a one component model with a cross-validated r^2 (q^2) of 0.395 with a correlation coefficient (r^2) of 0.514. The steric and electrostatic contributions amounted to 48% and 52%, respectively. Although a low q^2 was obtained, it is still significantly better than chance correlation, indicating that the model had some predictability.[30] One possible reason for the low q^2 values obtained was most likely due to the lack of specific interactions between the P1′ substituents and the amino acid residues in the S1′ site.

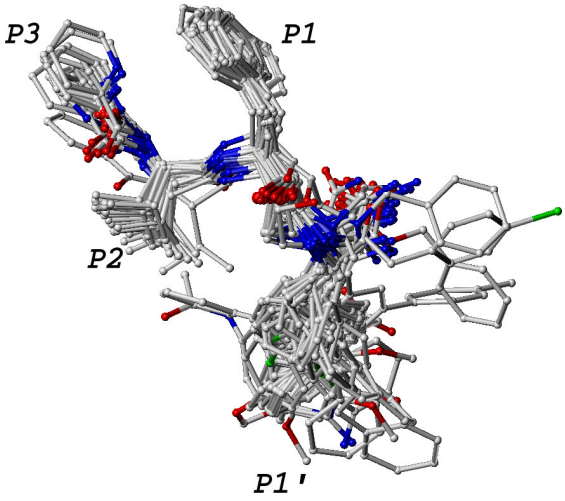


Figure 3. Docked alignment of the 37 compounds employed for CoMFA. Atoms are colored by atom type and hydrogen atoms are omitted for clarity.

Superimposition of the steric CoMFA contours onto the Connolly surface complemented each other. Greater activity is correlated with more bulk in the green contours. In Figure 4, the green contour lies close to the open end of the S1′ enclosed by Leu292, Asp293, and Phe294. This is in accordance with the observation made earlier concerning the C-substituted inhibitors. Regions where bulk is disfavored, corresponding to yellow contours, coincide with the Connolly surface, suggesting that no further bulk can be accommodated as seen in Figure 4. A similar observation was made concerning the electrostatic contours (blue favors more positive charge), which showed (Fig. 4) that more positive charge could be accommodated around residues Ser37, Asn39, Asn76, and Tyr77. This CoMFA model reinforced the qualitative results concerning the structure–activity relationships obtained from docking.

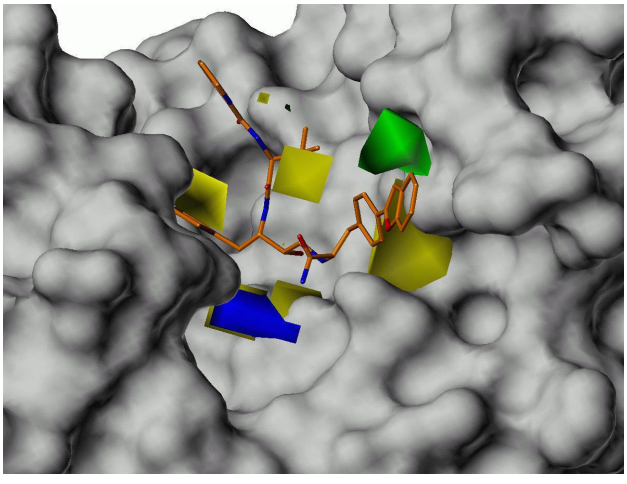


Figure 4. Steric fields (green, steric bulk favored 80%; yellow, steric bulk disfavored 20%) and electrostatic fields (blue, more positive charge favored 80%; red, more negative charge favored 20%) superimposed on the active pocket, represented by a Connolly surface (gray). The ligand is colored orange except for N (blue) and O (red) atoms. Residues Tyr77, Val78, Ser79, and hydrogen atoms are omitted for clarity.

3. Conclusion

A series of compounds with the generic structure **5** comprising a basic sp^3-hybridized nitrogen, predicted to be protonated in the transition state mimicking scaffold, have been prepared by classical and microwave assisted organic synthesis. Surprisingly, when the large biphenyl P1′ substituent was transferred from C-α to the basic nitrogen all Plm II activity was lost. This was in contrast to our previous findings, which demonstrated that large extended P1′ side chains are necessary for higher activity. The docking studies suggested that the small and large P1′ groups on the N-substituted inhibitors occupy a different region of the S1′ site, which could explain their differences in activities. The hybrid **19** did not show any improvement in inhibitory activity as compared to **3** and **11b**, indicating that no synergism was achieved upon having P1′ substituents in both positions. Finally, it has been shown that small variations can greatly affect the selectivity ratio between Cat D and the plasmepsins as inferred from the activities of compounds **11a** and **11b**, suggesting that selective Plm I and Plm II inhibitors could be synthesized.

4. Experimental

4.1. Plasmepsin assay and K_i determination

Pro-plasmepsin II was a generous gift from Helena Danielson (Department of Biochemistry, Uppsala University, Uppsala, Sweden) and the expression and purification of Plm I will be published elsewhere (manuscript in preparation). Human liver cathepsin D was purchased from Sigma–Aldrich (Sweden). The activities of Plm I, Plm II, and Cat D were measured using a total reaction volume of 100 µl as described earlier.[15] The concentration of pro-Plm II was 3 nM, the amount of Plm I was adjusted to give similar catalytic activity, and 50 ng/mL pro-cathepsin D was used. The prosequence of Plm II was cleaved off by preincubation in assay reaction buffer [100 mM sodium acetate buffer (pH 4.5), 10% glycerol, and 0.01% Tween 20] at room temperature for 40 min, and Cat D was activated by incubation in the same reaction buffer at 37 °C for 20 min. The reaction was initiated by the addition of 3 µM substrate (DABCYL-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-EDANS, AnaSpec Inc., San Jose, CA, USA), and hydrolysis was recorded as an increase in fluorescence intensity over a 10-min time period, during which the rate increased linearly with time. Stock solutions of inhibitors were serially diluted in DMSO and added directly before the addition of the substrate, giving a final DMSO concentration of 1%.

IC_{50} values were obtained by assuming competitive inhibition and fitting a Langmuir isotherm [$v_i/v_o = 1/(1+[I]/IC_{50})$] to the dose–response data (Grafit), where v_i and v_o are the initial velocities for the inhibited and uninhibited reactions, respectively, and [I] is the inhibitor concentration.[31] The K_i was subsequently calculated by using $K_i = IC_{50}/(1 + [S]/K_m)$[32] and a K_m value determined according to Michaelis–Menten.

The Caco-2 cell penetration assay was performed as described by Artursson and Karlsson.[25]

4.2. Chemistry

4.2.1. General procedures. All microwave reactions were conducted in heavy-walled glass Smith process vials sealed with aluminum crimp caps fitted with a silicon septum. The microwave heating was performed in a Smith Synthesizer single-mode microwave cavity producing continuous irradiation at 2450 MHz (Biotage AB, Uppsala, Sweden). Reaction mixtures were stirred with a magnetic stirring bar during the irradiation. The temperature, pressure, and irradiation power were monitored during the course of the reaction. After irradiation was completed, the reaction tube was cooled with high-pressure air until the temperature had fallen below 39 °C. ^{1}H and ^{13}C NMR spectra were recorded either on a JEOL JNM-EX 270 spectrometer at 270.2 and 67.8 MHz, respectively, or on a JEOL JNM-EX400 spectrometer at 399.8 and 100.5 MHz, respectively. Chemical shifts were reported as δ values (ppm) indirectly referenced to TMS via the solvent residual signal. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Specific rotations ($[\alpha]_D$) were reported in deg/dm, and the concentration (c) was given in g/100 mL in the specified solvent. Elemental analyses were performed by Mikro Kemi AB (Uppsala, Sweden). Flash column chromatography was performed on Merck silica gel 60, 0.04–0.063 mm. Thin-layer chromatography was performed using aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm; E. Merck) and visualized with UV light and ninhydrin. Analytical RP-LC–MS was performed on a Gilson HPLC system with a Zorbax SB-C8, 5 μM 4.6 × 50 mm (Agilent Technologies) column, with a Finnigan AQA quadropole mass spectrometer, at a flow rate of 1.5 mL/min. Preparative RP-LC–MS was performed on a Gilson HPLC system with a Zorbax SB-C8, 5 μM 21.2 × 150 mm (Agilent Technologies) column, with a Finnigan AQA quadropole mass spectrometer, at a flow rate of 15 mL/min.

4.2.2. (2*S*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(isobutylamino)-4-phenylbutan-2-ol (8a). (2*R*,3*S*)-3-[*N*-(*tert*-Butyloxycarbonyl)amino]-1,2-epoxy-4-phenylbutane (**6a**, 50 mg, 0.190 mmol)[22] was dissolved in 5 mL isopropanol. Isobutylamine (**7a**, 0.194 mL, 1.9 mmol) was added and the reaction mixture was stirred at 50 °C overnight. After evaporation, the product was dried under vacuum for 20 h to remove excess isobutylamine. The product was recrystallized from hexane to give compound **8a** (30 mg, 47%). Compound **8a**: mp 88.5–89.5 °C; $[\alpha]_D^{22}$ −36.6 (c 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz): δ 0.88 (d, J = 6.6 Hz, 6H), 1.20 (br s, 1H), 1.35 (s, 9H), 1.56–1.77 (m, 1H), 2.30–2.45 (m, 2H), 2.50–2.65 (m, 2H), 2.75–3.00 (m, 2H), 3.60–3.71 (m, 1H), 3.73–3.80 (m, 1H), 4.98 (br d, J = 9.6 Hz, 1H), 7.10–7.30 (m, 5H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 20.4, 20.5, 28.1, 38.9, 51.9, 53.4, 57.1, 67.6, 79.5, 126.0, 128.1, 129.2, 138.1, 155.7. Anal. ($C_{19}H_{32}N_2O_3$) C, H, N.

4.2.3. (5*S*,6*R*)-3-Aza-6-[(*tert*-butyloxycarbonyl)amino]-3-isobutyl-5-hydroxy-7-phenylheptanoyl amide (9a). Compound **8a** (0.10 g, 0.297 mmol) was dissolved in dry DMF (1.5 mL). K_2CO_3 (41 mg, 0.30 mmol) and methyl bromoacetate (56.2 μmol) was added and the mixture was stirred overnight. Water (5 mL) was added and the mixture was extracted twice with ethyl acetate (5 mL). The organic phase was dried, concentrated, and purified by column chromatography (ethyl acetate/isohexane, 1:4) to give a mixture of (5*S*,6*R*)-methyl-3-aza-6-(*tert*-butyloxycarbonyl)amino-3-isobutyl-5-hydroxy-7-phenylheptanoate and (5*S*)-3-aza-5-{[1-(*tert*-butyloxycarbonyl)amino-2-phenyl]-ethyl}-3-isobutyl-*S*-valerolactone in an approximate 1:1 mixture as determined by the mass signal of the analytical LC–MS. This mixture was stirred in a saturated solution of ammonia in methanol overnight to give after evaporation compound **9a** (98 mg, 84%). Compound **9a**: $[\alpha]_D^{22}$ −35.0 (c 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz): δ 0.82 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 6.5 Hz, 3H), 1.34 (br s, 1H), 1.37 (s, 9H), 1.53–1.72 (m, 1H), 2.09 (dd, J = 7.9, 12.7 Hz, 1H), 2.21 (dd, J = 6.5, 12.6 Hz, 1H), 2.40–2.58 (m, 2H), 2.78–2.95 (m, 2H), 3.02–3.10 (m, 2H), 3.55–3.85 (m, 2H), 5.10 (d, J = 9.5 Hz, 1H), 5.82 (br s, 1H), 7.07 (br s, 1H), 7.12–7.35 (m, 5H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 21.0, 21.1, 26.8, 28.6, 39.3, 54.1, 59.8, 60.2, 64.6, 68.6, 79.7, 126.7, 128.8, 129.7, 138.6, 156.3, 175.1. Anal. ($C_{21}H_{35}N_3O_4 \cdot 0.7H_2O$) C, H, N.

4.2.4. (5*S*,6*R*)-3-Aza-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-3-isobutyl-5-hydroxy-7-phenylheptanoyl amide (10a). Compound **9a** (0.10 g, 0.25 mmol) was dissolved in ethyl acetate (5 mL). Saturated HCl in ethyl acetate (20 mL) was added quickly under vigorous stirring. After the mixture was stirred for 1 h at room temperature, the solvents were evaporated. The residue was dissolved in DMF (3 mL). Boc-L-valine (54.3 mg, 0.25 mmol), TBTU (79.9 mg, 0.25 mmol), and diisopropylethylamine (87 μL, 0.50 mmol) were added and the mixture was stirred for 1 h. Saturated aqueous $NaHCO_3$ (10 mL) was added and the mixture was extracted twice with ethyl acetate (10 mL). The combined organic phases were dried, filtered, and evaporated, and the residue was purified by silica gel flash column chromatography (ethyl acetate) to give compound **10a** (0.12 g, 99%) as a white solid. Compound **10a**: $[\alpha]_D^{22}$ −66.8 (c 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$, 270 MHz): δ 0.77–0.93 (m, 12H), 1.44 (s, 9H), 1.57–1.80 (m, 1H), 2.02–2.35 (m, 3H), 2.42–2.60 (m, 2H), 2.88–3.03 (m, 2H), 3.03 (d, J = 16.8 Hz, 1H), 3.13 (d, J = 16.8 Hz, 1H), 3.68–3.80 (m, 1H), 3.83 (dd, J = 6.5, 8.5 Hz, 1H), 4.07–4.31 (m, 1H), 6.16 (br s, 1H), 6.54 (d, J = 8.6 Hz, 1H), 7.10–7.38 (m, 5H), 7.60 (d, J = 9.6 Hz, 1H). ^{13}C NMR (CD_3OD, 67.8 MHz): δ 16.1, 17.8, 18.9, 19.1, 25.3, 26.6, 29.5, 36.7, 52.0, 58.0, 58.6, 59.8, 63.4, 67.2, 78.4, 125.1, 127.2, 128.2, 137.5, 155.7, 172.0, 174.8. Anal. ($C_{26}H_{44}N_4O_5$) C, H, N.

4.2.5. (5*S*,6*R*)-3-Aza-3-isobutyl-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (11a). Compound **10a** (0.10 g, 0.25 mmol) was dissolved in ethyl acetate (5 mL). Saturated HCl in ethyl acetate (20 mL) was added quickly under vigorous stirring. After the mixture was stirred for 1 h at room temperature, the solvents were evaporated. Saturated aqueous $NaHCO_3$ (20 mL) was added and the mixture was extracted twice

with ethyl acetate (10 mL). The combined organic phases were dried, filtered, and evaporated. The residue was dissolved in DMF (5 mL). Picolinic acid (25 mg, 0.20 mmol), TBTU (65 mg, 0.20 mmol), and diisopropylethylamine (71 μL, 0.41 mmol) were added and the mixture was stirred for 1 h. Saturated aqueous $NaHCO_3$ (10 mL) was added and the mixture was extracted four times with ethyl acetate (10 mL). The combined organic phases were dried, filtered, and evaporated, and the residue was purified by silica gel flash column chromatography (ethyl acetate/methanol, 10:1) to give compound **11a** (74 mg, 73%) as a white solid. Compound **11a**: $[\alpha]_D^{22}$ −36.4 (*c* 1.0, CH_3OH); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.68–1.05 (m, 12H), 1.64–1.79 (m, 1H), 1.84–2.40 (m, 5H), 2.72–3.09 (m, 3H), 3.42–3.62 (m, 1H), 4.19–4.36 (m, 1H), 4.36–4.60 (m, 2H), 6.37–6.58 (m, 1H), 7.06–7.38 (m, 6H), 7.38–7.57 (m, 1H), 7.80–7.98 (m, 1H), 8.09–8.22 (m, 1H), 8.23–8.39 (m, 1H), 8.52–8.68 (m, 1H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.5, 19.7, 20.6, 20.8, 25.3, 29.8, 37.7, 38.7, 50.7, 51.9, 55.6, 59.6, 65.6, 122.5, 126.8, 128.7, 129.3, 129.4, 137.0, 137.6, 148.4, 137.6, 165.1, 168.5, 171.1. Anal. ($C_{27}H_{39}N_5O_4 \cdot 1.1H_2O$) C, H, N.

4.2.6. (2*S*,3*S*)-1-(Benzylamino)-3-[(*tert*-butyloxycarbonyl)amino]-4-phenylbutan-2-ol (8b). Compound **8b** (0.61 g, 82%) was prepared from epoxide **6a** (0.53 g, 2.013 mmol) and benzylamine (**7b**, 2.23 mL, 20.1 mmol) according to the method for the preparation of **8a**, except that it was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1) to give **8b** as a colorless glue. Compound **8b**: $[\alpha]_D^{22}$ −25.2 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.31 (br s, 1H), 1.38 (s, 9H), 1.41–1.55 (m, 1H), 2.40–2.75 (m, 3H), 2.75–2.99 (m, 2H), 3.50–3.62 (m, 1H), 3.63–3.84 (m, 3H), 4.93–5.07 (m, 1H), 7.10–7.40 (m, 10H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 28.3, 38.9, 51.8, 53.4, 53.6, 68.2, 79.2, 126.2, 127.2, 128.1, 128.3, 128.4, 129.4, 138.2, 139.1, 155.9. Anal. ($C_{22}H_{30}N_2O_3 \cdot 1.5H_2O$) C, H, N.

4.2.7. (5*S*,6*R*)-3-Aza-3-benzyl-6-[(*tert*-butyloxycarbonyl)amino]-5-hydroxy-7-phenylheptanoyl amide (9b). Compound **9b** (0.43 g, 70%) was prepared from substance **8b** (0.53 g, 1.43 mmol) according to the method for the preparation of **9a**. Compound **9b**: $[\alpha]_D^{22}$ −41.0 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.34 (br s, 1H), 1.37 (s, 9H), 2.50–2.69 (m, 2H), 2.78–3.00 (m, 2H), 3.05 (d, *J* = 16.5 Hz, 1H), 3.14 (d, *J* = 16.5 Hz, 1H), 3.52 (d, *J* = 13.3 Hz, 1H), 3.64 (d, *J* = 13.3 Hz, 1H), 3.64–3.90 (m, 2H), 5.01 (d, *J* = 9.7 Hz, 1H), 5.70 (br s, 1H), 6.98 (br s, 1H), 7.15–7.30 (m, 10H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 28.3, 38.8, 53.7, 58.1, 58.9, 59.5, 68.1, 79.5, 126.3, 127.9, 128.5, 128.6, 128.9, 129.3, 137.3, 138.1, 156.0, 173.9. Anal. ($C_{24}H_{33}N_3O_4 \cdot 0.4H_2O$) C, H, N.

4.2.8. (5*S*,6R)-3-Aza-3-benzyl-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-5-hydroxy-7-phenylheptanoyl amide (10b). Compound **10b** (0.19 g, 78%) was prepared from substance **9b** (0.20 g, 0.468 mmol) according to the method for the preparation of **10a**, except that for the acid-mediated deprotection TFA (30% in CH_2Cl_2) was used. Compound **10b**: $[\alpha]_D^{22}$ −47.9 (*c* 0.6, CH_3OH); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.74 (d, *J* = 6.4 Hz, 3H), 0.86 (d, *J* = 6.3 Hz, 3H), 1.43 (s, 9H), 1.90–2.20 (m, 1H), 2.42–2.75 (m, 2H), 2.83–2.94 (m, 1H), 3.06 (d, *J* = 16.0 Hz, 1H), 3.17 (d, *J* = 16.0 Hz, 1H), 3.30–4.00 (m, 5H), 4.00–4.27 (m, 1H), 4.94 (br d, *J* = 6.3 Hz, 1H), 5.69 (br s, 1H), 6.54 (br d, *J* = 7.3 Hz, 1H), 6.68 (br s, 1H), 7.01–7.49 (m, 10H). ^{13}C NMR (CD_3OD, 67.8 MHz): δ 17.3, 19.4, 28.3, 38.5, 38.6, 52.3, 57.8, 58.6, 59.4, 60.3, 67.8, 80.1, 126.5, 127.6, 128.6, 129.0, 129.3, 137.3, 137.9, 155.9, 171.6, 173.7. Anal. ($C_{29}H_{42}N_4O_5 \cdot 1.6H_2O$) C, H, N.

4.2.9. (5*S*,6*R*)-3-Aza-3-benzyl-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (11b). Compound **11b** (51 mg, 74%) was prepared from substance **10b** (70 mg, 0.13 mmol) according to the method for the preparation of **11a**, except that for the acid-mediated deprotection TFA (30% in CH_2Cl_2) was used. Compound **11b**: $[\alpha]_D^{22}$ −62.8 (*c* 0.6, CH_3OH); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.86 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 3H), 2.02–2.38 (m, 1H), 2.42–2.78 (m, 2H), 2.78–2.97 (m, 2H), 2.97–3.33 (m, 2H), 3.40–4.00 (m, 3H), 4.17 (dd, *J* = 8.1, 16.5 Hz, 1H), 4.24–4.62 (m, 2H), 6.05 (br s, 1H), 6.80–7.40 (m, 11H), 7.40–7.60 (m, 1H), 7.75–7.97 (m, 1H), 8.05–8.30 (m, 1H), 8.30–8.52 (m, 1H), 8.52–8.70 (m, 1H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.9, 19.6, 30.4, 38.5, 52.4, 57.8, 58.8, 59.2, 59.3, 67.9, 122.2, 126.7, 126.5, 128.3, 128.5, 129.1, 129.2, 137.4, 137.9, 148.3, 149.0, 164.60, 170.9, 174.0. Anal. ($C_{30}H_{37}N_5O_4 \cdot 0.5H_2O$) C, H, N.

4.2.10. (2*S*,3*S*)-1-(Benzylamino)-3-[(*tert*-butyloxycarbonyl)amino]-4-phenylbutan-2-ol (8c). Compound **8c** (56 mg, 85%) was prepared from (2*S*,3*S*)-3-[*N*-(*tert*-butyloxycarbonyl)amino]-1,2-epoxy-4-phenylbutane (**6b**)[19] (47 mg, 0.18 mmol) and benzylamine (**7c**, 0.29 mL, 1.69 mmol) according to the method for the preparation of **8a**, except that it was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1) to give **8c** as a colorless glue. Compound **8c**: $[\alpha]_D^{22}$ +2.1 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.24 (br s, 1H), 1.34 (s, 9H), 2.58–2.88 (m, 2H), 2.96 (dd, *J* = 4.7, 14.1 Hz, 1H), 3.42–3.63 (m, 3H), 3.75–3.86 (m, 1H), 3.80 (d, *J* = 13.3 Hz, 1H), 3.83 (d, *J* = 13.3 Hz, 1H), 4.78 (d, *J* = 9.2 Hz, 1H), 7.10–7.42 (m, 10H). ^{13}C NMR ($CDCL_3$, 100.5 MHz): δ 28.4, 36.7, 50.9, 53.7, 54.3, 70.87, 79.6, 126.4, 127.5, 128.5, 128.7, 128.7, 129.6, 137.9, 138.9, 156.1. Anal. ($C_{22}H_{30}N_2O_3$) C, H, N.

4.2.11. (5*S*,6*R*)-3-Aza-3-benzyl-6-[(*tert*-butyloxycarbonyl)amino]-5-hydroxy-7-phenylheptanoyl amide (9c). Compound **9c** (0.150 g, 96%) was prepared from substance **8c** (0.136 g, 0.37 mmol) according to the method for the preparation of **9a**. Compound **9c**: $[\alpha]_D^{22}$ +10.6 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.20 (br s, 1H), 1.31 (s, 9H), 2.50–2.93 (m, 4H), 3.03–3.20 (m, 1H), 3.20–3.35 (m, 1H), 3.49–3.72 (m, 1H), 3.72–3.94 (m, 3H), 4.90–5.09 (m, 1H), 6.28 (br s, 1H), 7.05–7.40 (m, 10H), 7.50 (br s, 1H). ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 28.4, 35.9, 55.2, 57.9, 58.3, 59.7, 71.3, 79.7, 126.5, 127.7, 128.5, 128.7, 129.2, 129.4, 137.6, 138.1, 156.3, 174.8. Anal. ($C_{24}H_{33}N_3O_4 \cdot 0.2H_2O$) C, H, N.

4.2.12. (5*S*,6*R*)-3-Aza-3-benzyl-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-5-hydroxy-7-phenylheptanoyl amide (10c). Compound **10c** (132 mg, 84%) was prepared from substance **9c** (128 mg, 0.30 mmol) according to the method for the preparation of **10a**, except that for the acid-mediated deprotection TFA (30% in CH_2Cl_2) was used. Compound **10c**: $[\alpha]_D^{22}$ −11.3 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.71 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 6.3 Hz, 3H), 1.39 (br s, 1H), 1.43 (s, 9H), 1.87–2.10 (m, 1H), 2.50–2.95 (m, 4H), 3.02–3.35 (m, 2H), 3.53–3.92 (m, 4H), 4.04–4.30 (m, 1H), 4.79–5.09 (m, 1H), 5.90 (br s, 1H), 6.59 (br s, 1H), 7.00–7.45 (m, 11H). ^{13}C NMR (CD_3OD, 100.5 MHz): δ 17.5, 19.3, 28.4, 30.4, 35.2, 53.2, 54.1, 58.0, 59.9, 60.7, 71.1, 80.3, 126.6, 127.7, 128.6, 128.7, 129.2, 129.4, 137.6, 137.8, 156.0, 172.1, 174.2. Anal. ($C_{29}H_{42}N_4O_5$) C, H, N.

4.2.13. (5*S*,6*R*)-3-Aza-3-benzyl-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (11c). Compound **11c** (45 mg, 39%) was prepared from substance **10c** (113 mg, 0.21 mmol) according to the method for the preparation of **11a**, except that for the acid-mediated deprotection TFA (30% in CH_2Cl_2) was used. Compound **11c**: $[\alpha]_D^{22}$ −27.9 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$/CD_3OD, 9:1, 400 MHz): δ 0.68 (d, J = 6.9 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H), 1.93–2.11 (m, 1H), 2.39–2.86 (m, 2H), 2.55 (dd, J = 10.2, 14.2 Hz, 1H), 2.86–3.21 (m, 2H), 3.39–3.80 (m, 4H), 3.95–4.22 (m, 2H), 6.76–6.90 (m, 1H), 6.90–7.09 (m, 4H), 7.09–7.35 (m, 5H), 7.35–7.50 (m, 1H), 7.67–7.88 (m, 1H), 7.96–8.11 (m, 1H), 8.45–8.58 (m, 1H). ^{13}C NMR ($CDCl_3$–CD_3OD, 9:1, 100.5 MHz): δ 17.6, 19.3, 30.5, 35.1, 53.8, 53.9, 57.8, 59.0, 59.6, 70.7, 122.3, 126.2, 126.7, 127.6, 128.2, 128.6, 129.0, 129.0, 129.2, 137.6, 137.8, 148.4, 148.9, 164.9, 171.4, 175.4. Anal. ($C_{30}H_{37}N_5O_4 \cdot 0.6H_2O$) C, H, N.

4.2.14. (2*S*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-4-phenyl-1-[(2-phenylethyl)amino]- butan-2-ol (8d). Compound **8d** (0.68 g, 88%) was prepared from epoxide **6a** (0.53 g, 2.013 mmol) and 2-phenylethylamine (**7d**, 2.6 mL, 20.1 mmol) according to the method for the preparation of **8a**, except that it was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1) to give **8d** as a white powder. Compound **8d**: $[\alpha]_D^{22}$ −29.7 (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.33 (br s, 1H), 1.40 (s, 9 H), 2.45–2.65 (m, 2H), 2.65–3.13 (m, 7H), 3.55 (dd, J = 4.1, 9.3 Hz, 1H), 3.71 (dd, J = 8.1, 16.5 Hz, 1H), 5.06 (br d, J = 9.5 Hz, 1H), 7.02–7.45 (m, 10H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 28.3, 36.1, 39.1, 50.5, 52.0, 53.6, 67.9, 79.2, 126.2, 128.3, 128.5, 128.6, 129.0, 129.4, 138.3, 139.4, 155.8. Anal. ($C_{23}H_{32}N_2O_3 \cdot 0.1H_2O$) C, H, N.

4.2.15. (5*S*,6*R*)-3-Aza-6-[(*tert*-butyloxycarbonyl)amino]-5-hydroxy-7-phenyl-3-(2-phenylethyl)-heptanoyl amide (9d). Compound **9d** (0.43 g, 70%) was prepared from substance **8c** (0.53 g, 1,43 mmol) according to the method for the preparation of **9a**. Compound **9d**: $[\alpha]_D^{22}$ −23.0 (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.34 (br s, 1H), 1.39 (s, 9H), 2.50–2.65 (m, 2H), 2.65–2.85 (m, 4H), 2.80 (dd, J = 8.4, 13.5 Hz, 1H), 2.91 (dd, J = 6.8, 13.3 Hz, 1H), 3.06 (d, J = 16.7 Hz, 1H), 3.18 (d, J = 16.7 Hz, 1H), 3.53–3.67 (m, 1H), 3.75–3.90 (m, 1H), 4.96 (d, J = 9.6 Hz, 1H), 5.55 (br s, 1H), 6.80 (br s, 1H), 7.20–7.40 (m, 10H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 28.3, 33.3, 38.6, 53.4, 56.9, 58.2, 58.5, 67.7, 79.5, 126.4, 126.4, 128.5, 128.5, 128.7, 129.2, 138.1, 139.7, 155.9, 174.0. Anal. ($C_{25}H_{35}N_3O_4$) C, H, N.

4.2.16. (5*S*,6*R*)-3-Aza-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-5-hydroxy-7-phenyl-3-(2-phenylethyl)-heptanoyl amide (10d). Compound **10d** (0.45 g, 69%) was prepared from substance **9d** (0.53 g, 1.2 mmol) according to the method for the preparation of **10a**. Compound **10d**: $[\alpha]_D^{22}$ −56.3 (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.77 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H), 1.55 (s, 9H), 2.08–2.32 (m, 1H), 2.56–2.76 (m, 2H), 2.76–2.94 (m, 3H), 2.94–3.12 (m, 2H), 3.12–3.46 (m, 2H), 3.68–3.90 (m, 2H), 3.90–4.08 (m, 1H), 4.15–4.35 (m, 1H), 5.09 (br s, 1H), 5.72 (br s, 1H), 6.61–6.90 (m, 1H), 7.12–7.49 (m, 10H). ^{13}C NMR (CD_3OD, 67.8 MHz): δ 17.3, 19.3, 28.2, 30.3, 33.1, 38.4, 52.1, 57.1, 58.1, 58.5, 60.4, 67.8, 80.1, 126.4, 126.5, 128.5, 128.5, 128.7, 129.1, 137.4, 139.4, 155.8, 171.5, 173.1. Anal. ($C_{30}H_{44}N_4O_5 \cdot 0.3H_2O$) C, H, N.

4.2.17. (5*S*,6*R*)-3-Aza-5-hydroxy-7-phenyl-3-(2-phenylethyl)-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (11d). Compound **11d** (51 mg, 74%) was prepared from substance **10d** (70 mg, 0.13 mmol) according to the method for the preparation of **11a**, except that for the acid-mediated deprotection TFA (30% in CH_2Cl_2) was used. Compound **11d**: $[\alpha]_D^{22}$ −60.5 (*c* 1.0, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.86 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H), 2.17–2.36 (m, 1H), 2.49–2.82 (m, 6H), 2.82–3.01 (m, 2H), 3.01–3.43 (m, 2H), 3.73 (br s, 1H), 4.08–4.30 (m, 1H), 4.37 (dd, J = 7.3, 9.2 Hz, 1H), 6.05 (br s, 1H), 6.88–7.32 (m, 12H), 7.32–7.51 (m, 1H), 7.71–7.90 (m, 1H), 8.04–8.21 (m, 1H), 8.37–8.50 (m, 1H), 8.50–8.68 (m, 1H). ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 17.9, 19.4, 30.4, 32.6, 38.3, 38.4, 52.3, 57.3, 58.6, 59.1, 68.1, 122.1, 126.0, 126.3, 128.1, 128.3, 128.5, 129.0, 129.2, 137.2, 137.8, 139.3, 148.1, 148.9, 164.3, 170.8, 174.4. Anal. ($C_{31}H_{39}N_5O_4 \cdot 0.8H_2O$) C, H, N.

4.2.18. (2*S*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-4-phenyl-1-[(3-phenylpropyl)amino]-butan-2-ol (8e). Compound **8e** (0.59 g, 78%) was prepared from epoxide **6a** (0.50 g, 1.9 mmol) and 3-phenylpropylamine (**7e**, 2.75 mL, 19.0 mmol) according to the method for the preparation of **8a**, except that it was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1) to give **8e** as a white powder. Compound **8e**: $[\alpha]_D^{22}$ −29.2 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.33 (br s, 1H), 1.40 (s, 9H), 1.78 (dq, J = 7.2, 7.3 Hz, 2H), 2.41–2.75 (m, 7H), 2.75–2.98 (m, 2H), 3.46–3.61 (m, 1H), 3.61–3.80 (dd, J = 8.1, 16.2 Hz, 1H), 4.93–5.14 (br d, J = 9.5 Hz, 1H), 6.97–7.40 (m, 10H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 28.3, 31.3, 33.4, 39.1, 48.9, 52.2, 53.7, 68.1, 79.3, 125.9, 126.2, 128.4, 128.4, 129.4, 129.4, 138.26, 141.6, 155.9. Anal. ($C_{24}H_{34}N_2O_3$) C, H, N.

4.2.19. (5*S*,6*R*)-3-Aza-6-[(*tert*-butyloxycarbonyl)amino]-5-hydroxy-7-phenyl-3-(3-phenylpropyl)-heptanoyl amide

(9e). Compound **9e** (0.45 g, 82%) was prepared from substance **8e** (0.48 g, 1.4 mmol) according to the method for the preparation of **9a**. Compound **9e**: $[\alpha]_D^{22}$ −23.0 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.32 (br s, 1H), 1.37 (s, 9H), 1.75–1.90 (m, 2H), 2.55–2.70 (m, 6H), 2.85–3.05 (m, 2H), 3.05–3.25 (m, 2H), 3.70–3.80 (m, 1H), 3.80–3.95 (m, 1H), 4.93 (d, J = 9.6 Hz, 1H), 5.55 (br s, 1H), 7.10 (br s, 1H), 7.25–7.40 (m, 10H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 28.2, 33.2, 38.9, 53.4, 53.5, 55.1, 58.5, 59.0, 68.1, 79.67, 126.00, 126.4, 128.2, 128.4, 128.5, 129.2, 138.1, 141.4, 155.0, 172.6. Anal. ($C_{26}H_{37}N_3O_4 \cdot 0.1H_2O$) C, H, N.

4.2.20. (5*S*,6*R*)-3-Aza-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-5-hydroxy-7-phenyl-3-(3-phenylpropyl)-heptanoyl amide (10e). Compound **10e** (0.17 g, 70%) was prepared from substance **9e** (0.20 g, 0.44 mmol) according to the method for the preparation of **10a**. Compound **10e**: $[\alpha]_D^{22}$ −38.0 (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.75 (d, J = 6.9 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H), 1.55 (s, 9H), 1.75–1.90 (m, 2H), 2.10–2.25 (m, 1H), 2.58–2.70 (m, 6H), 2.98–3.05 (m, 2H), 3.10–3.32 (m, 2H), 3.70–3.80 (m, 1H), 3.90–4.00 (m, 1H), 4.20–4.32 (m, 1H), 4.95 (br d, J = 9.5 Hz, 1H), 5.75 (br s, 1H), 6.65–6.72 (m, 1H), 6.99 (br s, 1H), 7.25–7.40 (m, 10H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.8, 19.8, 28.8, 30.7, 33.8, 39.0, 52.7, 52.7, 55.7, 58.95, 59.4, 60.8, 68.6, 80.7, 126.2, 127.1, 128.7, 128.9, 129.0, 129.7, 138.3, 141.9, 156.3, 167.9, 172.0. Anal. ($C_{31}H_{46}N_4O_5$) C, H, N.

4.2.21. (5*S*,6*R*)-3-Aza-5-hydroxy-6-[(picolyl-L-valinyl)amino]-7-phenyl-3-(3-phenylpropyl)-heptanoyl amide (11e). Compound **11e** (88 mg, 79%) was prepared from substance **10e** (0.11 g, 0.198 mmol) according to the method for the preparation of **11a**, except that for the acid-mediated deprotection TFA (30% in CH_2Cl_2) was used. Compound **11e**: $[\alpha]_D^{22}$ −46.5 (*c* 0.7, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.84 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 1.86–2.04 (m, 1H), 2.29–2.48 (m, 2H), 2.61–2.75 (m, 2H), 2.75–2.95 (m, 4H), 2.95–3.16 (m, 2H), 3.33–3.53 (m, 2H), 3.60–3.70 (m, 1H), 3.87 (br s, 1H), 4.18 (dd, J = 8.7, 16.4 Hz, 1H), 4.34 (dd, J = 6.9, 8.6 Hz, 1H), 6.19 (br s, 1H), 7.09–7.50 (m, 12H), 7.53–7.70 (m, 1H), 7.91–8.09 (m, 1H), 8.21–8.33 (m, 1H), 8.50–8.66 (m, 1H), 8.70–8.81 (m, 1H). ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 17.8, 19.5, 27.9, 30.3, 33.2, 38.2, 52.6, 55.5, 57.6, 59.0, 59.3, 68.1, 122.3, 126.1, 126.3, 126.5, 128.2, 128.3, 128.4, 129.4, 137.4, 137.8, 141.2, 148.3, 149.1, 164.7, 171.26, 173.1. Anal. ($C_{32}H_{41}N_5O_4 \cdot 0.5H_2O$) C, H, N.

4.2.22. (2*S*,3*S*)-1-[(*p*-Bromobenzyl)amino]-3-[(*tert*-butyloxycarbonyl)amino]-4-phenylbutan-2-ol (8f). Compound **8f** (1.63 g, 83%) was prepared from epoxide **6a** (1.15 g, 4.37 mmol) and *p*-bromobenzylamine (**7f**, 2.43 g, 13.1 mmol) according to the method for the preparation of **8a**, except that it was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1) to give **8f** as a colorless glue. Compound **8f**: $[\alpha]_D^{22}$ −24.0 (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.36 (br s, 1H), 1.41 (s, 9H), 2.48–2.77 (m, 2H), 2.75–3.03 (m, 2H), 3.53–4.00 (m, 4H), 5.06–5.23 (br d, J = 9.4 Hz, 1H), 7.03–7.18 (m, 2H), 7.18–7.37 (m, 5H), 7.37–7.60 (m, 2H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 30.2, 40.7, 53.8, 54.7, 55.6, 57.4, 70.5, 81.1, 122.8, 128.1, 130.2, 131.2, 131.7, 133.3.140.2, 157.8. Anal. ($C_{22}H_{29}BrN_2O_3 \cdot 0.2H_2O$) C, H, N.

4.2.23. (5*S*,6*R*)-3-Aza-3-(*p*-bromobenzyl)-6-[(*tert*-butyloxycarbonyl)amino]-5-hydroxy-7-phenylheptanoyl amide (9e). Compound **9e** (1.20 g, 65%) was prepared from substance **8e** (1.63 g, 3.63 mmol) according to the method for the preparation of **9a**. Compound **9e**: $[\alpha]_D^{22}$ −31.6 (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.38 (br s, 1H), 1.40 (s, 9H), 2.44–2.79 (m, 2H), 2.79–3.01 (m, 2H), 3.01–3.28 (m, 2H), 3.50 (d, J = 13.8 Hz, 1H), 3.61 (d, J = 13.8 Hz, 1H), 3.67–3.95 (m, 2H), 5.22 (br d, J = 9.6 Hz, 1H), 6.19 (br s, 1H), 6.96–7.18 (m, 2H), 7.18–7.38 (m, 5H), 7.38–7.55 (m, 2H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 28.2, 38.7, 53.8, 57.9, 58.7, 58.8, 67.9, 79.4, 121.3, 126.3, 128.4, 129.2, 130.5, 131.5, 136.4, 138.1, 156.0, 174.4. Anal. ($C_{24}H_{32}BrN_3O_4$) C, H, N.

4.2.24. (5*S*,6*R*)-3-Aza-3-(*p*-bromobenzyl)-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-5-hydroxy-7-phenylheptanoyl amide (10f). Compound **10f** (1.10 g, 82%) was prepared from substance **9e** (1.12 g, 2.21 mmol) according to the method for the preparation of **10a**, except that for the acid-mediated deprotection TFA (25% in CH_2Cl_2) was used. Compound **10f**: $[\alpha]_D^{22}$ −43.2 (*c* 1.6, CH_3OH); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.82 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 1.44 (br s, 1H), 1.46 (s, 9H), 1.90–2.11 (m, 1H), 2.42–2.72 (m, 2H), 2.85–2.99 (m, 2H), 3.06 (d, J = 16.7 Hz, 1H), 3.17 (d, J = 16.7 Hz, 1H), 3.51 (d, J = 13.4 Hz, 1H), 3.68 (d, J = 13.5 Hz, 1H), 3.63–4.00 (m, 2H), 4.09–4.29 (m, 1H), 5.37 (d, J = 8.1 Hz, 1H), 6.50 (br s, 1H), 6.91–7.35 (m, 9H), 7.35–7.50 (m, 2H). ^{13}C NMR (CD_3OD, 67.8 MHz): δ 17.6, 19.3, 28.2, 30.6, 38.2, 52.4, 57.6, 58.5, 58.7, 60.2, 67.6, 79.8, 121.2, 126.3, 128.3, 129.1, 130.5, 131.4, 136.3, 137.9, 155.9, 171.8, 174.3. Anal. ($C_{29}H_{41}BrN_4O_5$) C, H, N.

4.2.25. (5*S*,6*R*)-3-Aza-3-(*p*-bromobenzyl)-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (11f). Compound **11f** (0.96 g, 88%) was prepared from substance **10f** (1.08 g, 1.78 mmol) according to the method for the preparation of **11a**, except that for the acid-mediated deprotection TFA (25% in CH_2Cl_2) was used. Compound **11f**: $[\alpha]_D^{22}$ −44.0 (*c* 1.0, CH_3OH); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.86 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.4 Hz, 3H), 2.01–2.37 (m, 1H), 2.48–2.85 (m, 2H), 2.85–3.01 (m, 2H), 3.01–3.36 (m, 2H), 3.40–3.90 (m, 3H), 4.03–4.22 (m, 1H), 4.22–4.62 (m, 1H), 6.06 (br s, 1H), 6.80–7.30 (m, 9H), 7.30–7.60 (m, 3H), 7.75–7.95 (m, 1H), 8.03–8.25 (m, 1H), 8.34–8.52 (m, 1H), 8.52–8.70 (m, 1H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.9, 19.3, 30.4, 38.4, 52.5, 57.6, 57.8, 58.7, 59.3, 67.9, 121.5, 122.3, 126.4, 126.6, 128.4, 129.2, 130.8, 131.6, 136.2, 137.4, 137.8, 148.3, 149.0, 164.7, 171.0, 173.7. Anal. ($C_{30}H_{36}BrN_5O_4$) C, H, N.

4.2.26. (5*S*,6*R*)-3-Aza-5-hydroxy-7-phenyl-3-(*p*-phenylbenzyl)-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (12a).

Compound **11f** (53 mg, 87 μmol), phenylboronic acid (32 mg, 0.26 mmol), Cs_2CO_3 (85 mg, 0.26 mmol), and $Pd(PPh_3)_2Cl_2$ (6 mg, 9 μmol) were dissolved in a mixture of DME and ethanol (3:1, 2 mL) in a Smith vial. The vial was heated to 130 °C for 20 min by means of microwave irradiation. LC–MS analysis of the product mixture showed that the primary amide had been hydrolyzed in the process, thus, the mixture was filtered through a silica plug and eluted with CH_2Cl_2/methanol (4:1), the elute was concentrated in vacuo, and the residue was dissolved in methanol acidified with HCl. After stirring the mixture overnight, the solvent was removed by evaporation and the residue was extracted with aqueous K_2CO_3 and ethyl acetate. The organic phase was dried, filtered, and evaporated, and the residue was dissolved in methanol saturated with ammonia. After stirring the mixture overnight, the solvent was removed by evaporation and the residue was purified on a silica gel column using a mixture of CH_2Cl_2 and methanol (39:1) as mobile phase to give the title compound (50 mg, 95%). Compound **12a**: $[\alpha]_D^{22}$ −35.7 (*c* 0.4, CH_3OH); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.86 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H), 2.00–2.24 (m, 1H), 2.50–3.07 (m, 4H), 3.06–3.50 (m, 2H), 3.50–4.02 (m, 3H), 4.02–4.23 (m, 1H), 4.23–4.51 (m, 1H), 6.19 (br s, 1H), 6.80–7.65 (m, 17H), 7.65–7.90 (m, 1H), 7.90–8.22 (m, 1H), 8.35–8.76 (m, 2H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.9, 19.5, 30.5, 38.4, 52.7, 57.3, 58.7, 58.9, 59.3, 67.8, 68.0, 122.3, 125.2, 126.3, 126.5, 127.0, 127.3, 128.2, 128.3, 128.7, 129.0, 129.2, 129.8, 137.4, 137.8, 140.4, 140.7, 148.3, 149.0, 164.7, 171.1, 171.3. Anal. ($C_{36}H_{41}N_5O_4 \cdot H_2O$) C, H, N.

4.2.27. (2*S*,3*S*)-1-[(*m*-Bromobenzyl)amino]-3-[(*tert*-butyloxycarbonyl)amino]-4-phenylbutan-2-ol (8g). Compound **8g** (0.88 g, 86%) was prepared from epoxide **6a** (0.60 g, 2.3 mmol) and *m*-bromobenzylamine (**7g**, 0.84 g, 4.5 mmol) according to the method for the preparation of **8a**, except that it was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1) to give **8g** as a colorless glue. Compound **8g**: $[\alpha]_D^{22}$ −24.6 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$ (1% CD_3OD), 270 MHz): δ 1.15 (br s, 1H), 1.25 (s, 9H), 2.47–2.67 (m, 2H), 2.67–2.88 (m, 2H), 3.55–3.94 (m, 4H), 7.013–7.25 (m, 7H), 7.25–7.35 (m, 1H), 7.35–7.42 (m, 1H). ^{13}C NMR ($CDCl_3$ (1% CD_3OD), 67.8 MHz): δ 28.0, 38.1, 51.2, 52.1, 53.8, 68.3, 79.5, 122.5, 126.2, 127.2, 128.2, 129.1, 130.1, 130.9, 131.6, 137.9, 138.6, 156.3. Anal. ($C_{22}H_{29}BrN_2O_3$) C, H, N.

4.2.28. (5*S*,6*R*)-3-Aza-3-(*m*-bromobenzyl)-6-[(*tert*-butyloxycarbonyl)amino]-5-hydroxy-7-phenylheptanoyl amide (9g). Compound **9g** (0.49 g, 59%) was prepared from substance **8g** (0.73 g, 1.6 mmol) according to the method for the preparation of **9a**. Compound **9g**: $[\alpha]_D^{22}$ −26.6 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$ (1% CD_3OD), 270 MHz): δ 1.19 (br s, 1H), 1.25 (s, 9H), 2.32–2.65 (m, 2H), 2.65–2.83 (m, 2H), 2.83–3.14 (m, 4H), 3.30–3.75 (m, 4H), 6.94–7.40 (m, 9H). ^{13}C NMR ($CDCl_3$ (1% CD_3OD), 67.8 MHz): δ 28.0, 38.4, 53.3, 53.7, 57.4, 58.6, 67.6, 79.4, 122.3, 126.2, 127.4, 127.4, 128.2, 129.0, 129.9, 130.5, 131.7, 138.0, 139.7, 156.1, 170.4. Anal. ($C_{24}H_{32}BrN_3O_4 \cdot 0.7H_2O$) C, H, N.

4.2.29. (5*S*,6*R*)-3-Aza-3-(*m*-bromobenzyl)-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-5-hydroxy-7-phenylheptanoyl amide (10g). Compound **10g** (214 mg, 60%) was prepared from substance **9g** (300 mg, 0.59 mmol) according to the method for the preparation of **7a**, except that for the acid-mediated deprotection TFA (25% in CH_2Cl_2) was used. Compound **10g**: $[\alpha]_D^{22}$ −43.4 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.73 (d, *J* = 7.0 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H), 1.42 (s, 9H), 1.97–2.11 (m, 1H), 2.48–2.67 (m, 2H), 2.79–2.98 (m, 2H), 3.06(d, *J* = 17.2 Hz, 1H), 3.15 (d, *J* = 17.4 Hz, 1H), 3.48–3.52 (d, *J* = 14.0 Hz, 1H), 3.52–3.79 (m, 2H), 3.83 (dd, *J* = 5.9, 8.1 Hz, 1H), 4.05–4.21 (m, 1H), 4.88–5.04 (m, 1H), 5.74 (br s, 1H), 6.61 (br d, *J* = 9.8 Hz, 1H), 6.80 (br s, 1H), 7.09–7.50 (m, 9H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.3, 19.4, 28.3, 30.3, 38.4, 52.3, 57.5, 58.8, 58.8, 60.4, 68. 0, 80.2, 122.6, 126.6, 127.6, 128.6, 129.2, 130.1, 130.7, 131.8, 137.8, 139.9, 156.0, 171.7, 173.5. Anal. ($C_{29}H_{41}BrN_4O_5 \cdot 0.9H_2O$) C, H, N.

4.2.30. (5*S*,6*R*)-3-Aza-3-(*m*-bromobenzyl)-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (11g). Compound **11g** (160 mg, 48%) was prepared from substance **10g** (214 mg, 0.35 mmol) according to the method for the preparation of **11a**, except that for the acid-mediated deprotection TFA (25% in CH_2Cl_2) was used. Compound **11g**: $[\alpha]_D^{22}$ −56.5 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ ^{13}C NMR ($CDCl_3$, 270 MHz): δ 0.87 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 2.09–2.30 (m, 1H), 2.48–2.70 (m, 2H), 2.76–2.98 (m, 2H), 3.04 (d, *J* = 16.3 Hz, 1H), 3.16 (d, *J* = 16.5 Hz, 1H), 3.53 (d, *J* = 13.7 Hz, 1H), 3.68 (d, *J* = 13.9 Hz, 1H), 3.73–3.85 (m, 1H), 4.08–4.23 (m, 1H), 4.32 (d, *J* = 6.8, 9.0 Hz, 1H), 4.50 (br s, 1H), 5.97 (br s, 1H), 6.90 (d, *J* = 9.2 Hz, 1H), 6.95–7.50 (m, 10H), 7.77–7.91 (m, 1H), 8.08–8.20 (m, 1H), 8.38–8.50 (m, 1H), 8.50–8.63 (m, 1H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.9, 19.5, 30.4, 38.5, 52.5, 57.8, 58.8, 59.0, 59.3, 68.0, 122.3, 122.6, 126.3, 126.5, 127.6, 128.4, 129.2, 130.0, 130.6, 131.8, 137.4, 137.8, 140.0, 148.3, 149.0, 164.7, 170.9, 173.9. Anal. ($C_{30}H_{36}BrN_5O_4$) C, H, N.

4.2.31. (5*S*,6*R*)-3-Aza-5-hydroxy-7-phenyl-3-(*m*-phenylbenzyl)-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (12b). Compound **11g** (43 mg, 70 μmol), phenylboronic acid (50 mg, 0.41 mmol), $NaHCO_3$ (46 mg, 0.55 mmol), and $Pd(PPh_3)_2Cl_2$ (3 mg, 5 μmol) were dissolved in a mixture of DME and ethanol (3:1, 2 mL) in a Smith vial. The vial was heated to 140 °C for 20 min by means of microwave irradiation. The mixture was filtered through a celite plug, the solvent was removed by evaporation, and the residue was purified on a silica gel column using a mixture of CH_2Cl_2 and methanol (39:1) as mobile phase. The fractions containing the product were pooled and further purified using preparative HPLC (Zorbax C8, 20 × 150 mm, particle size 5 μm) using a gradient (H_2O/AcCN (0.1% formic acid) 95:5 → 4:6) over a period of 30 min. The fractions containing the pure product (measured with MS) were pooled and freeze dried to give title compound (4.3 mg, 10%). Compound **12b**: $[\alpha]_D^{22}$ −34.8 (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.86 (d, *J* = 6.9 Hz, 3H), 0.97 (d,

J = 7.0 Hz, 3H), 2.40–2.58 (m, 1H), 2.65–2.98 (m, 2H), 2.98–3.10 (m, 1H), 3.15 (d, J = 16.6 Hz, 1H), 3.24–3.51 (m, 2H), 3.75 (d, J = 13.7 Hz, 1H), 3.91 (d, J = 16.3 Hz, 3H), 4.01–4.33 (m, 2H), 4.37 (dd, J = 4.6, 6.5 Hz, 2H), 5.35–5.67 (m, 2H), 6.70–6.88 (m, 1H), 6.88–7.00 (m, 1H), 7.00–7.20 (m, 2H), 7.33–7.52 (m, 4H), 7.52–7.74 (m, 3H), 7.74–7.96 (m, 2H), 7.96–8.08 (m, 1H), 8.08–8.22 (m, 1H), 8.37–8.52 (m, 1H), 8.52–8.68 (m, 1H), 8.68–8.83 (m, 1H), ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 17.1, 19.7, 29.7, 38.8, 50.0, 60.4, 61.2, 65.5, 71.8, 12.7, 126.25, 126.9, 127.3, 127.6, 127.9, 128.6, 128.9, 129.2, 129.6, 130.2, 133.0, 133.8, 135.6, 137.7, 140.2, 142.2, 148.5, 148.8, 165.7, 170.9, 171.6. Anal. ($C_{36}H_{41}N_5O_4 \cdot 1.2H_2O \cdot HCOOH$) C, H, N.

4.2.32. (2*S*,3*S*)-1-{[2-(*p*-Bromophenyl)ethyl]amino}-3-[(*tert*-butyloxycarbonyl)amino]-4-phenylbutan-2-ol (8h). Compound **8h** (0.39 g, 96%) was prepared from epoxide **6a** (0.23 g, 0.87 mmol) and 2-(*p*-bromophenyl)ethylamine (**7h**, 0.35 g, 1.8 mmol) according to the method for the preparation of **8a**, except that it was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1) to give **8h** as a white powder. Compound **8h**: $[\alpha]_D^{22}$ −20.9 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$ 270 MHz): δ 1.36 (br s, 1H), 1.41 (s, 9H), 2.40–2.95 (m, 8H), 3.34–3.55 (m, 1H), 3.55–3.80 (m, 1H), 5.03 (br d, J = 9.1 Hz, 1H), 6.96–7.03 (m, 2H), 7.03–7.23 (m, 5H), 7.23–7.42 (m, 2H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 18.96, 28.28, 35.57, 39.0, 50.3, 52.1, 53.6, 68.2, 79.2, 120.0, 126.2, 128.3, 129.3, 130.3, 131.5, 138.3, 138.4, 155.8. Anal. ($C_{23}H_{31}BrN_2O_3 \cdot 0.3H_2O$) C, H, N.

4.2.33. (5*S*,6*R*)–3-Aza-3-[2-(*p*-bromophenyl)ethyl]-6-[(*tert*-butyloxycarbonyl)amino]-5-hydroxy-7-phenylheptanoyl amide (9h). Compound **9h** (0.35 g, 84%) was prepared from substance **8h** (0.37 g, 0.80 mmol) according to the method for the preparation of **9a**. Compound **9h**: $[\alpha]_D^{22}$ −30.8 (c 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.28 (s, 1H), 1.38 (s, 9H), 2.44–2.77 (m, 6H), 2.77–2.98 (m, 2H), 2.98–3.20 (m, 2H), 3.60–3.80 (m, 1H), 3.80–3.97 (m, 1H), 5.15–5.23 (m, 1H), 6.00–6.14 (m, 1H), 6.83–6.97 (m, 2H), 6.97–7.12 (m, 1H), 7.12–7.33 (m, 4H), 7.33–7.45 (m, 2H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 28.5, 32.7, 39.0, 53.8, 57.1, 58.7, 58.8, 68.1, 79.6, 120.1, 126.5, 128.6, 129.4, 130.5, 131.6, 138.4, 138.6, 156.2, 174.9. Anal. ($C_{25}H_{34}BrN_3O_4$) C, H, N.

4.2.34. (5*S*,6*R*)-3-Aza-3-[2-(*p*-bromophenyl)ethyl]-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-5-hydroxy-7-phenylheptanoyl amide (10h). Compound **10h** (270 mg, 65%) was prepared from substance **9h** (320 mg, 0.62 mmol) according to the method for the preparation of **10a**, except that for the acid-mediated deprotection TFA (25% in CH_2Cl_2) was used. Compound **10h**: $[\alpha]_D^{22}$ −22.4 (c 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz): δ 0.82–1.10 (m, 7H), 1.44 (s, 9H), 2.00–2.21 (m, 1H), 2.42–2.77 (m, 5H), 2.77–3.00 (m, 3H), 3.00–3.27 (m, 2H), 3.62–4.00 (m, 3H), 5.10–5.32 (m, 1H), 5.60–5.93 (m, 1H), 6.62–6.85 (m, 1H), 6.85–7.08 (m, 2H), 7.08–7.33 (m, 5H), 7.33–7.56 (m, 2H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.2, 19.0, 28.0, 30.4, 31.7, 37.8, 52.4, 57.0, 57.5, 58.2, 60.3, 67.5, 79.9, 119.9, 126.3, 128.3, 129.0, 130.3, 131.3, 137.8, 156.1, 167.8, 172.3. Anal. ($C_{30}H_{43}BrN_4O_5 \cdot 0.5H_2O$) C, H, N.

4.2.35. (5*S*,6*R*)-3-Aza-3-[2-(*p*-bromophenyl)ethyl]-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (11h). Compound **11h** (73 mg, 31%) was prepared from substance **10h** (240 mg, 0.39 mmol) according to the method for the preparation of **11a**, except that for the acid-mediated deprotection TFA (25% in CH_2Cl_2) was used. Compound **11h**: $[\alpha]_D^{22}$ −49.5 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz): δ 0.72–1.12 (m, 6H), 2.16–2.40 (m, 1H), 2.40–2.80 (m, 6H), 2.80–3.00 (m, 2H), 3.00–3.30 (m, 2H), 3.68–3.80 (m, 1H), 4.12–4.28 (m, 1H), 4.28–5.46 (m, 1H), 5.90 (br s, 1H), 6.82–7.09 (m, 5H), 7.09–7.21 (m, 4H), 7.30–7.41 (m, 2H), 7.41–7.59 (m, 1H), 7.76–7.96 (m, 1H), 8.04–8.24 (m, 1H), 8.40–8.33 (m, 1H), 8.33–8.51 (m, 1H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 18.1, 19.7, 30.4, 32.6, 38.7, 52.4, 57.2, 58.7, 58.9, 59.5, 68.5, 120.1, 122.4, 126.5, 126.7, 128.5, 129.3, 130.6, 131.6, 137.6, 138.0, 138.7, 148.5, 149.1, 164.9, 171.0, 174.4. Anal. ($C_{31}H_{38}BrN_5O_4$) C, H, N.

4.2.36. (5*S*,6*R*)-3-Aza-5-hydroxy-7-phenyl-3-[2-(*p*-phenylphenyl)ethyl]-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (12c). Compound **12c** (3.2 mg, 31%) was prepared from substance **11h** (33 mg, 53 μmol) according to the method for the preparation of **12b**. Compound **12c**: $[\alpha]_D^{22}$ −31.2 (c 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz): δ 0.75–1.03 (m, 6H), 2.22–2.41 (m, 1H), 2.41–2.55 (m, 1H), 2.55–2.70 (m, 1H), 2.70–2.96 (m, 6H), 3.04–3.15 (m, 1H), 3.15–3.30 (m, 1H), 3.57–3.72 (m, 1H), 4.10–4.25 (m, 1H), 4.29–4.40 (m, 1H), 5.20–5.45 (m, 1H), 6.43–6.65 (m, 2H), 7.00–7.12 (m, 1H), 7.12–7.24 (m, 5H), 7.31–7.39 (m, 1H), 7.39–7.62 (m, 7H), 7.72–7.91 (m, 1H), 8.03–8.19 (m, 1H), 8.46–8.56 (m, 1H), 8.60–8.74 (m, 1H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.7, 19.7, 30.1, 33.0, 38.7, 52.0, 57.2, 58.4, 58.6, 59.6, 68.1, 122.5, 126.6, 126.7, 127.0, 127.3, 127.5, 128.6, 128.9, 129.3, 129.4, 137.6, 137.8, 139.0, 139.4, 140.7, 148.5, 149.2, 165.0, 170.9, 173.6. Anal. ($C_{37}H_{43}N_5O_4 \cdot 1.5H_2O \cdot 2\ HCOOH$) C, H, N.

4.2.37. (2*S*,3*S*)-1-[(*N*-Benzyl-*N*-benzyloxycarbonyl)amino]-3-[(*tert*-butyloxycarbonyl)amino]-4-phenylbutan-2-ol (13). Compound **8b** (0.53 g, 1.4 mmol) was dissolved in CH_2Cl_2 (100 mL), a solution of K_2CO_3 (0.41 g, 3.0 mmol) in water (100 mL) and benzylchloroformate (0.30 μL, 2.13 mmol) was added, and the mixture was stirred for 2 h. The layers were separated and the water layer was extracted with CH_2Cl_2 (30 mL). The combined organic phases were dried, filtered and evaporated. The residue was purified by column chromatography (toluene/EtOAc, 3:1) to give compound **13** (0.38 mg, 52%). Compound **13**: $[\alpha]_D^{22}$ ±0 (c 1.2, $CHCl_3$); ^{1}H NMR (CD_3OD, 270 MHz, rotamers): δ 1.02–1.39 (m, 9H), 2.58–2.83 (m, 2H), 3.00–3.30 (m, 2H), 3.52–3.74 (m, 1H), 3.74–3.90 (m, 1H), 4.39–4.58 (m, 2H), 4.90–5.09 (m, 2H), 6.91–7.32 (m, 15H). Anal. ($C_{29}H_{36}N_2O_5$) C, H, N.

4.2.38. (2*S*,3*S*)-1-[(*N*-Benzyl-*N*-benzyloxycarbonyl)amino]-3-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-4-phenylbutan-2-ol (14). Compound **14** (0.19 g, 50%) was prepared from substance **13** (0.32 g, 0.63 mmol) according to the method

for the preparation of **10a**, except that for the acid-mediated deprotection TFA (25% in CH_2Cl_2) was used. Compound **14**: $[\alpha]_D^{22}$ −26.2 (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz, rotamers): δ 0.60–1.05 (m, 6H), 1.43 (s, 9H), 1.89–2.20 (m, 1H), 2.75–2.97 (m, 2H), 2.97–3.26 (m, 1H), 3.44–4.08 (m, 4H), 4.35–4.70 (m, 2H), 4.93–5.54 (m, 3H), 6.32–6.69 (m, 1H), 6.98–7.41 (m, 15H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.4, 19.3, 28.3, 30.5, 38.2, 52.3, 53.1, 53.5, 60.2, 67.7, 70.8, 79.9, 124.2, 126.4, 127.4, 127.5, 127.9, 129.1, 128.4, 128.6, 129.2, 136.0, 136.9, 137.7, 155.8, 158.1, 171.6. Anal. ($C_{34}H_{45}N_3O_6$) C, H, N.

4.2.39. (2*S*,3*S*)-1-[(*N*-Benzyl-*N*-benzyloxycarbonyl)amino]-4-phenyl-3-[(picolyl-L-valinyl)amino]-butan-2-ol (15). Compound **15** (0.12 g, 74%) was prepared from substance **14** (0.17 g, 0.28 mmol) according to the method for the preparation of **11a**, except that for the acid-mediated deprotection TFA (25% in CH_2Cl_2) was used. **15**: $[\alpha]_D^{22}$ −32.3 (*c* 0.8, $CHCl_3$); ^1H NMR [$CDCl_3$ (1% CD_3OD), 270 MHz, rotamers]: δ 0.60–1.09 (m, 6H), 1.90–2.23 (m, 1H), 2.62–2.92 (m, 2H), 2.92–3.22 (m, 1H), 3.22–3.58 (m, 2H), 3.67–3.88 (m, 1H), 3.88–4.12 (m, 1H), 4.32–4.68 (m, 2H), 4.88–5.19 (m, 2H), 6.80–7.35 (m, 15H), 7.35–7.55 (m, 1H), 7.70–7.84 (m, 1H), 7.84–8.20 (m, 1H), 8.36–8.62 (m, 2H). ^{13}C NMR [$CDCl_3$ (1% CD_3OD), 67.8 MHz, rotamers]: δ 17.7, 19.3, 30.6, 37.9, 51.8, 52.7, 53.2, 58.9, 67.5, 70.2, 122.3, 126.1, 126.5, 127.2, 127.3, 127.6, 127.7, 128.0, 128.1, 128.4, 128.9, 129.0, 136.0, 137.0, 137.7, 147.9, 148.7, 156.9 (minor), 157.6 (major), 164.3, 171.1. Anal. ($C_{36}H_{40}N_4O_5 \cdot H_2O$) C, H, N.

4.2.40. (2*S*,3*S*)-1-(Benzylamino)-4-phenyl-3-[(picolyl-L-valinyl)amino]-butan-2-ol (16). Compound **15** (70 mg, 0.12 mmol) and anisol (0.13 mg, 1.2 mmol) were dissolved in CH_2Cl_2 (30 mL). Triflic acid (0.2 mL, 2.3 mmol) was added slowly under stirring and the mixture was stirred for an additional 15 min. The reaction mixture was suspended to a silica gel column and this was flushed several times with CH_2Cl_2. The product was eluted with a mixture of CH_2Cl_2 and MeOH (9:1) and further purified with flash column chromatography (CH_2Cl_2/MeOH, 29:1) to give the pure secondary amine **16** (21 mg, 38%). Compound **16**: $[\alpha]_D^{22}$ −51.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz): δ 0.84 (d, *J* = 6.8 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 2.10–2.30 (m, 1H), 2.60–2.79 (m, 2H), 2.79–2.98 (m, 2H), 3.75–3.95 (m, 3H), 4.05–4.20 (m, 1H), 4.30–4.78 (m, 2H), 4.38 (dd, *J* = 6.5, 8.8 Hz, 1H), 6.82–6.98 (m, 1H), 6.98–7.38 (m, 9H), 7.38–7.53 (m, 1H), 7.78–7.95 (m, 1H), 8.09–8.22 (m, 1H), 8.35–8.50 (m, 1H), 8.53–8.65 (m, 1H). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 17.9, 19.6, 30.6, 38.3, 51.2, 52.8, 52.9, 59.3, 68.0, 122.4, 126.4, 126.6, 128.1, 128.5, 128.8, 129.0, 129.4, 137.5, 137.9, 148.4, 149.3, 164.8, 171.3. Anal. ($C_{28}H_{34}N_4O_3 \cdot H_2O$) C, H, N.

4.2.41. (2*S*,5*S*,6*R*)-3-Aza-3-benzyl-2-(*p*-bromobenzyl)-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (18). (2*S*,5*S*,6*R*)-3-Aza-2-(*p*-bromobenzyl)-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]- heptanoyl amide (**17**, 41 mg, 67 μmol) and Cs_2CO_3 (22 mg, 84 μmol) were suspended in DMF (2 mL). Benzyl bromide (20 μL, 0.17 mmol) was added and the mixture was stirred for overnight at 50 °C. The solvent was evaporated and the product was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1) to give the pure tertiary amine **18** (30 mg, 64%). Compound **18**: $[\alpha]_D^{22}$ −52.2 (*c* 0.9, $CHCl_3$); ^1H NMR [$CDCl_3$ (1% CD_3OD), 270 MHz]: δ 0.79 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H), 2.02–2.22 (m, 1H), 2.46–2.64 (m, 2H), 2.64–2.86 (m, 3H), 2.92 (dd, *J* = 7.3, 13.9 Hz, 1H), 3.35–3.48 (m, 2H), 3.50–3.62 (d, *J* = 13.9 Hz, 1H), 3.67–3.80 (d, *J* = 13.7 Hz, 1H), 3.98–4.13 (m, 1H), 4.13–4.25 (m, 1H), 6.82–6.91 (m, 2H), 6.91–7.04 (m, 3H), 7.04–7.18 (m, 6H), 7.20–7.34 (m, 3H), 7.38–7.48 (m, 1H), 7.72–7.84 (m, 1H), 7.98–8.07 (m, 1H), 8.52–8.60 (m, 1H). ^{13}C NMR [$CDCl_3$ (1% CD_3OD), 67.8 MHz]: δ 17.4, 19.3, 30.2, 34.0, 38.1, 51.9, 54.6, 55.5, 59.2, 65.2, 67.8, 119.9, 122.1, 126.1, 126.6, 127.1, 128.8, 128.2, 128.6, 129.0, 130.7, 131.2, 137.2, 137.4, 137.7, 138.6, 148.3, 148.6, 164.9, 171.0, 173.9. Anal. ($C_{37}H_{42}BrN_5O_4$) C, H, N.

4.2.42. (2*S*,5*S*,6*R*)-3-Aza-3-benzyl-2-(*p*-phenylbenzyl)-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (19). Compound **18** (23 mg, 33 μmol), phenylboronic acid (24 mg, 0.20 mmol), Cs_2CO_3 (32 mg, 98 μmol), and $Pd(PPh_3)_2Cl_2$ (1.8 mg, 5 μmol) were dissolved in a mixture of DME and ethanol (3:1, 0.7 mL) in a Smith vial. The vial was heated to 130 °C for 20 min by means of microwave irradiation. The mixture was filtered through a celite plug, the solvent was removed by evaporation, and the residue was purified on a silica gel column using a mixture of CH_2Cl_2 and methanol (19:1) as mobile phase to give compound **19** (18 mg, 79%) as a white powder. **19**: $[\alpha]_D^{22}$ 29.8 (*c* 0.4, $CHCl_3$); ^1H NMR [$CDCl_3$ (5% CD_3OD), 400 MHz]: δ 0.85 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 6.8 Hz, 3H), 1.90–2.13 (m, 1H), 2.51–2.80 (m, 4H), 3.00 (dd, *J* = 7.0, 13.8 Hz, 1H), 3.10 (dd, *J* = 8.1, 13.6 Hz, 1H), 3.43–3.49 (m, 1H), 3.58 (d, *J* = 14.2 Hz, 1H), 3.70 (dd, *J* = 7.0, 8.0 Hz, 1H), 3.98 (d, *J* = 14.1 Hz, 1H), 4.12–4.20 (m, 1H), 4.21–4.29 (m, 1H), 6.90–6.96 (m, 1H), 6.98–7.23 (m, 8H), 7.26–7.36 (m, 4H), 7.38–7.46 (m, 2H), 7.46–7.63 (m, 5H), 7.89–8.01 (m, 1H), 8.04–8.13 (m, 1H), 8.60–8.65 (m, 1H). Anal. ($C_{43}H_{47}N_5O_7 \cdot 0.3H_2O$) C, H, N.

4.3. Computational procedure

4.3.1. General procedure. Molecular modeling calculations were performed on a dual Intel Xeon 2.4 GHz CPU workstation and a 2.6 GHz Pentium 4 workstation running Red Hat Linux 9. Statistical analyses and visualizations were performed on Silicon Graphics Octane workstations with two 500 MHz IP35 processors and R14000 processor chips. The different suite of programs employed for modeling studies were SYBYL v.6.9[33] for data set preparation and 3D comparative molecular field analysis (CoMFA),[34] Maestro v.3.0, and Macromodel v.7.1[35] for enzyme refinement. Flexible docking was performed using FLO.[26]

4.3.2. Enzyme preparation. The X-ray crystal structure of Plm II from *Plasmodium falciparum* in complex with inhibitor rs370 was obtained under the accession code 1LF2[27] from the Protein Data Bank (PDB).[36] This

329-residue structure was resolved at 1.80 Å and possessed an *R*-value of 0.195. Crystallographic waters were removed and hydrogens added via an all-atom treatment with no atoms having lone pairs. The structural refinement was performed using the BatchMin algorithm of Macromodel v.7.1, employing the AMBER*[37] all-atom force field with a dielectric constant of 78. The extended nonbonded cutoff distances for van der Waals and Coulombic interactions were set to 3.5 and 6.0 Å, respectively, while the hydrogen-bonding cutoff distance was kept at 4.0 Å. These cutoffs have been shown to give optimal results in terms of speed and accuracy.[38] The protein was minimized with a total of 200 iterations employing the Polak–Ribiere conjugate gradient (PRCG) method, and convergence was monitored with the derivative convergence criteria using a cutoff value of 0.05 kJ/Å mol. A pocket around the inhibitor (rs370) was marked and extracted for docking calculations. This pocket constituted the crucial amino acids lining the active site. The inhibitor rs370 was removed from the active pocket and the protein was subjected to docking studies. As Plm II is found to exist in an acidic food vacuole having a pH of 5, arginines, lysines, aspartic, and glutamic acids were kept positively and negatively charged, respectively.

4.3.3. Data set. The data set used for the docking and CoMFA studies comprises a series of 37 analogues with a basic hydroxyethylamine transition state isostere scaffold as the central fragment. All compounds were protonated at the basic hydroxyethylamine nitrogen.

4.3.4. Alignment of the inhibitors. A CoMFA study requires that the 3D structures of the molecules to be analyzed be aligned according to their bioactive conformation. It was decided to dock the inhibitors and use the docked frames as the alignment for CoMFA.

4.3.5. Docking protocol. The FLO suite of programs was used to dock the database of molecules into the pocket. FLO search algorithms are derived from the method of Monte Carlo perturbation with energy minimization in Cartesian space. It uses a modified version of the AMBER force field. Partial charges were calculated using bond dipole moments. All compounds were manually positioned in the active site and were thereafter subjected to 500 steps of local Monte Carlo perturbation. It has been well documented that the binding cavity of Plm II is flexible,[28,39] for example, the residues in proximity to the S1′ site have been shown to move and therefore these residues Ile290-Val296 together with other residues known to be flexible (Tyr77-Ser79 and Gly216-Ser218) were allowed to move under constraints. All other residues in the pocket were kept fixed.

4.3.6. 3D QSAR studies. The docked poses were used to generate the CoMFA alignment. The CoMFA fields were generated using default settings as implemented in Sybyl 6.9. A 3D cubic lattice was created with a grid spacing of 2 Å and extending 4 Å units beyond the docked inhibitors in all directions. An sp^3 carbon with +1 charge was used as probe atom to produce steric and electrostatic field energies. Cutoffs for both steric and electrostatic fields were set to 30 kcal/mol, employing a distance-dependent dielectric ($1/r^2$) for calculating electrostatics and a 6–12 Lennard–Jones potential for sterics. The CoMFA region was focused and the resulting focused CoMFA descriptor fields were used as independent variables in the partial least squares (PLS)[40] regression analysis with measured p*K*i ($-\log K_i$) values as the dependent variable. A K_i value of 3600 nM was used for all compounds that had no exact value determined. Column filtering with a leave-one-out (LOO) validation, and a maximum of six components was used for all cross-validation calculations to determine the q^2 (cross-validated correlation coefficient) and the PRESS value (standard error of prediction). Since CoMFA was performed only to rationalize the qualitative results obtained from docking, no test set was used.

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