Evaluation of P_1'-Diversified Phosphinic Peptides Leads to the Development of Highly Selective Inhibitors of MMP-11

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Phosphinic peptides were previously reported to be potent inhibitors of several matrixins (MMPs). To identify more selective inhibitors of MMP-11, a matrixin overexpressed in breast cancer, a series of phosphinic pseudopeptides bearing a variety of P_1'-side chains has been synthesized, by parallel diversification of a phosphinic template. The potencies of these compounds were evaluated against a set of seven MMPs (MMP-2, MMP-7, MMP-8, MMP-9, MMP-11, MMP-13, and MMP-14). The chemical strategy applied led to the identification of several phosphinic inhibitors displaying high selectivity toward MMP-11. One of the most selective inhibitors of MMP-11 in this series, compound **22**, exhibits a K_i value of 0.23 μM toward MMP-11, while its potency toward the other MMPs tested is 2 orders of magnitude lower. This remarkable selectivity may rely on interactions of the P_1'-side chain atoms of these inhibitors with residues located at the entrance of the S_1'-cavity of MMP-11. The design of inhibitors able to interact with residues located at the entrance of MMPs' S_1'-cavity might represent an alternative strategy to identify selective inhibitors that will fully differentiate one MMP among the others.

Introduction

Matrix metalloproteases (MMPs) have been considered to be attractive targets for cancer therapy on the basis of their overexpression and activation in malignant tissues and their unique ability to degrade all components of the extracellular matrix.[1] Unfortunately, the results of clinical trials performed with synthetic MMP inhibitors were disappointing. Current understanding of the wide range of biological activities mediated by MMPs—cell death, proliferation, differentiation, tumor-associated angiogenesis, malignant conversion—may explain the lack of efficacy of MMP inhibitors in anticancer therapy.[2−4] Indeed, such studies suggest the assignment of unique functions to individual MMPs; however, the exact role of each MMP during tumor progression is still poorly understood. Clearly, there is a need for the development of highly selective inhibitors for the different MMPs in order to determine the specific role of each MMP along the different stages of tumor progression. This knowledge may lead to the reconsideration of the use of MMP inhibitors in cancer clinical trials and could also be of great value for their use in other pathologies associated with MMPs' overexpression.[5,6]

The development of highly selective inhibitors of MMPs, able to differentiate between the 25 members of this protease family, is a particularly difficult task, since the topology and the nature of the residues in the enzyme active site are highly conserved among the different MMPs.[7] Moreover, due to the occurrence of some conformational flexibility in the S_1'-specificity loop, the size and the shape of the MMPs' S_1'-specificity pocket change upon inhibitor binding.[8] Such flexibility in the S_1'-specificity pocket has been frequently proposed to explain the lack of specificity of numerous inhibitors toward MMPs.[7,8] Among the members of the MMP family, stromelysin-3 (MMP-11), which was first described to be expressed by fibroblastic cells surrounding invasive breast carcinoma,[9] displays a number of unusual features: mature forms of MMP-11 are unable to degrade any major extracellular matrix proteins, and whereas most of the MMPs should be activated extracellularly, MMP-11 is secreted in active form.[10,11] Recent in vivo studies demonstrated that MMP-11 exhibits a new and unexpected role for an MMP during tumorogenesis, acting as a cancer cell survival factor, instead of increasing neo-angiogenesis or cancer cell proliferation, suggesting that MMP-11 is more involved in tumor formation than in tumor growth.[12]

We previously reported the development of the first potent inhibitors of MMP-11, based on phosphinic peptide chemistry, e.g. RXP03, (Scheme 1).[13] Whereas the developed compounds displayed weak potencies toward MMP-1 and MMP-7, they turned out also to be very potent inhibitors of MMP-8, MMP-2, and MMP-9. This lack of specificity precludes further use of these inhibitors in animal models to evaluate the effect of MMP-11-specific inhibition on tumor growth. To develop a new series of phosphinic pseudopeptide inhibitors of MMP-11, the core structure of RXP03 was retained, since this compound has proven to be active in vivo.[14] Furthermore, like other phosphinic peptides,[15,16] RXP03 was observed to be metabolically stable in vivo.[17] In addition, previous structure−activity studies have demonstrated the positive contribution to the inhibitory

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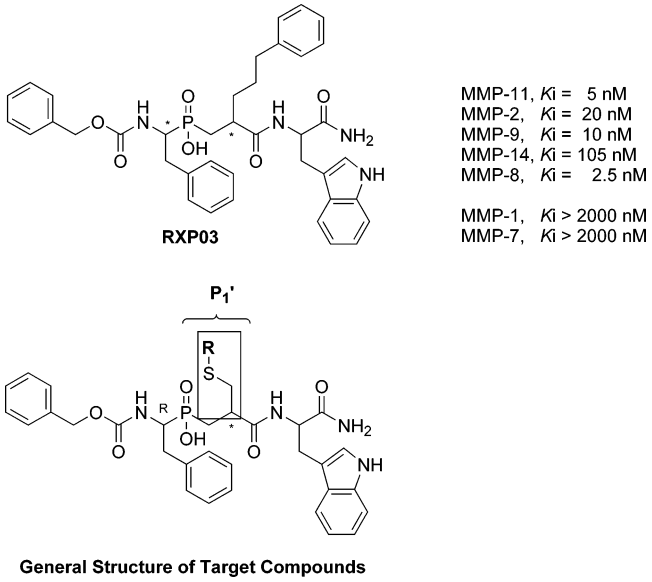
Scheme 1. Stucture of Phosphinic Peptide Inhibitors of MMPs



potency of a sulfur atom, when present in the P_1'-side chain of phosphinic peptide inhibitors of MMPs. Such sulfur-containing compounds can be prepared by parallel chemical diversification of a common dehydroalaninyl precursor. Therefore, phosphinic peptides displaying the general structure depicted in Scheme 1 were selected for this study.

Results

Chemistry. The classical approach to prepare a series of P_1'-diversified phosphinic peptides would be rather inconvenient, since the preparation of the 24 inhibitors reported herein would demand four synthetic steps, as well as three column purifications for each inhibitor. To avoid using this low-yielded, cumbersome, and time-consuming procedure, a new chemical strategy has been applied. As shown in Scheme 2, according to previously applied procedures,[13] 2-(bromomethyl)acrylic ester **I** is converted to the pseudocysteine precursors of type **II**. Phosphinic synthons of type **III** are usually obtained by a Michael addition of the silyl ester[18,19] of Z-protected phenylalanine phosphinic analogue[20] to acrylate **II**.[21] Cleavage of carboxylic ester **III** and coupling of **IV** with L-tryptophanylamide could afford the target inhibitors **4**−**26** in low to moderate yields. It is worth mentioning that such couplings, where the hydroxyphosphinyl function is unprotected, proceed in moderate to good yields, but they are strongly influenced by the size and nature of the P_1'-side chain.[22] This factor introduces an additional disadvantage to this method, given the diversity of the P_1'-side chains of the target phosphinic inhibitors. Protection of the hydroxyphosphinyl group would be a safer choice,[21] but it would render the procedure even lengthier and would probably create additional restrictions in terms of orthogonal protection of all functional groups present at the target molecules.

To overcome these drawbacks, we developed a new synthetic strategy, which allows fast and easy diversification of the P_1'-position of phosphinic peptides.[23] As shown in Scheme 2 (steps e−h), 2-(bromomethyl)acrylic ester **I** is subjected to Michael addition of Z-protected silyl phosphonite, leading to dehydroalaninyl phosphinic synthon **1**, via a tandem Arbuzov addition/allylic rearrangement. Removal of the *tert*-butyl ester (compound **2**) and coupling with L-tryptophanylamide affords the dehydroalaninyl precursor **3**, in 65% overall yield. Compound **3** was used as a template for parallel diversification of the P_1'-position, with a wide variety of side chains, as shown in Table 1, using commercially available thiols, leading to the target pseudocysteine phosphinic peptide inhibitors **4**−**26**, in good to excellent yields (Table 1).

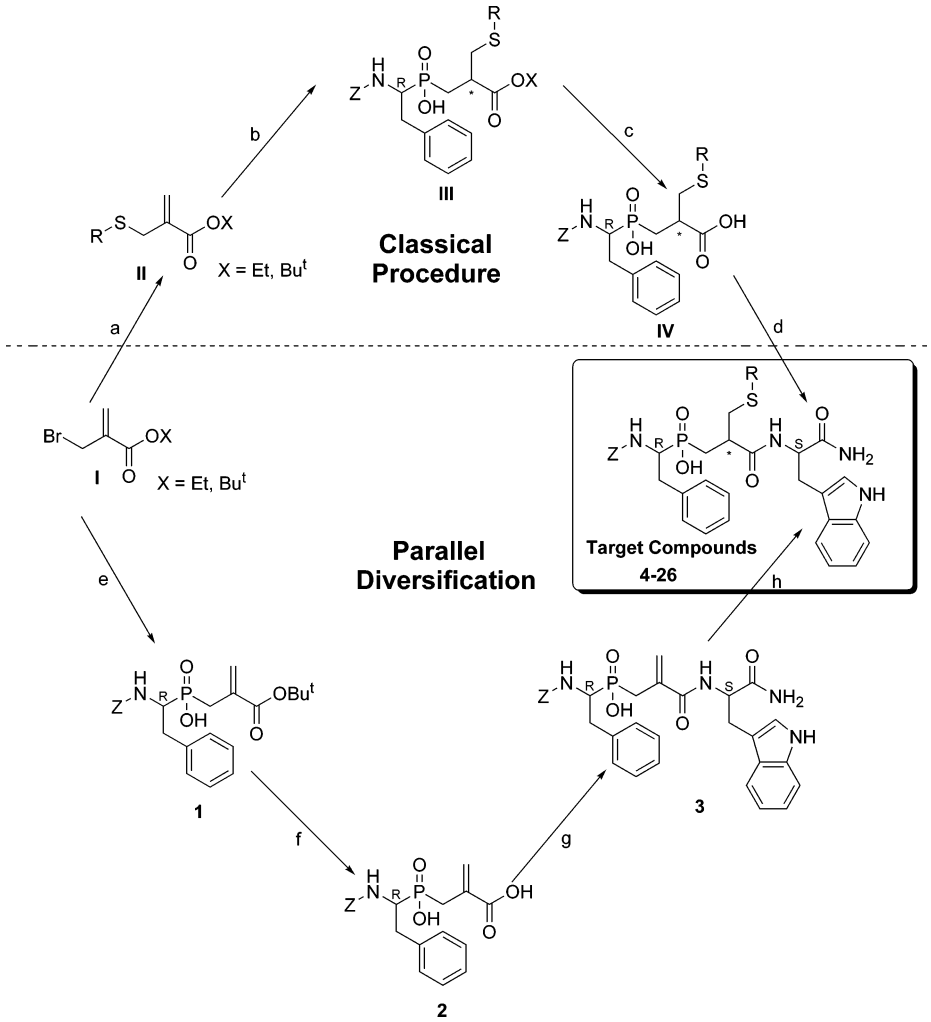
On the basis of the common precursor **3**, this strategy allowed the synthesis of the inhibitors of the present study in a parallel manner, expeditiously and cost-effectively. Yield and reaction time for thiol additions are given in Table 1.

Phosphinic inhibitor **27** was prepared from **25** by Suzuki arylation, using phenylboronic acid, as shown in Scheme 3, in 81% yield.

As an additional perspective of this strategy, it was examined whether the synthetic procedure described above can be applied in solid-phase synthesis (Scheme 4). Therefore, the possibility to directly diversify the dehydroalaninyl phosphinic template **3** while anchored to a solid support has been checked. Coupling of synthon **2** with the amino terminus of L-tryptophan, attached to Rink amide resin, can be achieved by applying the procedure used for the solution methodology. Interestingly, a single coupling step with 1 equiv of the phosphinic block **2** was sufficient, as indicated by a negative Kaiser test and proved by HPLC. Thiol addition can be performed under identical conditions with those in solution. The procedure was applied for the nucleophilic addition of an aromatic thiol, **8**, and an aliphatic compound, **10**, giving results identical with those created using solution methodology. Conclusively, it has been established that parallel diversification of phosphinic peptides can also take place on a solid support, providing an interesting alternative with regard to automatization and/or even for the preparation of longer sequences.

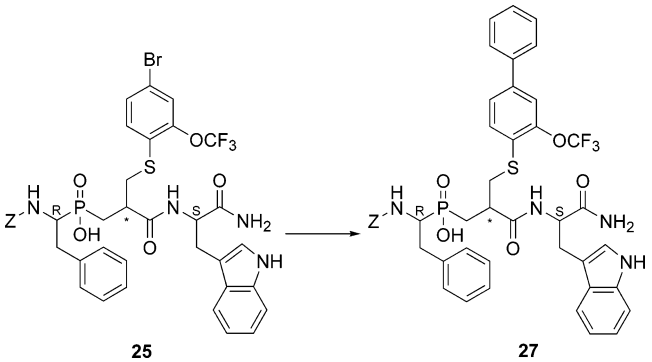
Inhibitory Potencies. Screening of the first set of compounds was performed with phosphinic peptides in which the side chain in the P_1'-position possesses both *S*- and *R*-configuration. Indeed, we previously reported that in this family of phosphinic peptides, both configurations of the P_1'-side chain provide potent inhibitors of MMPs.[14] As shown in Figure 1, compounds in which the sulfur atom is connected with arylalkyl (compounds **4**−**7**) are rather potent inhibitors of MMP-11 and MMP-8. The lack of potency of the examined compounds for MMP-7 is consistent with previous observations and is in agreement with the presence of a small S_1'-pocket in the active site of this MMP.[7] Connection of the sulfur atom with either aliphatic side chains (compounds **11**−**13**) or aliphatic side chains bearing a carboxylic ester functionality (compounds **14**−**17**) provided inhibitors exhibiting lower potencies. Surprisingly, the cyclic aliphatic compound **10**, bearing a cyclohexyl group, displayed a remarkable selectivity for MMP-11. Considerable selectivity for MMP-11 was also observed with compound **9**, bearing a phenyl group. Remarkably, the presence of a naphthyl group in compound **8** led to an

Scheme 2. General Synthetic Procedures[a]



[a] Reagents, conditions, and yields: (a) RSH (1.1 equiv), MeONa/MeOH (1.1 equiv) in Et_2O, −15 °C, to room temperature, 2−3 h; (b) Z(R)PhePO_2H_2 (0.77 equiv), HMDS (4 equiv), 110 °C, 4 h, under argon, then EtOH; (c) cleavage of carboxylic ester, TFA/DCM 50% or saponification; (d) L-TrpNH_2 (1 equiv), EDC·HCl (4 equiv), HOBt (1 equiv), DIPEA (2 equiv), in DCM, rt, 1 h; (e) same as step b, X = Bu^t, 94%; (f) TFA/DCM 50%, rt, 1 h, quantitative; (g) same as step d, 69%; (h) RSH (4 equiv), R given in Table 1, EtONa/EtOH or MeONa/MeOH for compounds **14** and **16** (2 equiv), in THF, rt, 3−40 h, 62−98%.

Scheme 3. Synthesis of Compound **27**[a]



[a] Reagents and conditions: $PhB(OH)_2$ (1.2 equiv), $Pd[P(Ph_3)]_4$ (0.05 equiv), 2 M Na_2CO_3 (2 equiv), reflux in toluene, under argon, 48 h, 81%.

inhibitor that did not differentiate between MMP-11 and MMP-8. Compound **8** turned out also to be a potent inhibitor of MMP-8, as compared to compound **9**. The rather high potency and selectivity displayed by compound **9** toward MMP-11 led us to develop a series of analogues in which the phenyl ring was modified by different kinds of substituents. Given the lack of potency of compound **9** for MMP-7, this MMP was removed from the set of MMPs taken under consideration. All these compounds were purified by HPLC, under conditions allowing the separation of the two diastereomeric forms of these compounds. Data that are reported in Figure 2 are those corresponding to the diastereomers eluting first from the HPLC column. Indeed, evaluation of the two HPLC fractions obtained for each compound revealed that the first eluting fraction displayed higher potency toward MMP-11 than the second one. Inspection of Figure 2 demonstrates that most of the selected phenyl ring modifications dramatically increase the selectivity of the inhibitors toward MMP-11. However, this gain seems to correlate with a loss of potency of the compounds toward MMP-11 by 1 order of magnitude. The substitution on the phenyl ring in the ortho-position seems to play a key role on the selectivity, since meta-modification provided inhibitors with lower selectivity, as indicated by the potencies of compounds **22** and **26**. Finally, to improve the potency of the inhibitor, compound **27**, which contains a longer side chain and retains the ortho-substitution for selectivity control, was prepared. Indeed, as exemplified by compound **5**, in-

Table 1. Yields and Reaction Time for Compounds **4**−**26**

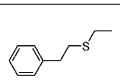
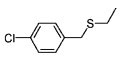
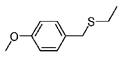
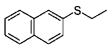
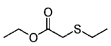
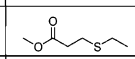
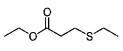
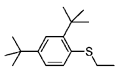
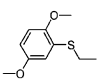
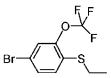
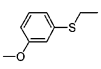
No	P_1' side chain	Yield (%)	Reaction time (h)
4		92	3
5		88	7
6		89	8
7		98	3
8		71	40
9		89	8
10		96	1
11		97	1
12		98	1
13		95	1
14		75	15
15		82	15
16		80	15
17		79	15
18		87	20
19		93	5
20		94	5
21		88	5
22		67	20
23		62	20
24		70	20
25		70	20
26		69	20

Table 2. K_i Values and Selectivity Factors of Compounds **18**, **22**, and **24** toward MMPs

no.		MMP-11	MMP-14	MMP-2	MMP-9	MMP-13	MMP-8
18	K_i (μM)	0.11	30.1	4.65	3.91	4.7	18.4
	S[a]	1	266	41	35	42	163
22	K_i (μM)	0.23	160	30	35.6	15.7	38.9
	S[a]	1	705	132	157	69	171
24	K_i (μM)	0.41	30	200	148	200	200
	S[a]	1	73	484	358	484	484

[a] $S = K_i$(MMP-X)/K_i(MMP-11).

creasing the size of the P_1'-side chains provided compounds with higher inhibitory potencies. Unfortunately, this novel compound did not display higher potency toward MMP-11. K_i values and selectivity factors of the most discriminating compounds are reported in Table 2. Compound **22** displays a K_i value of 0.23 μM toward MMP-11 and its potency is 2 orders of magnitude lower against other MMPs.

Modeling Studies. In an attempt to find a structural basis for the unusual selectivity of the compounds reported in Table 2, models of interaction between the compound **18** and the active site of three MMPs (MMP-11, MMP-8, and MMP-14) were developed. The selected inhibitor displayed a potency of 110 nM toward MMP-11 and was 260 and 160 times less potent toward MMP-14 and MMP-8, respectively (Table 2). For each model, the interaction energy between the P_1'-side chain and the residues of the MMP catalytic domain was calculated. On the basis of these calculations, the residues displaying an interaction energy lower than a threshold value of 0.1 kcal/mol were identified (Figures 3 and 4). As shown in Figure 3, the P_1'-side chain of compound **18** interacts with a limited set of MMP residues, belonging to the so-called S_1'-specificity pocket. Inspection of Figure 4 reveals that the interaction energy values between the P_1'-side chain and these residues

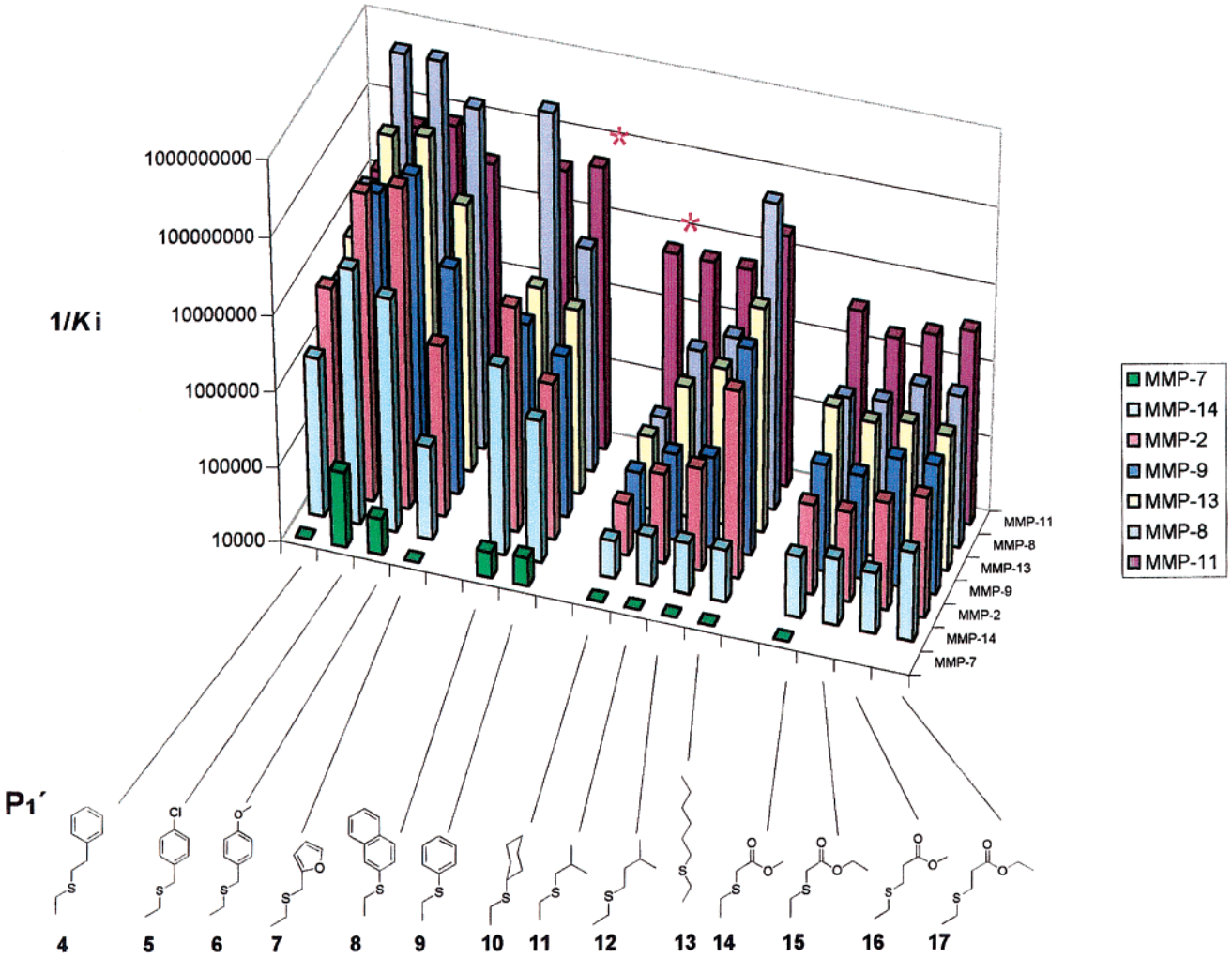
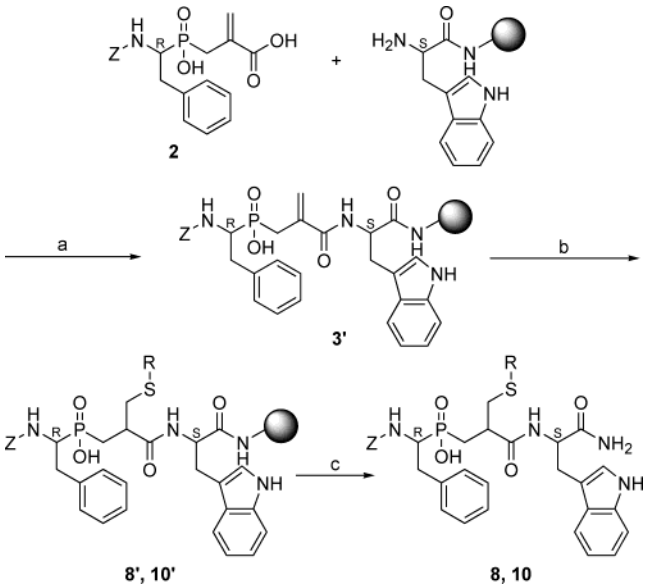


Figure 1. Graphic representation of inhibitory potencies of compounds **4**−**17** toward MMPs. The compounds were tested as a mixture of diastereoisomers.

Scheme 4. Solid Phase Diversification[a]



[a] Reagents and conditions: (a) **2** (1 equiv), EDC·HCl (4 equiv), HOBt (1 equiv), DIPEA (2 equiv), in DCM, rt, 1.5 h; (b) cyclohexyl or naphthyl thiol (4 equiv), 1 M EtONa/EtOH (2 equiv), in THF, rt; (c) TFA/TIS/H_2O 95:2.5:2.5.

are not significantly different in the three models. This applies even for residues not strictly conserved in the three MMPs, positions 338, 340, 342 of the S_1'-cavity (residues squared in Figure 4). Thus, these models did not offer an explanation for the different affinities displayed by the compound **18** toward the selected MMPs.

Discussion

The aim of this study was to develop compounds able to selectively inhibit MMP-11 over other MMPs coexpressed with MMP-11 in cancer tissues.[3−5,9] Resolution of the crystal structure of the catalytic domain of MMP-11, in interaction with the phosphinic peptide inhibitor RXP03, has revealed that MMP-11 belongs to the group of MMPs characterized by the presence of a very deep S_1'-cavity in their active sites.[24] This observation corroborates the striking ability of MMP-11 to cleave efficiently synthetic substrates harboring an unusually long side chain in their P_1'-position[25] and to interact tightly with inhibitors displaying the same structural features.[13] The presence of such S_1'-cavity in MMP-11 explains also why the development of selective inhibitors of this MMP has been a tedious task.[13] Indeed, with the exception of MMP-1 and MMP-7, which possess a small S_1'-cavity, most of the MMPs display a S_1'-cavity similar to the one observed in MMP-11.[7] In connection with this, it should be remarked that many of the inhibitors of MMPs that claim to be highly selective are actually

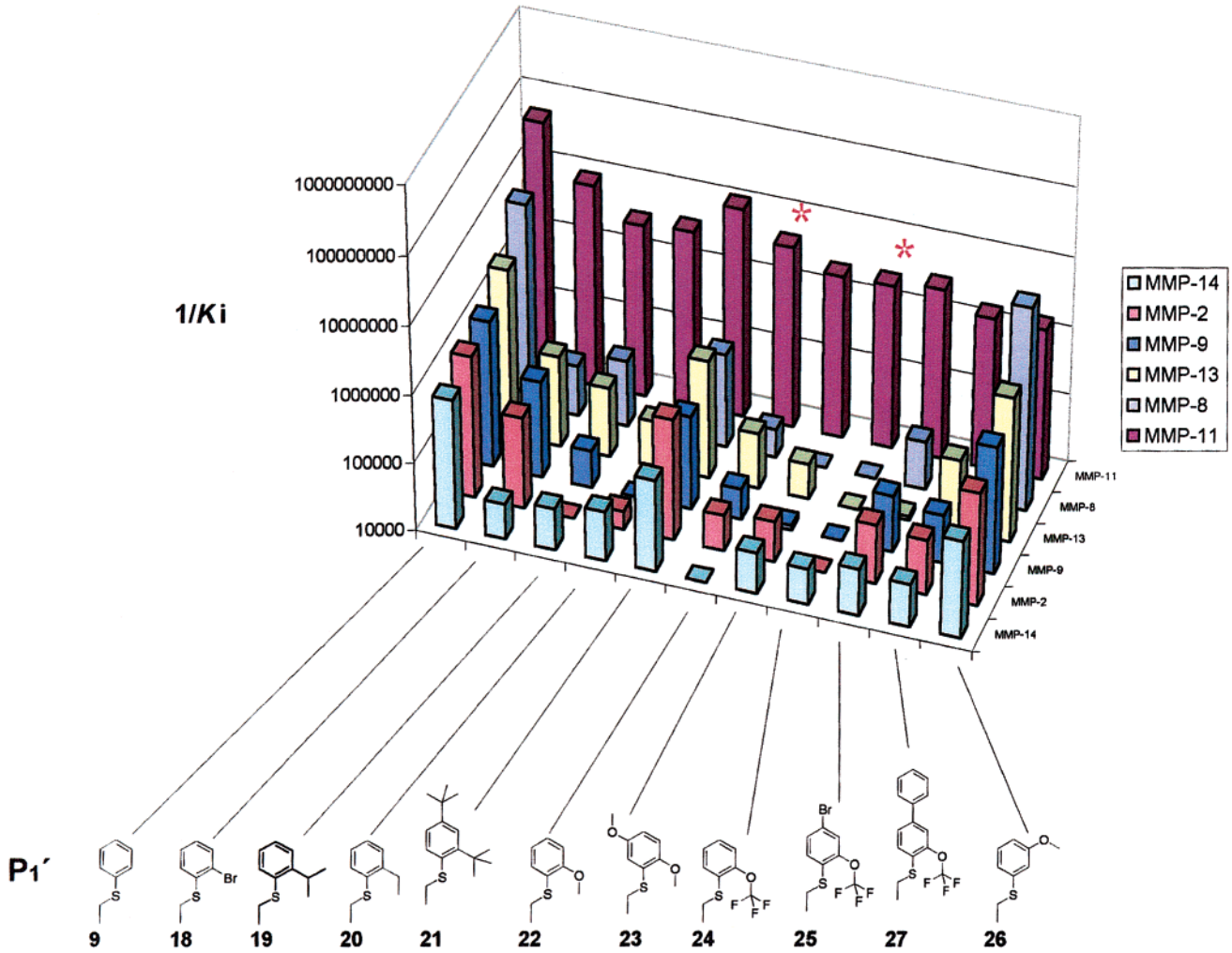


Figure 2. Graphic representation of inhibitory potencies of compounds **18**−**27** toward MMPs.

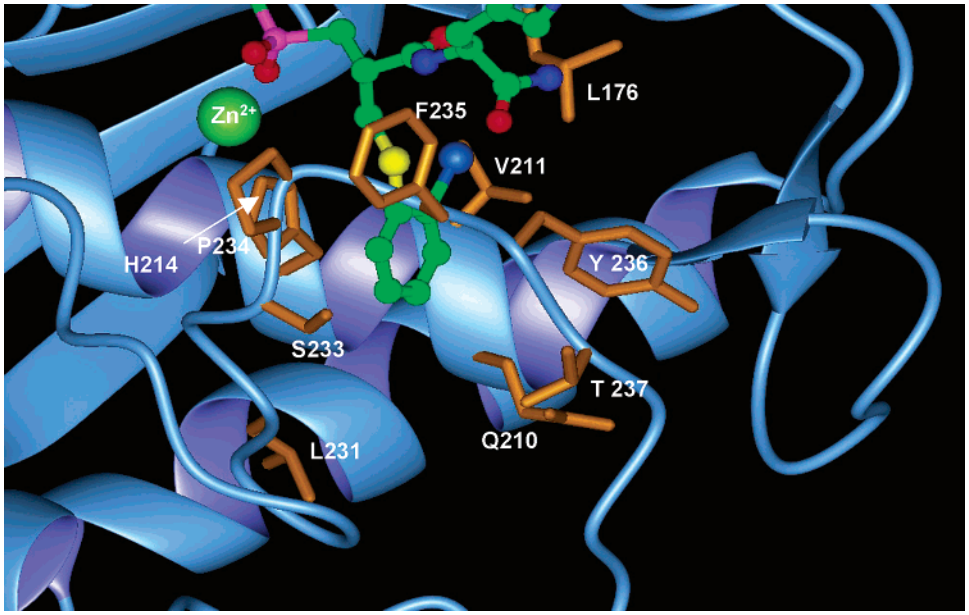


Figure 3. Model of interaction of compound **18** and the S_1'-subsite of MMP-11. Created with CHARMM.[31]

compounds that essentially discriminate MMPs according to the size of their S_1'-cavity. For example, AG-3340 or prinomastat, one of the most cited selective inhibitor of MMPs, differentiates MMP-2 (K_i = 50 pM) from MMP-1 and MMP-7 with a selectivity factor of 166 and 1080, respectively.[26] However, toward MMP-3 and MMP-13, two MMPs with a deep S_1'-cavity, the selectivity factor of prinomastat is only 6 and 0.6, respectively (MMP-2 as reference).[26] Thus, to our knowledge, compounds **22** and **24** are the most selective inhibitors of

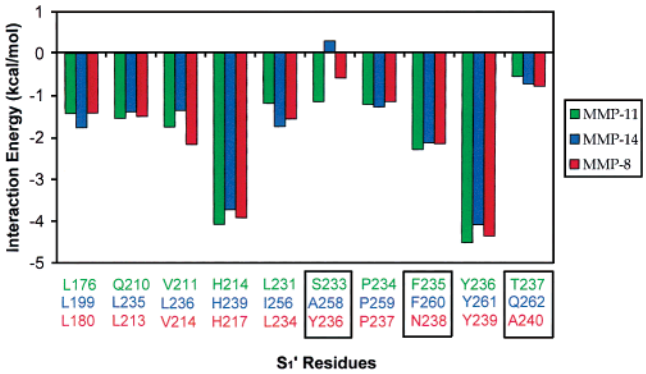


Figure 4. Interaction energies in kcal/mol of the P_1'-side chain of compound **18** with different residues of the S_1'-subsite of MMP-11, MMP-14, and MMP-8, calculated using the molecular models of interaction of compound **18** with the catalytic domains of MMP-11, MMP-14, and MMP-8 as described in the Experimental Section. The residue names correspond to those of MMP-11.

an MMP reported to date, which is able to differentiate MMP-11 between other examined MMPs that share a deep S_1'-cavity. As far as the bound form of the enzyme (EI complex) is considered, modeling and computational studies performed with compound **18** did not offer explanations for the better potency displayed by this inhibitor toward MMP-11. These simulations indicated that the interaction of compound **18** with the active sites of MMPs do not imply steric hindrance nor noticeable conformational shift of the residues surrounding the P_1'-side chain of this compound, when the RXP03−MMP-11 complex is taken as reference.[24] These results were in agreement with the observation that the inhibitors harboring bulkier groups (compounds **19**−**25**) than a Br atom displayed potency and selectivity similar to compound **18**. A better agreement between experimental and calculated data may require taking into account other contributions to the binding free energy, such as entropy or solvation energy. The role of water molecules observed in the S_1'-cavity of MMPs, in specific positions, remains also to be determined.[24] As previously discussed, the development of accurate models in the specific case of MMPs suffers from the inherent flexibility of the S_1'-loop.[7,8] It should be remarked that the occurrence of conformational changes at the S_1'-loop level was mostly observed upon binding of inhibitors bearing a very long P_1'-side chain and were suggested to take place in order to avoid steric clash.[7,8] Our models showed that the binding of compound **18** involves no steric clash and the P_1'-side chain of this inhibitor does not enter deeply in the S_1'-cavity. Thus, the inherent flexibility involving residues located in the bottom part of the S_1'-loop (bottom of the S_1'-cavity) is not expected to play any important role during the binding of compound **18**. However, we could not exclude the occurrence of conformational changes in other parts of the enzyme active site upon inhibitor binding. Most of the available X-ray structures of MMPs correspond to forms of MMPs complexed to inhibitors. Little information is available regarding the conformational states of MMPs free of inhibitors. Therefore, development of accurate models to describe these states represents a difficult task. As recently illustrated, better understanding of the molecular motions involved in the MMP−inhibitor interactions will be greatly assisted by the information provided by experimental approaches, such as NMR, designed to probe the mobility of proteins.[27]

Previous design of selective inhibitors of MMPs was mainly based on the development of compounds bearing very long, unusual P_1'-side chains able to interact with residues located at the bottom of the MMP S_1'-subsite.[7,28] This strategy provides potent inhibitors of MMPs, but as exemplified by compounds **4**, **5**, and **6**, such inhibitors do not display high selectivity, for the reasons discussed above. The most selective compounds reported in this study are characterized by the presence of groups, at the ortho-position of the phenyl ring, that interact with residues of MMPs located at the entrance of the S_1'-cavity. These results suggest that the development of compounds able to probe the entrance of the S_1'-cavity, rather than its bottom part, might represent an alternative strategy to obtain more selective inhibitors of MMPs. This proposal is supported by the observation that the upper segment of the S_1'-loop, located at the entrance of the S_1'-cavity, displays high sequence variability. Furthermore, comparison of the X-ray structure of the catalytic domains of different MMPs shows that the conformation of the upper part of the S_1'-loop is highly conserved, suggesting that this segment of the S_1'-loop is less flexible than its lower part.[29,30] The selectivity of the reported compounds for MMP-11 represents a significant progress. Further increase of their potencies by developing inhibitors bearing P_1'-side chains able to probe the entrance of the S_1'-cavity and fill this cavity may represent attractive future approaches. Compound **27**, developed according to this proposal, did not display the expected increase in potency. This result clearly shows the limit of classical structure-based design to discover highly selective inhibitors of MMPs. Better understanding of the factors governing the potency and selectivity of inhibitors toward MMPs is of critical importance in order to realize further significant progress in this field.

Conclusions

Implementation of a chemical strategy to produce rapidly P_1'-diversified phosphinic pseudopeptides led to the identification of inhibitors able to discriminate MMP-11 from other MMPs with a two-order magnitude of selectivity. These results confirm the efficacy of phosphinic peptide chemistry for the development of highly selective inhibitors of zinc−metalloproteases. Further development of the phosphinic peptide chemistry, conjugated to experimental approaches providing information on mobility of MMP−inhibitor complexes, should contribute to the discovery of the expected selective MMP inhibitors.

Experimental Section

General Procedures. All of the compounds, for which analytical and spectroscopic data are quoted, were homogeneous by TLC. TLC analyses were performed using silica gel plates (E. Merck silica gel 60 F-254) and components were visualized by the following methods: ultraviolet light absorbance and charring after spraying with a solution of $(NH_4)HSO_4$ and ninhydrin spray. The solvent systems used for TLC developments were (a) $CHCl_3$–MeOH−AcOH (7:2:1), (b) $CHCl_3$–MeOH−AcOH (7:0.5:0.5), (c) 1-butanol−AcOH−H_2O (4:1:1), (d) petroleum ether (bp 40−60 °C)∠AcOEt−AcOH (3:3:0.2). Column chromatography was carried out on silica gel (E. Merck, 70−230 mesh), height 35 cm, diameter 1.2 cm. Melting

points provided are those of the diastereoisomeric mixtures—where present—and are not corrected.

The purity of phosphinic peptides was established by analytical HPLC and mass spectrometry; ESMS spectra were recorded on a Micromass Platform II instrument using negative ionization mode by Atheris Laboratories, 314 CH-1233 Bernex, Geneva, Switzerland.

HPLC analyses and preparative purifications were carried out on a MZ-Analytical Column 250 × 4 mm, Kromasil, 100, C18, 5μm (column 1), at a flow rate of 0.5 mL/min, an analytical Hypersil C18 (column 2), at a flow rate of 1 mL/min, and a semipreparative VYDAC C18 (column 3), at a flow rate of 3 mL/min. Solvent A: 10% CH_3CN, 90% H_2O, 0.1% TFA. Solvent B: 90% CH_3CN, 10% H_2O, 0.09% TFA. The following gradients were used: Gradient 1: $t = 0$ min (0% B), $t = 10$ min (25% B), $t = 45$ min (75% B). Gradient 2: $t = 0$ min (0% B), $t = 15$ min (45% B), $t = 45$ min (60% B). Gradient 3: $t = 0$ min (0% B), $t = 10$ min (15% B), $t = 45$ min (40% B). Gradient 4: $t = 0$ min (0% B), $t = 15$ min (35% B), $t = 45$ min (60% B). Gradient 5: $t = 0$ min (0% B), $t = 15$ min (35% B), $t = 45$ min (65% B). Gradient 6: $t = 0$ min (0% B), $t = 15$ min (50% B), $t = 45$ min (75% B). Gradient 7: $t = 0$ min (0% B), $t = 15$ min (30% B), $t = 45$ min (65% B). Gradient 8: $t = 0$ min (0% B), $t = 15$ min (35% B), $t = 45$ min (70% B). Gradient 9: $t = 0$ min (0% B), $t = 10$ min (35% B), $t = 45$ min (55% B). Eluted peaks were detected at 280, 254, and 230 nm. Given times correspond to two diastereoisomeric forms and are counted in minutes.

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a 200 MHz Mercury Varian spectrometer. All NMR experiments were carried at 298 K. ^{13}C and ^{31}P NMR spectra are fully proton decoupled. ^{31}P chemical shifts are reported on δ scale, in ppm, downfield from 85% H_3PO_4.

Abbreviations: AcOH, acetic acid; AcOEt, ethyl acetate; Bu^t, *tert*-butyl; DCM, dichloromethane; DIC, disopropylcarbodiimide; DIPEA, diisopropylethylamine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDC·HCl, 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide·HCl; Et_2O, diethyl ether; EtOH, ethanol; HMDS, 1,1,1,3,3,3-hexamethyldisilazane; HOBt, 1-hydroxybenzotriazole; MeOH, methanol; PE (40−60), petroleum ether (bp 40−60 °C); $PhB(OH)_2$, phenylboronic acid; Pd-$[P(Ph)_3]_4$, tetrakis(triphenylphosphine)palladium; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIS, triisopropylsilane; Z, benzyloxycarbonyl.

Chemistry. (*R*)-2-[(1-Benzyloxycarbonylamino-2-phenylethyl)hydroxyphosphinoylmethyl]acrylic Acid *tert*-Butyl Ester (1). A suspension of (*R*)-(1-benzyloxycarbonylamino-2-phenylethyl)phosphinic acid (10 mmol, 3.19 g) and HMDS (50 mmol, 4.8 g, 6.3 mL) was heated at 110 °C for 1 h under argon atmosphere. Acrylate **I** (X = Bu^t) (13 mmol, 2.87 g) was added at this temperature dropwise for 15 min and the reaction mixture was stirred for 3 h. Then, absolute EtOH (30 mL) was added dropwise. After cooling at room temperature, the mixture was evaporated in vacuo. The residue was dissolved in AcOEt (200 mL) and the resulting suspension was washed with HCl (1 N, 2 × 50 mL). Purification by column chromatography, using $CHCl_3$–MeOH−AcOH (7:0.4:0.4) as eluent, afforded the product (4.3 g, 94%) as white solid: mp 141−145 °C; TLC R_f(b) 0.53, R_f(d) 0.37; ^{1}H NMR (200 MHz, $CDCl_3$/TFA-d_1 = 99.5/0.5) δ 1.47 (s, 9H, C(CH_3)$_3$), 2.60−3.05 (m, 3H, PCH_2, PhCHH), 3.15−3.37 (m, 1H, PhCHH), 4.16−4.35 (m, 1H, PCH), 4.85−5.06 (br s, 2H, OCH_2Ph), 5.70−5.88 (m, 2H, NH, C═CHH), 6.23 (s, 1H, C═CHH), 7.02−7.32 (m, 10H, aryl); ^{13}C NMR (50 MHz, $CDCl_3$/TFA-d_1 = 99.5/0.5) δ 27.8 (C(CH_3)), 30.8 (d, $^1J_{PC}$ = 94.6 Hz, PCH_2), 34.3 (CH_2Ph), 51.3 (d, $^1J_{PC}$ = 107.5 Hz, PCH), 66.5 (OCH_2Ph), 81.7 (C(CH_3)), 126.1, 127.6, 128.2, 129.1, 133.5, 136.5, 137.8, 138.1 (aryl, vinyl), 156.8 (OCONH), 167.3 (COOBut); ^{31}P NMR (81 MHz, $CDCl_3$/TFA-d_1 = 99.5/0.5) δ 49.53; ESMS *m*/*z* calcd for $C_{24}H_{29}NO_6P$ $(M - H)^-$ 458.2, found 458.5.

(*R*)-2-[(1-Benzyloxycarbonylamino-2-phenylethyl)hydroxyphosphinoylmethyl]acrylic Acid (2). To a solution of compound **1** (1 mmol, 0.46 g) in DCM (5 mL) was added TFA (5 mL). The reaction mixture was stirred for 1 h at room temperature and then evaporated to dryness. The residue was treated with DCM and a solid precipitated. Filtration afforded the product quantitatively (0.4 g) as a white solid: mp 179−181 °C; TLC R_f(a) 0.57, R_f(c) 0.74; HPLC (column 1, gradient 1) 25.46; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.45−2.86 (m, 3H, PCH_2, PhCHH), 3.03−3.18 (m, 1H, PhCHH), 3.85−4.04 (m, 1H, PCH), 4.82−5.01 (br s, 2H, OCH_2Ph), 5.78 (s, 1H, C═CHH), 6.16 (s, 1H, C═CHH), 7.12−7.64 (m, 11H, aryl, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 29.3 (d, $^1J_{PC}$ = 85.2 Hz, PCH_2), 32.9 (CH_2Ph), 51.8 (d, $^1J_{PC}$ = 105.1 Hz, PCH), 65.1 (OCH_2Ph), 126.2, 127.1, 127.6, 128.2, 129.0, 132.8, 137.3, 138.3, 138.6 (aryl, vinyl), 156.0 (d, $^3J_{PC}$ = 4.2 Hz, OCONH), 167.6 (d, $^3J_{PC}$ = 4.1 Hz, COOH); ^{31}P NMR (81 MHz, DMSO-d_6) δ 42.38; ESMS *m*/*z* calcd for $C_{20}H_{21}NO_6P$ $(M - H)^-$ 402.1, found 402.1.

[(*R*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]allyl}-phosphinic Acid (3). To a suspension of compound **2** (1 mmol, 0.40 g) in DCM (20 mL) were added DIPEA, (1 mmol, 0.13 g, 0.17 mL), L-tryptophanylamide (1 mmol, 0.20 g), HOBt (1 mmol, 0.13 g), EDC·HCl, (4 mmol, 0.77 g), and finally another 1 mmol of DIPEA. The reaction mixture was stirred for 1 h at room temperature. Then it was diluted with DCM (60 mL) and washed with a solution of 1 N HCl (3 × 5 mL), a saturated solution of NH_4HCO_3 (3 × 1 mL), 1 N HCl to pH 1 and brine (10 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography using $CHCl_3$-MeOH−AcOH (7:1:0.4), as eluent afforded the product (0.41 g, 69%) as a white solid, mp 176−180 °C. TLC R_f(a) 0.55, R_f(c) 0.73; HPLC (column 1, gradient 1) 30.59; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.20−3.42 (m, 6H, indolylCH_2, PCH_2, PhCH_2), 3.73−4.02 (m, 1H, PCH), 4.33−4.56 (m, 1H, indolylCH$_2$CH), 4.75−5.05 (br s, 2H, OCH_2Ph), 5.17 (s, 1H, C═CHH), 5.44 (s, 1H, C═CHH), 6.92−7.68 (m, 17H, aryl, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 27.9 (d, $^1J_{PC}$ = 78.6 Hz, PCH_2), 33.6 (CH_2Ph), 50.5 (d, $^1J_{PC}$ = 103.9 Hz, PCH), 54.7 (indolylCH$_2$$C$H), 64.9 (O$CH_2$Ph), 111.1, 118.3, 121.1, 123.9, 126.6, 127.4, 128.1, 129.3, 136.1, 137.5, (aryl, vinyl), 139.9, 155.9 (OCONH), 168.6 (CH_2═CCONH), 174.6 (CONH$_2$); ^{31}P NMR (81 MHz, DMSO-d_6) δ 29.37; ESMS *m*/*z* calcd for $C_{31}H_{32}N_4O_6P$ $(M - H)^-$ 587.2, found 587.4.

General Procedure for Thiol Addition, in Solution. To a solution of compound **3** (0.05 mmol, 0.029 g) in freshly distilled THF (3 mL) were added the appropriate thiol (0.2 mmol) and a 1 M solution of EtONa/EtOH (0.1 mmol, 0.1 mL), except in the case of compounds **14** and **16**, where MeONa/MeOH was used instead of EtONa/EtOH. The reaction mixture was stirred at room temperature (for reaction time, see Table 1) and then evaporated to dryness. After the addition of some drops of H_2O in the residue, $CHCl_3$ (5 mL) was added and the resulting solution was washed with a solution of 1 N HCl to pH 1. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Dissolution of the mixture in the minimum volume of $CHCl_3$, precipitation with PE (40−60), filtration, and washings of the residue with PE (40−60) afforded the products as white solids. Further purification of the products can be achieved by column chromatography using $CHCl_3$–MeOH−AcOH (7:0.7:0.4) as eluent system. Yields are given in Table 1.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-phenethylsulfanylpropyl}phosphinic acid (4): TLC R_f-(a) 0.55/0.69, R_f(c) 0.74/0.79; HPLC (column 2, gradient 1) 29.08/31.53; ESMS *m*/*z* calcd for $C_{39}H_{42}N_4O_6PS$ $(M - H)^-$ 725.3, found 725.9; relative intensity of the molecular ion, 98%.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(4-chlorobenzylsulfanyl)propyl}phosphinic acid (5): TLC R_f(a) 0.60/0.71, R_f(c) 0.78/0.84; HPLC (column 2, gradient 1) 30.17/32.61; ESMS *m*/*z* calcd for $C_{38}H_{39}ClN_4O_6PS$ $(M - H)^-$ 745.2, found 745.8; relative intensity of the molecular ion, 98%.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(4-methoxybenzylsulfanyl)propyl}phosphinic acid (6): TLC R_f(a) 0.65/0.76, R_f(c) 0.80/0.85; HPLC (column 2, gradient

1) 27.65/28.90; ESMS *m/z* calcd for $C_{39}H_{42}N_4O_7PS$ $(M - H)^-$ 741.3, found 741.9; relative intensity of the molecular ion, 98%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(furan-2-ylmethylsulfanyl)propyl}phosphinic acid (7): TLC R_f(a) 0.60/0.67, R_f(c) 0.73/0.75; HPLC (column 2, gradient 1) 25.17/27.30; ESMS *m/z* calcd for $C_{36}H_{38}N_4O_7PS$ $(M - H)^-$ 701.2, found 702.0; relative intensity of the molecular ion, 95%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(naphthelene-2-ylsulfanyl)propyl}phosphinic acid (8): TLC R_f(a) 0.53/0.65, R_f(c) 0.73/0.77; HPLC (column 1, gradient 1) 39.23/43.47, (column 2, gradient 9) 29.76/32.81; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.98−2.31 (m, 2H, PCH_2), 2.61−3.39 (m, 7H, SCH_2, PCH$_2$CH, PhCH_2, indolylCH_2), 3.82−4.18 (m, 1H, PCH) 4.25−4.58 (m, 1H, indolylCH$_2$CH), 4.84−5.01 (br s, 2H, OCH_2Ph), 6.93−7.89 (m, 24H, aryl, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 27.9 (d, $^1J_{PC}$ = 78.3 Hz, PCH$_2$), 32.9 (*C*H$_2$-Ph), 41.9 (PCH$_2$*C*H), 50.1 (d, $^1J_{PC}$ = 104.0 Hz, P*C*H), 53.9 (indolylCH$_2$*C*H), 65.2 (O*C*H$_2$Ph), 111.1, 112.8, 118.6, 121.0, 123.3, 123.4, 126.4, 127.4, 128.3, 129.3, 136.1, 137.3, 138.6, (aryl), 156.1 (O*C*ONH), 172.1 (CHC*C*ONH), 173.8 (*C*ONH$_2$); ^{31}P NMR (81 MHz, DMSO-d_6) δ 42.60, 43.28; ESMS *m/z* calcd for $C_{41}H_{40}N_4O_6PS$ $(M - H)^-$ 747.2, found 747.9; relative intensity of the molecular ion, 97%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-phenylsulfanylpropyl}phosphinic acid (9): TLC R_f(a) 0.60/0.67, R_f(c) 0.74/0.78; HPLC (column 2, gradient 1) 27.09/29.19, (column 3, gradient 4) 25.42/28.28; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.82−2.05 (m, 2H, PCH_2), 2.68−3.58 (m, 7H, SCH_2, PCH$_2$CH, PhCH_2, indolylCH_2), 3.73−4.07 (m, 1H, PCH) 4.35−4.45 (m, 1H, indolylCH$_2$CH), 4.83−4.99 (br s, 2H, OCH_2-Ph), 6.92−7.68 (m, 22H, aryl, NH); ESMS *m/z* calcd for $C_{37}H_{38}N_4O_6PS$ $(M - H)^-$ 697.2, found 697.6; relative intensity of the molecular ion, 99%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-cyclohexylsulfanylpropyl}phosphinic acid (10): TLC R_f(a) 0.71/0.80, R_f(c) 0.82/0.85; HPLC (column 1, gradient 1) 40.70/44.24, (column 2, gradient 1) 27.66/30.37; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.78−2.08 (m, 10H, CH_2 of cyclohexyl), 2.10−3.42 (m, 10H, CHSCH$_2$, CHSCH_2, PCH$_2$CH, PCH_2, PhCH_2, indolylCH_2), 3.80−4.16 (m, 1H, PCH) 4.28−4.56 (m, 1H, indolylCH$_2$CH), 4.85−5.05 (br s, 2H, OCH_2Ph), 6.80−7.78 (m, 17H, aryl, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 25.5 (*C*H$_2$ of cyclohexyl), 27.7 (d, $^1J_{PC}$ = 77.9 Hz, PCH$_2$), 33.1 (*C*H$_2$Ph), 42.5 (PCH$_2$*C*H), 51.3 (d, $^1J_{PC}$ = 102.9 Hz, P*C*H), 53.8 (indolylCH$_2$*C*H), 65.1 (O*C*H$_2$Ph), 111.1, 118.1, 121.0, 123.3, 123.6, 126.6, 127.4, 128.2, 128.9, 136.2, 137.3, 139.9, (aryl), 156.1 (O*C*ONH), 173.3 (CHC*C*ONH), 174.3 (*C*ONH$_2$); ^{31}P NMR (81 MHz, DMSO-d_6) δ 45.94, 46.85; ESMS *m/z* calcd for $C_{37}H_{44}N_4O_6PS$ $(M - H)^-$ 703.3, found 704.0; relative intensity of the molecular ion, 99%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-isobutylsulfanylpropyl}phosphinic acid (11): TLC R_f(a) 0.70/0.77, R_f(c) 0.82/0.85; HPLC (column 2, gradient 1) 26.70/29.29; ESMS *m/z* calcd for $C_{35}H_{42}N_4O_6PS$ $(M - H)^-$ 677.3, found 677.4; relative intensity of the molecular ion, 98%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(3-methylbutylsulfanyl)propyl}phosphinic acid (12): TLC R_f(a) 0.70/0.79, R_f(c) 0.82/0.86; HPLC (column 2, gradient 1) 28.29/31.15; ESMS *m/z* calcd for $C_{36}H_{44}N_4O_6PS$ $(M - H)^-$ 691.3, found 691.8; relative intensity of the molecular ion, 98%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-hexylsulfanylpropyl}phosphinic acid (13): TLC R_f(a) 0.71/0.80, R_f(c) 0.84/0.87; HPLC (column 2, gradient 1) 31.27/34.30; ESMS *m/z* calcd for $C_{37}H_{46}N_4O_6PS$ $(M - H)^-$ 705.3, found 705.5; relative intensity of the molecular ion, 99%.

[(*R*),(*R,S*),(*S*)]-{3-[(1-Benzyloxycarbonylamino-2-phenylethyl)hydroxyphosphinoyl]-2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]propylsulfanyl}acetic acid, methyl ester (14): TLC R_f(a) 0.64/0.71, R_f(c) 0.78/0.81; HPLC (column 2, gradient 1) 20.72/23.21, (column 3, gradient 2) 39.06/41.57; ESMS *m/z* calcd for $C_{34}H_{39}N_4O_8PS$ $(M - H)^-$ 693.2, found 694.0; relative intensity of the molecular ion, 97%.

[(*R*),(*R,S*),(*S*)]-{3-[(1-Benzyloxycarbonylamino-2-phenylethyl)hydroxyphosphinoyl]-2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]propylsulfanyl}acetic acid, ethyl ester (15): TLC R_f(a) 0.65/0.73, R_f(c) 0.78/0.82; HPLC (column 2, gradient 1) 24.78/26.49, (column 3, gradient 2) 42.60/46.15; ESMS *m/z* calcd for $C_{35}H_{40}N_4O_8PS$ $(M - H)^-$ 707.2, found 708.0; relative intensity of the molecular ion, 97%.

[(*R*),(*R,S*),(*S*)]-3-{3-[(1-Benzyloxycarbonylamino-2-phenylethyl)hydroxyphosphinoyl]-2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]propylsulfanyl}propionic acid, methyl ester (16): TLC R_f(a) 0.65/0.73, R_f(c) 0.78/0.82; HPLC (column 3, gradient 2) 38.82/44.39; ESMS *m/z* calcd for $C_{35}H_{40}N_4O_8PS$ $(M - H)^-$ 707.2, found 708.1; relative intensity of the molecular ion, 97%.

[(*R*),(*R,S*),(*S*)]-3-{3-[(1-Benzyloxycarbonylamino-2-phenylethyl)hydroxyphosphinoyl]-2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]propylsulfanyl}propionic acid, ethyl ester (17): TLC R_f(a) 0.65/0.73, R_f(c) 0.78/0.82; HPLC (column 2, gradient 1) 22.31/27.50; ESMS *m/z* calcd for $C_{36}H_{42}N_4O_8PS$ $(M - H)^-$ 721.3, found 722.0; relative intensity of the molecular ion, 97%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{3-(2-bromophenylsulfanyl)-2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]propyl}phosphinic acid (18): TLC R_f(a) 0.61/0.68, R_f(c) 0.74/0.77; HPLC (column 2, gradient 1) 28.40/31.05, (column 3, gradient 4) 34.40/38.51; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.89−2.28 (m, 2H, PCH_2), 2.58−3.40 (m, 7H, SCH_2, PCH$_2$CH, PhCH_2, indolylCH_2), 3.77−4.09 (m, 1H, PCH), 4.37−4.57 (m, 1H, indolylCH$_2$CH), 4.85−4.99 (br s, 2H, OCH_2Ph), 6.94−7.78 (m, 21H, aryl, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 27.7 (d, $^1J_{PC}$ = 77.1 Hz, PCH$_2$), 32.8 (*C*H$_2$-Ph), 42.1 (PCH$_2$*C*H), 51.0 (d, $^1J_{PC}$ = 103.5 Hz, P*C*H), 54.3 (indolylCH$_2$*C*H), 65.1 (O*C*H$_2$Ph), 111.2, 118.2, 120.7, 126.2, 127.5, 128.1, 129.0, 136.1, 137.2, 138.6, (aryl), 156.0 (O*C*ONH), 172.0 (CHC*C*ONH), 173.7 (*C*ONH$_2$); ^{31}P NMR (81 MHz, DMSO-d_6) δ 45.30; ESMS *m/z* calcd for $C_{37}H_{37}BrN_4O_6PS$ $(M - H)^-$ 775.1, found 775.5; relative intensity of the molecular ion, 99%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(2-isopropylphenylsulfanyl)propyl}phosphinic acid (19): TLC R_f(a) 0.60/0.67, R_f(c) 0.74/0.78; HPLC (column 3, gradient 4) 41.02/45.15; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.06−1.18 (m, 7H, CH(CH)$_3$, CH(CH)$_3$) 1.81−2.06 (m, 2H, PCH_2), 2.61−3.56 (m, 7H, SCH_2, PCH$_2$CH, PhCH_2, indolylCH_2), 3.68−4.08 (m, 1H, PCH), 4.39−4.52 (m, 1H, indolylCH$_2$CH), 4.80−4.98 (br s, 2H, OCH_2Ph), 6.95−7.72 (m, 21H, aryl, NH); ESMS *m/z* calcd for $C_{40}H_{44}N_4O_6PS$ $(M - H)^-$ 739.3, found 739.6; relative intensity of the molecular ion, 97%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(2-ethylphenylsulfanyl)propyl}phosphinic acid (20): TLC R_f(a) 0.60/0.67, R_f(c) 0.74/0.78; HPLC (column 3, gradient 5) 37.18/41.35.^{1}H NMR (200 MHz, DMSO-d_6) δ 1.01−1.13 (m, 5H, CH$_2$CH_3, CH_2CH$_3$), 1.85−2.10 (m, 2H, PCH_2), 2.59−3.50 (m, 7H, SCH_2, PCH$_2$CH, PhCH_2, indolylCH_2), 3.71−4.09 (m, 1H, PCH), 4.37−4.58 (m, 1H, indolylCH$_2$CH), 4.82−4.96 (br s, 2H, OCH_2Ph), 6.99−7.79 (m, 21H, aryl, NH); ESMS *m/z* calcd for $C_{39}H_{42}N_4O_6PS$ $(M - H)^-$ 725.3, found 725.6; relative intensity of the molecular ion, 98%.

[(*R*),(*R,S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoy l-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(2,4-di-*tert*-butylphenylsulfanyl)propyl}phosphinic acid (21): TLC R_f(a) 0.62/0.67, R_f(c) 0.75/0.78; HPLC (column 3, gradient 6) 26.39/29.82; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.25−1.37 (m, 18H, C(CH)$_3$, CH(CH)$_3$) 1.83−2.07 (m, 2H, PCH_2), 2.64−3.58 (m, 7H, SCH_2, PCH$_2$CH, PhCH_2, indolylCH_2), 3.74−

4.12 (m, 1H, PC*H*), 4.40−4.57 (m, 1H, indolylCH$_2$C*H*), 4.82−5.02 (br s, 2H, OC*H*$_2$Ph), 6.91−7.68 (m, 21H, aryl, N*H*); ESMS *m*/*z* calcd for $C_{45}H_{54}N_4O_6PS$ (M − H)$^-$ 809.4, found 809.7; relative intensity of the molecular ion, 97%.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(2-methoxyphenylsulfanyl)propyl}phosphinic acid (22): TLC R_f(a) 0.61/0.68, R_f(c) 0.74/0.77; HPLC (column 3, gradient 7) 34.72/37.28.^{1}H NMR (200 MHz, DMSO-d_6) δ 1.91−2.07 (m, 2H, PC*H*$_2$), 2.59−3.49 (m, 7H, SC*H*$_2$, PCH$_2$C*H*, PhC*H*$_2$, indolylC*H*$_2$), 3.86−4.01 (m, 4H, OC*H*$_3$, PC*H*), 4.41−4.58 (m, 1H, indolylCH$_2$C*H*), 4.88−5.00 (br s, 2H, OC*H*$_2$Ph), 6.92−7.75 (m, 21H, aryl, N*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ 27.4 (d, $^1J_{PC}$ = 77.5 Hz, P*C*H$_2$), 32.7 (*C*H$_2$Ph), 42.3 (PCH$_2$*C*H), 51.1 (d, $^1J_{PC}$ = 102.8 Hz, P*C*H), 53.9 (O*C*H$_3$), 54.3 (indolylCH$_2$*C*H), 65.0 (O*C*H$_2$Ph), 111.2, 118.2, 120.6, 126.2, 127.4, 128.1, 129.0, 136.1, 137.2, 138.6, (aryl), 150.1 (CH$_3$O*C*), 156.1 (O*C*ONH), 171.9 (CHC*C*ONH), 173.7 (*C*ONH$_2$); ^{31}P NMR (81 MHz, DMSO-d_6) δ 46.15; ESMS *m*/*z* calcd for $C_{38}H_{40}N_4O_7PS$ (M − H)$^-$ 727.2, found 727.6; relative intensity of the molecular ion, 99%.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(2,5-dimethoxyphenylsulfanyl)propyl}phosphinic acid (23): TLC R_f(a) 0.62/0.67, R_f(c) 0.75/0.77; HPLC (column 3, gradient 8) 30.96/33.34; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.89−2.05 (m, 2H, PC*H*$_2$), 2.59−3.39 (m, 7H, SC*H*$_2$, PCH$_2$C*H*, PhC*H*$_2$, indolylC*H*$_2$), 3.44−4.05 (m, 7H, OC*H*$_3$, PC*H*), 4.38−4.56 (m, 1H, indolylCH$_2$C*H*), 4.84−5.02 (br s, 2H, OC*H*$_2$Ph), 6.84−7.73 (m, 20H, aryl, N*H*); ESMS *m*/*z* calcd for $C_{39}H_{42}N_4O_8PS$ (M − H)$^-$ 757.3, found 757.6; relative intensity of the molecular ion, 99%.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(2-trifluoromethoxyphenylsulfanyl)propyl}phosphinic acid (24): TLC R_f(a) 0.60/0.65, R_f(c) 0.74/0.77; HPLC (column 3, gradient 4) 38.94/42.99; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.90−2.08 (m, 2H, PC*H*$_2$), 2.61−3.47 (m, 7H, SC*H*$_2$, PCH$_2$C*H*, PhC*H*$_2$, indolylC*H*$_2$), 3.87−4.00 (m, 1H, PC*H*), 4.40−4.58 (m, 1H, indolylCH$_2$C*H*), 4.87−5.03 (br s, 2H, OC*H*$_2$Ph), 6.90−7.76 (m, 21H, aryl, N*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.9 (d, $^1J_{PC}$ = 79.1 Hz, P*C*H$_2$), 32.7 (*C*H$_2$Ph), 42.1 (PCH$_2$*C*H), 52.8 (d, $^1J_{PC}$ = 99.6 Hz, P*C*H), 54.3 (indolylCH$_2$*C*H), 64.3 (O*C*H$_2$Ph), 110.4, 111.2, 116.3, 118.2, 120.6, 122.5, 126.2, 127.4, 128.1, 129.0, 132.5, 136.1, 137.2, 138.6, (aryl, *C*F$_3$), 155.8 (O*C*ONH), 172.7 (CHC*C*ONH), 173.5 (*C*ONH$_2$); ^{31}P NMR (81 MHz, DMSO-d_6) δ 45.83; ESMS *m*/*z* calcd for $C_{38}H_{37}F_3N_4O_7PS$ (M − H)$^-$ 781.2, found 781.5; relative intensity of the molecular ion, 99%.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{3-(4-bromo-2-trifluoromethoxyphenylsulfanyl)-2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-propyl}-phosphinic acid (25): TLC R_f(a) 0.60/0.65, R_f(c) 0.74/0.77; HPLC (column 1, gradient 1) 46.56/50.45, (column 3, gradient 2) 33.38/42.75; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.87−2.08 (m, 2H, PC*H*$_2$), 2.58−3.48 (m, 7H, SC*H*$_2$, PCH$_2$C*H*, PhC*H*$_2$, indolylC*H*$_2$), 3.86−4.02 (m, 1H, PC*H*), 4.38−4.58 (m, 1H, indolylCH$_2$C*H*), 4.86−5.03 (br s, 2H, OC*H*$_2$Ph), 6.87−7.78 (m, 20H, aryl, N*H*); ESMS *m*/*z* calcd for $C_{38}H_{36}BrF_3N_4O_7PS$ (M − H)$^-$ 859.1, found 859.4; relative intensity of the molecular ion, 97%.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(3-methoxyphenylsulfanyl)propyl}phosphinic acid (26): TLC R_f(a) 0.61/0.68, R_f(c) 0.74/0.77; HPLC (column 2, gradient 1) 26.50/28.53; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.90−2.07 (m, 2H, PC*H*$_2$), 2.57−3.46 (m, 7H, SC*H*$_2$, PCH$_2$C*H*, PhC*H*$_2$, indolylC*H*$_2$), 3.84−4.03 (m, 3H, OC*H*$_3$, PC*H*), 4.41−4.57 (m, 1H, indolylCH$_2$C*H*), 4.82−5.03 (br s, 2H, OC*H*$_2$Ph), 6.92−7.77 (m, 21H, aryl, N*H*); ESMS *m*/*z* calcd for $C_{38}H_{40}N_4O_7PS$ (M − H)$^-$ 727.2, found 727.9; relative intensity of the molecular ion, 98%.

[(*R*),(*R*,*S*),(*S*)]-(1-Benzyloxycarbonylamino-2-phenylethyl)-{2-[1-carbamoyl-2-(1*H*-indol-3-yl)ethylcarbamoyl]-3-(3-trifluoromethoxybiphenyl-4-ylsulfanyl)propyl}-phosphinic Acid (27). To a solution of compound **25** (0.1 mmol, 86 mg) and Pd[P(Ph$_3$)]$_4$ (0.005 mmol, 5.8 mg) in toluene (5 mL) was added a solution of 2 M Na_2CO_3 (0.2 mmol, 0.1 mL) and PhB(OH)$_2$ (0.12 mmol, 14.6 mg) under argon atmosphere. The reaction mixture was refluxed under argon, for 48 h. After cooling to room temperature, 2 N HCl was added (pH 1) and the reaction mixture was extracted with AcOEt (2 × 10 mL), washed with water and brine (5 mL each), dried over Na_2SO_4, and evaporated. Purification by flash column chromatography, using $CHCl_3$–MeOH−AcOH (7:0.3:0.3) as eluent afforded the product (76 mg, 81%) as a white solid: TLC R_f(a) 0.61/0.65, R_f(c) 0.75/0.77; HPLC (column 1, gradient 1) 51.01/53.00.; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.72−2.11 (m, 2H, PC*H*$_2$), 2.59−3.67 (m, 7H, SC*H*$_2$, PCH$_2$C*H*, PhC*H*$_2$, indolylC*H*$_2$), 3.74−4.12 (m, 1H, PC*H*), 4.32−4.49 (m, 1H, indolylCH$_2$C*H*), 4.84−5.01 (br s, 2H, OC*H*$_2$Ph), 6.94−7.81 (m, 26H, aryl, N*H*); ESMS *m*/*z* calcd for $C_{44}H_{41}F_3N_4O_7PS$ (M − H)$^-$ 857.2, found 857.3; relative intensity of the molecular ion, 95%.

General Procedure for Thiol Addition, on Solid Phase. Synthesis was carried out on Rink amide AM resin with a loading capacity 0.63 mmol/g. The resin was swollen using DMF and deprotected with 20% piperidine in DMF. Resin (50 mg) was swollen in 0.5 mL of DMF and a solution of 3 equiv of FmocTrpOH, 3 equiv of DIC, and 3 equiv of HOBt in 0.5 mL of DMF was added. Coupling was repeated if necessary. The resin was swollen using DMF, deprotected with 20% piperidine in DMF, and washed several times with DMF, DCM, and Et$_2$O. A solution of **2** (0.03 mmol, 1 equiv, 12.7 mg) DIPEA, (0.06 mmol, 10 μL), HOBt (0.03 mmol, 4 mg), and EDC·HCl (0.12 mmol, 23 mg) in DCM was added to the resin and allowed to stand at room temperature for 1.5 h. One coupling was sufficient (negative Kaiser test). The resin was washed several times with DCM, DMF, 2-propanol/water (8:2), 2-propanol, DCM, and Et$_2$O. Then freshly distilled THF (1 mL), 2-thionaphthol (for compound **8**), or cyclohexylthiol (for compound **10**) (0.12 mmol) and a 1 M solution of EtONa/EtOH (0.06 mmol, 0.06 mL) were added. The resin was allowed to stand at room temperature (40 and 1 h respectively) and was then filtered and washed several times with 2-propanol/water (8:2), 2-propanol, DCM, and Et$_2$O. Cleavage from the resin was achieved using TFA/TIS/H$_2$O 95:2.5:2.5. The residue was concentrated in vacuo and precipitated with Et$_2$O−PE (40−60) (1:1). Yields were 71% and 96%, respectively.

Inhibitor Potency. Enzyme assays and determination of the K_i values were performed as previously described in detail.[13]

Molecular Modeling. Molecular models of the interaction of the phosphinic inhibitor **18** with the catalytic domains of three different MMPs were achieved using a protocol based on molecular dynamics with the program CHARMM (version 27).[31] The CHARMM force field version 22 was used.[32] The 3D structures of the catalytic domains of MMP-11, MMP-14, and MMP-8 used in this work were taken from the PDB (code 1HV5, 1BQQ, and 1MMB, respectively).

Geometrical and nonbonded parameters for the phosphinic inhibitor were derived from ab initio quantum calculations with the program Gaussian98.[33] These calculations were done at the MP2 level of theory, using a 6-31+G(d, p) basis set.

The starting structure of each complex was defined using the coordinates of the complex of MMP-11 with a phosphinic inhibitor determined by X-ray diffraction (pdb code 1HV5).[24] As the present modeling study was aimed at defining the interaction of the P_1'-side chain of the inhibitor with the different MMPs, the Z−NH part of the phosphinic compound was omitted.

To preserve the structure of the protein during the relaxation of the complexes, harmonic restraints were applied to the atomic position of several sets of atoms. The harmonic constants were set to 30, 10, and 5 kcal mol^{-1} A^{-2} for the cations and the residues chelating the cations, the non-hydrogen backbone atoms, and the non-hydrogen side chain atoms, respectively. No harmonic restraints were applied to the residues located at a distance below 5 Å from the inhibitor.

During the calculations, the nonbonded interactions were modeled using a Lennard-Jones function and a Coulombic

electrostatic term with a nonbonded cutoff of 15 Å. The dielectric constant was set to 4.

The first step of the relaxation protocol consists of an initial 2000 cycle of adopted basis Newton−Raphson energy minimization. Then, 100 000 steps of molecular dynamics using the Verlet algorithm were undertaken. The integration step was set to 0.0005 ps. The temperature was gradually increased by 25 K each 100 steps to reach 450 K. This molecular dynamics was followed by 5000 cycles of energy minimization. The resulting structure was then analyzed and the interaction energy between the atoms of the P_1'-side chain and each residue of the MMP was calculated using the potential energy function of CHARMM.

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Supporting Information Available: Mass spectra and HPLC chromatograms of compounds **4**, **6**, **7**, **10**−**13**, and **18**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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