Phosphinic Pseudo-Tripeptides as Potent Inhibitors of Matrix Metalloproteinases: A Structure−Activity Study†

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Several phosphinic pseudo-tripeptides of general formula R-XaaΨ(PO_2-CH_2)Xaa′-Yaa′-NH_2 were synthesized and evaluated for their in vitro activities to inhibit stromelysin-3, gelatinases A and B, membrane type-1 matrix metalloproteinase, collagenases 1 and 2, and matrilysin. With the exception of collagenase-1 and matrilysin, phosphinic pseudo-tripeptides behave as highly potent inhibitors of matrix metalloproteinases, provided they contain in P_1' position an unusual long aryl−alkyl substituent. Study of structure−activity relationships regarding the influence of the R and Xaa′ substituents in this series may contribute to the design of inhibitors able to block only a few members of the matrix metalloproteinase family.

Introduction

Matrix metalloproteinases (MMPs), also known as matrixins, form a group of structurally related extracellular zinc endoproteinases collectively able to degrade all components of the extracellular matrix.[1] MMPs are believed to be mediators of both normal and pathological tissue-remodeling processes. Their overexpression has been observed and linked with a variety of diseases, including cancer, arthritis, multiple sclerosis, and arteriosclerosis.[2−8] Therefore, there has been a substantial interest in developing MMP synthetic inhibitors for a variety of therapeutic indications.[9,10]

Among the 15 members of the human MMP multigene family,[11] stromelysin-3 (MMP-11), which was first described in fibroblastic cells of invasive breast carcinoma,[12] displays a number of unusual features: mature forms of MMP-11 appear unable to degrade any major extracellular matrix component;[13,14] MMP-11 is secreted in an active form,[15,16] whereas most of the other secreted MMPs must be activated extracellularly; and finally while most MMPs possess a conserved leucine in their S_1' pocket, this residue is replaced by glutamine in MMP-11. These findings suggest that MMP-11 may have a unique role in tissue-remodeling processes, including those associated with tumor progression, and may represent a particular target in the development of MMP inhibitors. In this respect, it is worth noting that recent studies on the role of MMP-11 in cancer development led to the conclusion that MMP-11 may act as a cancer cell survival factor and could be more involved in tumor formation rather than in tumor growth.[17,18]

The development of an inhibitor program directed toward MMP-11 has been hampered by the lack of a simple test for routine assay of MMP-11. Recently, we described the first fluorogenic synthetic substrates that are cleaved efficiently by MMP-11,[19] allowing the development of an inhibitor screening program. Most of the MMP inhibitors developed over the last 10 years possess a zinc-binding function attached to a peptide framework, which binds to the primed regions of the catalytic active site. The vast majority of inhibitors described incorporate hydroxamate or carboxylate as zinc-binding groups.[20] Several studies suggest that the hydroxamate function is the best zinc-chelating group for development of potent inhibitors of MMPs. However, concerns about the unfavorable pharmacokinetics of hydroxamates, poor solubility, and potential for chronic toxicities arising from metabolic activation of the hydroxamate group have stimulated the development of MMPs inhibitors based on other zinc-chelating groups. Pseudo-peptides, containing a phosphinic group as a zinc-chelating function, may represent an interesting inhibitor design alternative. Recent progress in the solid-phase synthesis of phosphinic peptides makes it possible to use either parallel or combinatorial chemistry for rapid development of potent inhibitors of zinc-metalloproteases.[21−23] Furthermore, in contrast to peptidic inhibitors containing a thiol, a hydroxamate, or a carboxylate function, phosphinic peptides, as good mimics of the substrate in the transition state, interact with both the primed and unprimed side of the active site cleft.[24] This property can be exploited to optimize inhibitor selectivity.

In the present report, several phosphinic peptides were synthesized and evaluated as inhibitors of MMP-

† Abbreviations: MMP, matrix metalloproteinases; MMP-1, collagenase-1; MMP-2, gelatinase-A; MMP-7, matrilysin; MMP-8, collagenase-2; MMP-9, gelatinase-B; MMP-11, stromelysin-3; MMP-14, membrane type-1 matrix metalloproteinase.

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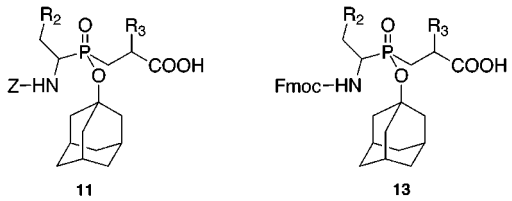
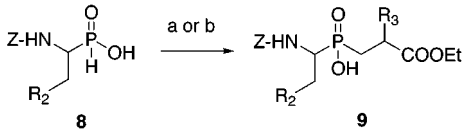
Chart 1. Phosphinic Synthons



Scheme 1[a]



[a] Reagents and conditions: (a) HMDS, 110 °C, $H_2C{=}C(R_3)COOC_2H_5$, EtOH, 70 °C; (b) TMSCl, 0 °C, rt, $H_2C{=}C(R_3)COOC_2H_5$, rt, EtOH.

11. The potencies of these inhibitors were also determined toward other MMPs in order to decipher some specificity rules, which could be used to develop compounds able to block only a subset of the MMP family. These inhibitors were designed according to some important structural elements present in the best substrates of MMP-11: an alanine or phenylalanine residue in the P_1 position and an unusual amino acid, containing a long aryl−alkyl side chain, in the P_1' position.

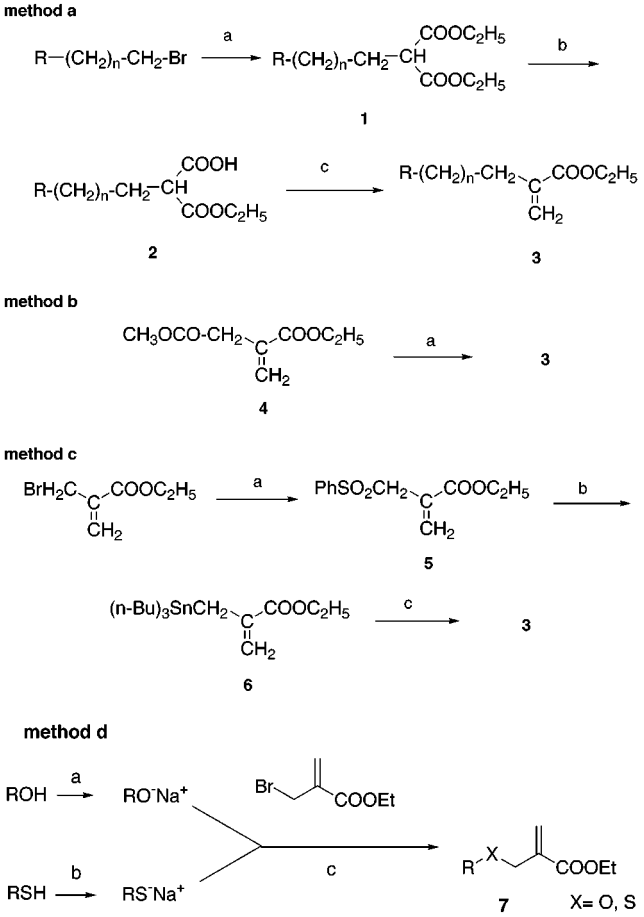
Chemistry

The inhibitors of the present study were prepared by conventional solid-phase peptide synthesis.[21] This strategy relies on the synthesis of two different types of phosphinic synthon (Chart 1). These two types of synthon were obtained from a common phosphinic synthon precursor (compound **9**). Compounds **9** were prepared via a Michael addition of α-substituted-acrylates to monosubstituted phosphinates **8** (Scheme 1). Four different methods were used to obtain the required α-substituted-acrylates (Scheme 2). In method a, appropriate bromides were added to diethyl malonate affording diethyl monosubstituted malonate derivatives type **1**, which gave the monoesters type **2** after saponification.[25] Ethyl α-substituted-acrylates **3a**−**e** were obtained by treatment of ethyl monosubstituted malonates **2** and paraformaldehyde, under Knoevenagel reaction. Compound **3f** was obtained by substitution of the acetoxy group of acrylate **4**, by nucleophilic attack of the appropriate Grignard reagent, in the presence of CuI (method b). To avoid simultaneous attack of the two esters in acrylate **4**, this reaction must take place at low temperature (−78 °C).[26]

Several attempts to prepare compound **3g** by method a or b were unsuccessful. This compound was finally prepared by method c,[27] outlined in Scheme 2: Ethyl α-bromomethylacrylate was converted to acrylate **5** by treatment with benzenesulfinic sodium salt. The sulfinic acrylate **5** was treated with tri-*n*-butyl-tin hydride, in the presence of a catalytic amount of AIBN, to afford acrylate **6**. Compound **3g** was obtained by refluxing acrylate **6** with bromoethylnaphthalene, in the presence of a catalytic amount of AIBN.

Ethyl alkoxy or alkylthio methacrylates type **7** were prepared by reaction of the corresponding alcohol or thiol anions with ethyl α-bromomethyl acrylate (Scheme 2, method d). Conversion of the appropriate alcohols or thiols to sodium salts gave white solids in high yields (85−95%). Substitution of ethyl α-bromomethyl acrylate by these sodium salts was easily achieved in dry ether.

Scheme 2[a]

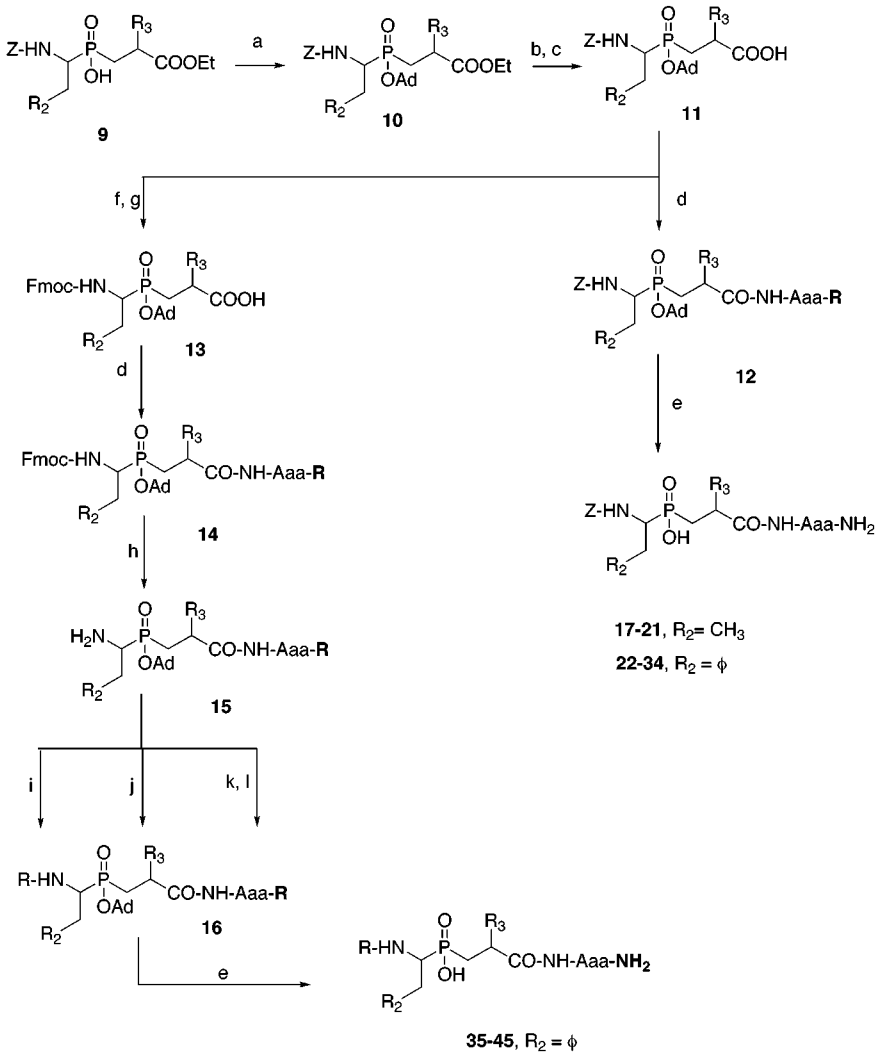


[a] Reagents and conditions. Method a: (a) $CH_2(COOC_2H_5)_2$, EtOH, Na; (b) KOH, EtOH, HCl; (c) paraformaldehyde, piperidine/pyridine. Method b: (a) $R(CH_2)_nMgBr$, abs. Et_2O, THF, cat. CuI, −78 °C. Method c: (a) $PhSO_2Na$, MeOH; (b) *n*-Bu_3SnH, AlBN, benzene; (c) $R(CH_2)_nBr$, AlBN, benzene. Method d: (a) Na, Et_2O; (b) MeO^-Na^+, MeOH; (c) Et_2O, −15 °C, rt, 2 h.

Phosphinic synthons type **9** were prepared according to methods previously described in the literature.[21,28] From compounds **9**, synthons **11** were prepared in two steps[21] (Scheme 3). In the first step, the silver salt of compound **9** was reacted with 1-adamantyl bromide to give the corresponding ester (compound **10**). The second step was the saponification of the ethyl ester which, despite the adamantyl ester, was completely selective. From synthons type **11**, compounds **17**−**34** were prepared by solid-phase synthesis, using a Rink amide resin as a solid support.

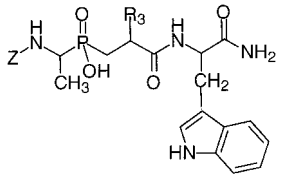
Synthon **13** was obtained from compound **11** by a two-step reaction, decarbobenzyloxylation of compound **11**, with ammonium formate as the hydrogen donor and palladium/carbon as the catalyst in methanol, and then introduction of the Fmoc protecting group. Using the synthon **13**, free amino phosphinic peptide compound **15** was prepared by classical Fmoc solid-phase peptide synthesis. This intermediate, still linked to the resin, was the common precursor to prepare compounds **35**−**45**.

Scheme 3[a]



[a] Reagents and conditions: (a) Ad−Br, Ag_2O, $CHCl_3$; (b) 0.6 NaOH, MeOH; (c) aq HCl; (d) H_2N-Aaa-Rink (**R**), HBTU, DIEA, NMP; (e) TFA/CH_2Cl_2/triisopropylsilane/H_2O 5:4:0.5:0.5; (f) $HCOO^-NH4^+$, 10% Pd/C, MeOH; (g) Fmoc−Cl, 20% Na_2CO_3, dioxane; (h) 20% piperidine/NMP; (i) R−OH, HBTU, DIEA, NMP; (j) R−Cl, DIEA, CH_2Cl_2; (k) $BrCH_2COBr$, DIEA, CH_2Cl_2; (l) aniline derivatives, DIEA, DMSO.

Table 1. Influence of the R_3 Substituent (P_1' Position)

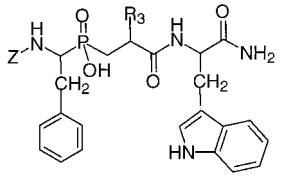


		K_i (nM)						
compd	R_3	MMP-11 mST3	MMP-2 gel-A	MMP-9 gel-B	MMP-14 MT1-MMP	MMP-1 HFC	MMP-7 matrilysin	MMP-8 HNC
17	CH_2-ϕ	350	250	280	2030	24% @ 2 μM	8% @ 2 μM	240
18	CH_2-CH_2-ϕ	51	80	60	270	23% @ 2 μM	3% @ 2 μM	20
19	CH_2-CH_2-CH_2-ϕ	100	31	23	92	30% @ 2 μM	4% @ 2 μM	8
20	CH_2-O-CH_2-ϕ	175	250	44	550	15% @ 2 μM	1% @ 2 μM	19
21	CH_2-S-CH_2-ϕ	36	14	6	26	45% @ 2 μM	2% @ 2 μM	<0.5

Results

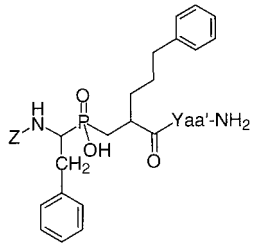
With the exception of MMP-7 and MMP-1, the results reported in Table 1 demonstrate that phosphinic pseudotripeptides can behave as highly potent inhibitors of MMPs, provided they contain in their P_1' position an unusual amino acid side chain. In the case of MMP-8, the lengthening of the side chain in the P_1' position, from a benzyl to a phenylpropyl group (compounds **17**−**19**), led to a 30-fold increase in inhibitory potency. In terms of selectivity, MMP-2, MMP-9, MMP-14, and MMP-8 preferentially bound inhibitor containing a phenylpropyl group, whereas MMP-11 accommodated a phenylethyl group better in this position. Comparison of compounds **19**, **20**, and **21** illustrated the influence

Table 2. Influence of the R_3 Substituent (P_1' Position)



		K_i (nM)						
compd	R_3	MMP-11 mST3	MMP-2 Gel-A	MMP-9 Gel-B	MMP-14 MT1-MMP	MMP-1 HFC	MMP-7 matrilysin	MMP-8 HNC
22	CH_3	2670	0% @ 2 μM	0% @ 2 μM	0% @ 10 μM	20% @ 2 μM	0% @ 2 μM	9% @ 1 μM
23	CH_2-CH-$(CH_3)_2$	22	202	65	192	45% @ 2 μM	210	40
24	CH_2-CH_2-ϕ	8.8	275	110	660	10% @ 2 μM	7% @ 2 μM	45
25	CH_2-CH_2-CH_2-ϕ	5	20	10	105	23% @ 2 μM	8% @ 2 μM	2.5
26	CH_2-CH_2-CH_2-CH_2-ϕ	33	145	70	580	5% @ 2 μM	7% @ 2 μM	4.3
27	CH_2-O-CH_2-ϕ	16	85	55	545	9% @ 2 μM	3% @ 2 μM	20
28	CH_2-S-CH_2-ϕ-OMe	2	6	3	22	13% @ 2 μM	20% @ 2 μM	0.7
29	2-CH_2-naphthyl	74	330	675	1350	0% @ 5 μM	1800	230
30	2-$(CH_2)_2$-naphthyl	12	30	55	125	0% @ 2 μM	2100	34
31	$(CH_2)_6$-CH_3	34	75	30	271	27% @ 2 μM	4% @ 2 μM	6

Table 3. Influence of the Yaa′ Residue (P_2' Position)



		K_i (nM)						
compd	Yaa′	MMP-11 mST3	MMP-2 Gel-A	MMP-9 Gel-B	MMP-14 MT1-MMP	MMP-1 HFC	MMP-7 matrilysin	MMP-8 HNC
32	Ala	20% @ 1 μM	31% @ 2 μM	45% @ 2 μM	2960	9% @ 2 μM	0% @ 2 μM	240
25	L-Trp	5	20	10	105	23% @ 2 μM	8% @2 μM	2.5
33	D-Trp	1% @ 1 μM	6% @ 2 μM	14% @ 2 μM	10% @ 10 μM	0% @ 2 μM	0% @2 μM	2% @ 1 μM
34	Dpa	27	260	245	1282	9% @ 2 μM	4% @ 2 μM	25

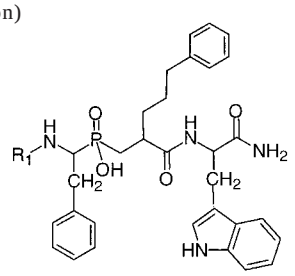
of a heteroatom in the γ position of the P_1' side chain. As compared to a carbon atom (compound **19**), the presence of an oxygen in this position decreased the inhibitory potency (compound **20**), while a sulfur significantly increased the potency of the inhibitor (compound **21**). The magnitude of these substitutions on the inhibitor affinity depended on the MMP.

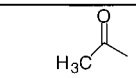
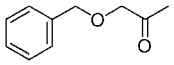
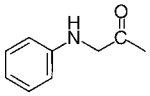
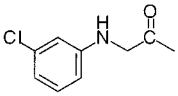
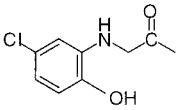
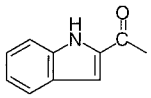
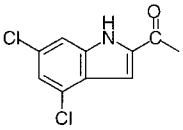
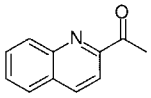
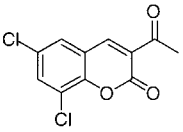
The potency of the phosphinic peptides could also be increased by substituting the P_1 side chain of the inhibitor with a benzyl group (Table 2, compounds **24**, **25**, **27**; as compared to compounds **18**, **19**, **20** in Table 1). In this series of compounds, the substitution of a phenylethyl group in the P_1' position by a phenylpropyl slightly increased the potency of the inhibitor for MMP-11, while more substantial effects on potency were observed for the other MMPs. This result is mainly due to the fact that MMP-11 very well accommodates a phenylethyl group, as compared to other MMPs. Accordingly, compound **24** is one of the most selective MMP-11 inhibitors of this series. Comparison of the potency of compounds **25** and **26** revealed that all MMPs preferred a phenylpropyl group to a butylphenyl one in the P_1' position. However, such a modification did not affect the inhibitor potency to the same extent with respect to the different MMPs. This explains the selectivity of compound **26** for MMP-8. The rather high potencies of compounds **29** and **30**, containing a naphthyl group, demonstrate that the S_1' pocket of some MMPs is large enough to accept such a bulky group. In terms of selectivity, MMP-11 preferred to bind a naphthylethyl rather than a linear heptyl group (compounds **30** and **31**), whereas the reverse was true for MMP-8 and MMP-9. With the exception of MMP-1 and MMP-7, the most potent inhibitor of MMPs in this series was compound **28**. The presence of a sulfur atom in the P_1' side chain of compound **28** accounts in part for its high potency. As compared to the potency of compounds containing either a methyl or an isobutyl side chain in their P_1' position (compounds **22** and **23**), the high potency of compound **28** illustrates the critical role of an unusual residue in the P_1' position in the affinity of phosphinic peptides for MMPs.

Substitution of tryptophan in the P_2' position of the inhibitor by alanine dramatically decreased potency (Table 3). The same effect was observed with modification of tryptophan chirality. However, the rather high potency displayed by compound **34** suggests that other aromatic side chains can be tolerated in this position, depending on the MMP.

As phosphinic peptide chemistry makes it possible to develop inhibitors which interact with the unprimed enzyme subsites, several inhibitors containing a variety of substituents in the P_2 position were synthesized in order to determine the influence of these modifications.

Table 4. Influence of the R_1 Substituent (P_2 Position)



Compounds	R_1	Ki (nM) MMP-11 mST3	MMP-2 Gel-A	MMP-9 Gel-B	MMP-14 MT1-MMP	MMP-1 HFC	MMP-7 Matrilysin	MMP-8 HNC
35		20	26	35	90	43%@2 μM	28%@2 μM	3.5
36		5	8	10	40	43%@2 μM	52%@2 μM	2.5
37		15	17	6	73	49%@2 μM	28%@2 μM	4.5
38		3.8	9	6	45	77%@2 μM	40%@2 μM	4
39		10	30	34	63	72%@2 μM	65%@2 μM	7.5
40		1.5	10	8	41	53	605	1.5
41		0.9	24	7	32	36	117	5
42		4.2	19	13	60	340	370	5
43		5	100	110	217	62%@2 μM	52%@2 μM	17
44	Z-Ala	8	11	10	41	40%@2 μM	33%@2 μM	5.5
45	Z-Leu	6	40	22	53	32%@2 μM	45%@2 μM	7

The presence of an acetyl group on the N-terminal side of the P_1 position of the inhibitor is sufficient to yield a potent inhibitor of MMP-8 (compound **35**, Table 4). Overall, the substitution of the acetyl by different bulky groups had a limited effect on the affinity of these molecules for MMP-8. In contrast, in this series of compounds, for MMP-11 the inhibitory potencies vary from 20 nM (compound **35**) to 0.9 nM (compound **41**). Compound **43**, an example of a P_2 modification influencing inhibitor selectivity, was less potent on MMP-2, MMP-9, and MMP-14 than on MMP-11 and MMP-8. Surprisingly, compounds **40** and **41** turned out to be the only inhibitors in this study able to block MMP-1 activity potently and MMP-7 activity less so.

Discussion

MMPs are increasingly implicated in a wide range of disease states and have therefore become the focus of considerable efforts aimed at developing potent inhibitors.[10,20] However, in the light of MMP involvements in the control of several important physiological functions, it became apparent that selective as well as broad-spectrum MMP inhibitors should be developed.

This goal turns out to be difficult to achieve, as the amino acid content of the MMP binding sites is highly conserved.[11] The resolution of the 3D-structure of some MMP catalytic domains has, nevertheless, provided interesting information, which has been used to classify the MMPs in terms of the size of their S_1' pocket.[29−35]

According to these structural studies, MMP-1 and MMP-7 both possess a "small S_1' pocket". The floor of the S_1' pocket of MMP-1 and MMP-7 is characterized by the presence, respectively, of the guanidinium group of Arg-214 and the phenol group of Tyr-214. In contrast, with the exception of MMP-11, most of the other MMPs are characterized by the presence of a leucine, at the position equivalent to Arg-214 in MMP-1 or Tyr-214 in MMP-7.[11] In these MMPs, the S_1' pocket corresponds to a large cavity, forming a channel that extends through the whole body of the enzyme catalytic domain. Based on the size of their S_1' pocket, MMP-1 and MMP-7 should prefer to accommodate an isobutyl side chain in the P_1' position of inhibitor, rather than a long aryl–alkyl side chain. The higher potency of compound **23** for MMP-1 and MMP-7, as compared to inhibitors containing a longer side chain (compounds **24**–**28**), is consistent with this proposal. However, the unexpectedly high potency of compound **41** toward MMP-1 and MMP-7 demonstrates that in some cases, inhibitors containing a long side chain in their P_1' position are able to interact potently with MMP-1 and MMP-7. The recent resolution of the crystal structure of a complex between MMP-7 and a carboxylate peptide inhibitor, containing in its P_1' position a biphenylpropyl side chain, offers an explanation of how the binding of such inhibitors to MMP-7 is possible.[36] The analysis of this complex reveals the occurrence of a dramatic conformational shift in the S_1' pocket of the enzyme, creating a large S_1' tunnel capable of accommodating the biphenylpropyl side chain of the inhibitor. The same conformational transition has also been observed in MMP-1, explaining the ability of these MMPs to interact with inhibitors bearing a long side chain in the P_1' position.[36] The existence of such conformational flexibility in the S_1' pocket of MMP-7 and MMP-1 makes it more difficult to predict the behavior of inhibitors toward these two MMPs and to develop selective inhibitors of MMPs.

It was important to determine the behavior of MMP-11, which is characterized by the presence of a glutamine at the floor of its S_1' pocket and is thus predicted to possess a "small S_1' pocket" like MMP-1 and MMP-7. Our present data demonstrate that MMP-11 is able to tightly bind inhibitors containing different types of unusual long side chains. MMP-11 thus behaves more like the MMPs harboring a leucine in their S_1' pocket than MMP-1 and MMP-7. This apparent discrepancy between a proposed model of MMP-11 and our observations could be resolved by suggesting that a conformational transition could also occur in MMP-11, allowing the size of the S_1' pocket of this enzyme to change from a small to a large tunnel cavity. However, the energetic cost of this conformational transition should be lower in MMP-11 than in MMP-1 and MMP-7. Consequently, the interaction of inhibitors containing a long side chain in their P_1' position is a more favorable process in MMP-11 than in MMP-1 and MMP-7. This property favors the development of potent MMP-11 inhibitors that do not block MMP-1 and MMP-7 but makes it much more difficult to develop potent MMP-11 inhibitors that do not interact with the MMPs containing a leucine in their S_1' pocket.

Conclusion

This work represents the first study aiming at exploring the potential of phosphinic peptide derivatives as inhibitors of MMP-11. This study has uncovered some preferences of MMP-11 for particular substitutions in the P_1' and P_2 positions of the inhibitor, which should be useful for the development of more selective MMP-11 inhibitors. The positive contribution of a sulfur atom in the γ position of the P_1' side chain of the inhibitor is an interesting observation which should be more systematically exploited in the future. The structure–activity relationships established from this work, together with the related contributions of other groups to MMP inhibitors,[37–39] should contribute to the development of inhibitors with specificity for small sets of MMPs. However, as discussed above, the presence of some flexibility at the level of the S_1' pocket of MMPs, in addition to the binding sites highly conserved between the different MMP members, will make this goal particularly difficult to accomplish.

Experimental Section

General Procedures. Commercially available reagents and starting materials were from Aldrich, Sigma, Novabiochem, and Lancaster. Solid-phase synthesis of the phosphinic peptides was performed in an Advanced Chemtech 357 multiple synthesizer on a Rink amide resin, using Fmoc strategy.

HPLC analyses and preparative purifications were carried out on a VYDAC C18 analytical column and a VYDAC C18 preparative column, utilizing the following methods. Solvent A = 0.1% TFA and 10% CH_3CN in water. Solvent B = 0.1% TFA and 90% CH_3CN in water. The following gradients were used. Gradient 1: t = 0 min (0% B), t = 10 min (30% B), t = 45 min (45% B). Gradient 2: t = 0 min (0% B), t = 10 min (30% B), t = 45 min (50% B). Gradient 3: t = 0 min (0% B), t = 10 min (35% B), t = 45 min (50% B). Gradient 4: t = 0 min (0% B), t = 10 min (35% B), t = 45 min (55% B). Gradient 5: t = 0 min (0% B), t = 10 min (35% B), t = 45 min (60% B). Gradient 6: t = 0 min (0% B), t = 10 min (40% B), t = 40 min (60% B). Gradient 7: t = 0 min (0% B), t = 10 min (45% B), t = 40 min (65% B). Gradient 8: t = 0 min (0% B), t = 10 min (45% B), t = 45 min (70% B). These purifications were performed on Gilson gradient system equipped with a variable wavelength detector. Compounds were detected at 280 and 230 nm.

^1H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker DPX 250 spectrometer. All NMR experiments were carried out at 298 K in $CDCl_3$. Chemical shifts of proton resonances and ^{13}C resonances were measured relative to the residual chloroform resonance at 7.26 ppm in proton spectra and 77.36 ppm in ^{13}C spectra. Chemical shift ^{31}P resonances were measured relative to the H_3PO_4 resonance at 0 ppm. ^1H and ^{13}C resonance assignments were made using a combination of homo- and heteronuclear experiments including COSY, TOCSY, HMQC, and HMBC experiments. For compounds of type 9, two ^{13}C resonances corresponding to two diastereoisomers were observed for the carbon atoms bound to the phosphorus atom (indicated by * in the NMR characterization).

Phosphinic peptides purities were established by analytical HPLC and mass spectroscopy. ES-MS spectra were recorded on a Micromass Platform II instrument (Micromass, Altrincham, U.K.).

General Procedures To Prepare α-Alkylacrylates. Three different methods were utilized depending on the nature of the α-substituent of the acrylate (Scheme 2; Table 5).

Method a (Scheme 2). Acrylates **3 a**–**e** were synthesized according to a procedure described previously.[25] Diethyl malonate (10 mmol) was added over a period of 10 min to a solution of sodium ethoxide (10 mmol of sodium in 11 mL of absolute ethanol). This solution was stirred at 50 °C for 1 h. Bromide (10 mmol) was added dropwise to this reaction

Table 5

compd **3**	R	*n*	method	bp (mmHg), °C	yield, %
a	phenyl	0	a	64−65 (0.01)[40]	80
b	phenyl	1	a	90−95 (0.7)[25]	76
c	phenyl	2	a	112−115 (0.6)[40]	87
d	phenyl	3	a	120−125 (0.005)[40]	78
e	naphthyl	0	a	oil	25
f	methyl	5	b	101−105 (8)	40
g	naphthyl	1	c	oil	13

mixture, which was stirred for 6 h, at 50 °C. Ethanol was removed under vacuum and diethyl ether was added. This solution was washed with water and brine, dried over Na_2SO_4, and concentrated to give an oily residue, which after distillation gave compounds type **1**, in yields ranging from 40 to 65%. A solution of KOH (10 mmol) in ethanol (8 mL) was added to a solution of the diester **1** (10 mmol) in ethanol (8 mL). This mixture was stirred for 16 h. After evaporation of the organic solvent, the residue was treated with water and extracted with diethyl ether. The aqueous phase was acidified with 6 N HCl and extracted twice with diethyl ether. The organic phases were dried over Na_2SO_4 and evaporated to give compounds type **2**, in yields ranging from 65 to 90%. Paraformaldehyde was added (6 mmol) to a solution of monoester **2** in pyridine (0.8 mL) and piperidine (0.05 mL). This mixture was stirred and heated at 50−55 °C for 3 h. After addition of diethyl ether, the organic phase was washed with water, 3 N HCl, dried over Na_2SO_4, and concentrated to give compounds type **3**, in yields ranging from 25 to 87%.

Method b, (Scheme 2). The acrylate **3f** was synthesized according to a procedure described previously.[26] Under argon atmosphere, a solution of $CH_3(CH_2)_5Br$ (1.65 g, 10 mmol) in absolute diethyl ether (15 mL) was added dropwise to a flask containing Mg (0.22 g, 11 mmol) and I_2 (catalyst), over a period of 90 min. This reaction mixture was refluxed for 1 h. After CuI (0.18 mmol) was added, the temperature of the mixture was lowered to −78 °C. A solution of compound **4**[26] (1.15 g, 6.7 mmol) in Et_2O:THF (10 mL) was then slowly added to this reaction mixture which was stirred for 30 min at room temperature. After treatment of the mixture with 0.5 N HCl, 5% $NaHCO_3$, and water, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum, and the resulting residue was purified by column chromatography, using petroleum ether (40−60 °C)/ether (13:1) as eluent. The pure acrylate, compound **3f**, was obtained in a yield of 40%.

Method c (Scheme 2). The acrylate **3g** was synthesized according to a procedure described previously.[27] A mixture of bromomethylacrylate[40] (1.9 g, 10 mmol) and benzenesulfinic sodium salt (3.3 g, 20 mmol) in methanol (40 mL) was refluxed for 12 h. The solvent was removed, and diethyl ether was added. The solution was washed with water and brine, dried over Na_2SO_4, and concentrated to give 2 g of compound **5** (80%) as an oil. Tri-*n*-butyltin hydride (4.7 g, 16.4 mmol) and 2.2′-azobisisobutyronitrile (AIBN) (0.15 g, 0.96 mmol) were added to a mixture of sulfinic acrylate **5** (2 g, 8 mmol) in dry benzene (40 mL). This mixture was refluxed for 1.5 h. Water was added, and the extracted organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography, using petroleum ether (40−60 °C)/ether (9:1) as eluent, to give 2.9 g of compound **6** (90%). The alkylstannane **6** (1.45 g, 3.6 mmol) and 2-bromoethylnaphthalene (0.39 g, 1.8 mmol) were dissolved in dry benzene (10 mL). After addition of AIBN (0.064 g, 0.39 mmol), the reaction mixture was refluxed for 2 h. After addition of water, the organic phase was separated, washed with water, dried over Na_2SO_4, and concentrated to dryness. Compound **3g** (0.06 g, 13%) was obtained after purification of the residue by column chromatography, using petroleum ether (40−60 °C)/ether (9:1) as eluent.

Synthesis of Ethyl α-Alkoxy or Alkylthio Methacrylates. Synthesis of ethyl α-alkoxy or alkylthio methacrylates was achieved by the reaction of the corresponding alcohol or thiol anions with ethyl α-bromomethyl acrylate (Scheme 2, method d; Table 6).

Table 6

compd **7**	R−X	yield, %
a	4-MeO-C_6H_4-CH_2-S	80
b	C_6H_4-CH_2-S	88
c	C_6H_4-CH_2-O	85

Table 7

compd **9**	R_2	R_3	method	yield, %	R_f [a]
a	H	CH_2-Ph	a	80	0.41
b	H	CH_2-CH_2-Ph	a	95	0.47
c	H	CH_2-CH_2-CH_2-Ph	a	83	0.57
d	H	CH_2-O-CH_2-Ph	a	61	0.69
e	H	CH_2-S-CH_2-Ph	a	60	0.48
f	Ph	CH_2-CH_2-Ph	a	85	0.77
g	Ph	CH_2-CH_2-CH_2-Ph	a	79	0.80
h	Ph	CH_2-CH_2-CH_2CH_2-Ph	a	75	0.82
i	Ph	CH_2-$(CH_2)_5$-CH_3	a	81	0.68
j	Ph	CH_2-O-CH_2-Ph	a	64	0.61
k	Ph	CH_2-S-CH_2-Ph-OMe	a	40	0.63
l	Ph	2-CH_2-naphthyl	b	60	0.66
m	Ph	2-$(CH_2)_2$-naphthyl	b	30	0.65

[a] $CHCl_3$/MeOH/AcOH (7:0.5:0.5).

Synthesis of Ethyl *S*-Alkyl Methacrylate, 7a and 7b. A solution of the appropriate thiol (10 mmol) in MeOH (15 mL) was added dropwise to a stirred ice-cold solution of sodium (9 mmol) in MeOH (20 mL) over 30 min. After the thiol was added, the mixture was concentrated to dryness and Et_2O was added. The precipitated salt was cooled in an ice−water bath. The product was filtered, washed with cold Et_2O, and dried over P_2O_5 to give the sodium salt in 85−95% yield. To a mixture of the sodium salt (10 mmol) suspended in dry Et_2O (40 mL) and cooled in an ice−water bath, a solution of α-bromomethyl acrylate (9 mmol) in Et_2O (20 mL) was added dropwise over 45 min. This solution was stirred for 30 min at 0 °C and 1−2 h at room temperature. This reaction mixture was diluted with water (20 mL), and the organic layer was washed with water, dried over Na_2SO_4, and evaporated. Compounds **7a** and **7b** were purified by column chromatography, using petroleum ether (40−60 °C)/Et_2O (8:2) as eluent.

Synthesis of Ethyl *O*-Alkyl Methacrylate, 7c. Small pieces of sodium (10 mmol) were added to dry Et_2O (20 mL) in a well-dried flask. A solution of benzyl alcohol (10 mmol) in dry Et_2O (10 mL) was added to this reaction mixture over 2 h, under gentle reflux. The mixture was refluxed for an additional 6 h. The white precipitate was cooled in an ice−water bath, filtered, washed with cold dry Et_2O, and dried over P_2O_5 to give the sodium salt in 85−95% yield. The reaction of this salt with α-bromomethyl acrylate and the purification of **7c** were similar to those described above.

General Procedure for the Preparation of Phosphinic Synthons Type 9 (Scheme 1). These phosphinic synthons were prepared according to two procedures described previously: method a[21] or method b[28] (Scheme 1, Table 7). Monosubstituted phosphinate derivatives were synthesized according to a procedure described previously.[41]

Synthesis of Phosphinic Synthons 9a−9k, Method a. A suspension of the *N*-benzyloxycarbonyl phosphinic acid **8** (1 mmol) and hexamethyldisilazane (5 mmol) was heated at 110 °C for 1 h, under nitrogen. The appropriate acrylate (1.3 mmol) was then added dropwise over 15 min. This reaction mixture was stirred for an additional 3 h at 110 °C, allowed to cool to 70 °C, and ethanol (3 mL) was added dropwise. After cooling to room temperature, the reaction mixture was concentrated. The residue was purified by column chromatography, using chloroform/methanol/acetic acid (7:0.5:0.5) as eluent, to give compounds **9** (yields reported in Table 7).

Synthesis of Phosphinic Synthons 9l, 9m, Method b. Diisopropylamine (2.1 mmol) and trimethylsilyl chloride (2.1 mmol) were added to an ice-cold solution of phosphinic acid **8** (1 mmol) in chloroform (2 mL), and the mixture was stirred at room temperature for 3 h. After cooling to 0 °C, the appropriate ethyl acrylate (1.4 mmol) was added, and the

reaction mixture was stirred at room temperature for 16 h. After EtOH was added dropwise, the solvents were removed under vacuum. Compounds **9l** and **9m** were obtained after a chromatography step, as described above.

NMR Characterization of Type 9 Phosphinic Synthons. 9a. ^{1}H NMR: δ 7.04−7.30 ($C_6H_5CH_2O$, $C_6H_5CH_2C$), 5.32 (N*H*), 5.12 ($C_6H_5CH_2O$), 4.11 ($COOCH_2CH_3$), 4.02 (CH_3CH), 2.88 ($C_6H_5CH_2CH$), 2.27/1.84 (PO_2CH_2), 1.91/1.79 ($C_6H_5CH_2CH$), 1.24 (CH_3CH), 1.23 ($COOCH_2CH_3$). ^{13}C NMR: 175.3 ($COOCH_2CH_3$), 156.1 ($C_6H_5CH_2OCO$), 126.2−140.6 ($C_6H_5CH_2O$, $C_6H_5CH_2C$), 45.8/46.2* (CH_3CH), 67.5 ($C_6H_5CH_2O$), 61.4 ($COOCH_2CH_3$), 40.05 ($C_6H_5CH_2CH$), 35.7 ($C_6H_5CH_2CH$)), 29.7/28.9* (PO_2CH_2), 14.5 (CH_3CH), 14.1 ($COOCH_2CH_3$). ^{31}P NMR: 54.1/54.32* (PO_2CH_2).

9b. ^{1}H NMR: δ 7.05−7.33 ($C_6H_5CH_2O$, $C_6H_5CH_2CH_2C$), 5.36 (N*H*), 5.12 ($C_6H_5CH_2O$), 4.10 ($COOCH_2CH_3$), 4.05 (CH_3CH), 2.85 ($C_6H_5CH_2CH_2CH$), 2.55 ($C_6H_5CH_2CH_2CH$), 2.25/1.84 (PO_2CH_2), 1.95/1.83 ($C_6H_5CH_2CH_2CH$), 1.28 (CH_3CH), 1.26 ($COOCH_2CH_3$). ^{13}C NMR: 174.9 ($COOCH_2CH_3$), 156.2 ($C_6H_5CH_2OCO$), 126.4−141.4 ($C_6H_5CH_2O$, $C_6H_5CH_2CH_2C$), 45.3/46.05* (CH_3CH), 67.6 ($C_6H_5CH_2O$), 61.4 ($COOCH_2CH_3$), 39.2 ($C_6H_5CH_2CH_2CH$), 35.9 ($C_6H_5CH_2CH_2CH$), 33.4 ($C_6H_5CH_2CH_2CH$), 28.8/28.5* (PO_2CH_2)), 14.6 (CH_3CH), 14.5 ($COOCH_2CH_3$). ^{31}P NMR: 54.45/54.23* (PO_2CH_2).

9c. ^{1}H NMR: δ 7.04−7.29 ($C_6H_5CH_2O$, $C_6H_5CH_2CH_2CH_2C$), 5.29 (N*H*), 5.1 ($C_6H_5CH_2O$), 4.10 ($COOCH_2CH_3$), 4.03 (CH_3CH), 2.92 ($C_6H_5CH_2CH_2CH_2CH$), 2.56 ($C_6H_5CH_2CH_2CH_2CH$), 2.27/1.84 (PO_2CH_2), 1.95/1.81 ($C_6H_5CH_2CH_2CH_2CH$), 1.52 ($C_6H_5CH_2CH_2CH_2CH$), 1.25 (CH_3CH), 1.20 ($COOCH_2CH_3$). ^{13}C NMR: 176 ($COOCH_2CH_3$), 156.4 ($C_6H_5CH_2OCO$), 126.6−141.3 ($C_6H_5CH_2O$, $C_6H_5CH_2CH_2CH_2C$), 46.3/45.1* (CH_3CH), 67.4 ($C_6H_5CH_2O$), 61.7 ($COOCH_2CH_3$), 39.2 ($C_6H_5CH_2CH_2CH_2CH$), 35.7 ($C_6H_5CH_2CH_2CH_2CH$), 32.9 ($C_6H_5CH_2CH_2CH_2CH$), 28.3 ($C_6H_5CH_2CH_2CH_2CH$), 29.2/28.3* (PO_2CH_2), 14.4 (CH_3CH), 14.1 ($COOCH_2CH_3$). ^{31}P NMR: 53.2/52.17* (PO_2CH_2).

9d. ^{1}H NMR: δ 7.26−7.32 ($C_6H_5CH_2OCO$, $C_6H_5CH_2OCH_2CH$), 5.41 (N*H*), 5.09 ($C_6H_5CH_2OCO$), 4.12 ($COOCH_2CH_3$), 4.48 ($C_6H_5CH_2OCH_2CH$), 4.05 (CH_3CH), 3.65 ($C_6H_5CH_2OCH_2CH$), 3.09 ($C_6H_5CH_2OCH_2CH$), 2.25/2.02 (PO_2CH_2), 1.35 (CH_3CH), 1.20 ($COOCH_2CH_3$). ^{13}C NMR: 173.2 ($COOCH_2CH_3$), 156.3 ($C_6H_5CH_2OCO$), 138.2−127.9 ($C_6H_5CH_2OCO$, $C_6H_5CH_2OCH_2CH$), 77.4 ($C_6H_5CH_2OCH_2CH$), 73.5 $C_6H_5CH_2OCH_2CH$), 67.64 ($C_6H_5CH_2OCO$), 61.77 ($COOCH_2CH_3$), 46.8/45.2 (CH_3CH), 40.13 ($C_6H_5CH_2OCH_2CH$), 25.8/24.2* (PO_2CH_2), 14.4 (CH_3CH), 14.1 ($COOCH_2CH_3$)). ^{31}P NMR: 54.3 (PO_2CH_2).

9e. ^{1}H NMR: δ 7.26−7.33 ($C_6H_5CH_2O$, $C_6H_5CH_2SCH_2CH$), 5.41 (N*H*), 5.12 ($C_6H_5CH_2O$), 4.15 ($COOCH_2CH_3$), 4.08 (CH_3CH), 3.65 ($C_6H_5CH_2SCH_2CH$), 2.75/2.50 ($C_6H_5CH_2SCH_2CH$), 3.0 ($C_6H_5CH_2SCH_2CH$), 2.25/2.02 (PO_2CH_2), 1.35 (CH_3CH), 1.22 ($COOCH_2CH_3$). ^{13}C NMR: 173.5 ($COOCH_2CH_3$), 156.2 ($C_6H_5CH_2OCO$), 138.1−127.4 ($C_6H_5CH_2O$, $C_6H_5CH_2SCH_2CH$), 67.8 ($C_6H_5CH_2OCO$), 61.7 ($COOCH_2CH_3$), 46.7/44.9* (CH_3CH), 39.2 ($C_6H_5CH_2SCH_2CH$), 36 ($C_6H_5CH_2SCH_2CH$), 34.56 ($C_6H_5CH_2OCH_2CH$), 28.2/26.8* (PO_2CH_2), 14.4 (CH_3CH), 14.1 ($COOCH_2CH_3$). ^{31}P NMR: 54.41/54.23* (PO_2CH_2).

9f. ^{1}H NMR: δ 7.0−7.3 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_6H_5CH_2CH_2CH$), 5.61 (N*H*), 5.0 ($C_6H_5CH_2O$), 4.15 ($COOCH_2CH_3$), 4.02 ($C_6H_5CH_2CHP$), 3.17/2.83 ($C_6H_5CH_2CHP$), 2.92 ($C_6H_5CH_2CH_2CH$), 2.55 ($C_6H_5CH_2CH_2CH$), 2.22/1.84 (PO_2CH_2), 1.97/1.83 ($C_6H_5CH_2CH_2CH$), 1.23 ($COOCH_2CH_3$). ^{13}C NMR: 177.6 ($COOCH_2CH_3$), 158.0 ($C_6H_5CH_2OCO$), 141−127.1 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_6H_5CH_2CH_2CH$), 53.5/52.1* ($C_6H_5CH_2CHP$), 68.5 ($C_6H_5CH_2OCO$), 63.05 ($COOCH_2CH_3$), 39.8 ($C_6H_5CH_2CH_2CH$), 35.5 ($C_6H_5CH_2CH_2CH$), 33.1 ($C_6H_5CH_2CH_2CH$), 28.9/27.7* (PO_2CH_2),14.1 ($COOCH_2CH_3$). ^{31}P NMR: 51.34 (PO_2CH_2).

9g. ^{1}H NMR: δ 7.14−7.26 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_6H_5CH_2CH_2CH_2CH$), 4.98 (N*H*), 4.94 ($C_6H_5CH_2O$), 4.13 ($COOCH_2CH_3$), 4.20 ($C_6H_5CH_2CHP$), 3.23/2.83 ($C_6H_5CH_2CHP$), 2.87 ($C_6H_5CH_2CH_2CH_2CH$), 1.51 ($C_6H_5CH_2CH_2CH_2CH$), 2.52 ($C_6H_5CH_2CH_2CH_2CH$), 2.21/1.75 (PO_2CH_2), 1.53/1.45 ($C_6H_5CH_2CH_2CH_2CH$), 1.21 ($COOCH_2CH_3$). ^{13}C NMR: 177.2 ($COOCH_2CH_3$), 157.7 ($C_6H_5CH_2OCO$), 142.3−126.6 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_6H_5CH_2CH_2CH_2CH$), 68.3 ($C_6H_5CH_2OCO$), 62.3 ($COOCH_2CH_3$), 53.3/51.75* ($C_6H_5CH_2CHP$), 39.7 ($C_6H_5CH_2CH_2CH_2CH$), 35.7 ($C_6H_5CH_2CH_2CH_2CH$), 33.6 ($C_6H_5CH_2CH_2CH_2CH$), 28.4 ($C_6H_5CH_2CH_2CH_2CH$, 29.1/28.0* (PO_2CH_2), 13.9 ($COOCH_2CH_3$). ^{31}P NMR: 53.1/52.2* (PO_2CH_2).

9h. ^{1}H NMR: δ 7.0−7.26 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_6H_5CH_2CH_2CH_2CH_2CH$), 5.40 (NH), 4.99 ($C_6H_5CH_2O$), 4.10 ($COOCH_2CH_3$), 4.25 ($C_6H_5CH_2CHP$), 3.26/2.82 ($C_6H_5CH_2CHP$), 2.80 ($C_6H_5CH_2CH_2CH_2CH_2CH$), 2.52 ($C_6H_5CH_2CH_2CH_2CH_2CH$), 2.23/1.75 (PO_2CH_2), 1.62/1.53 ($C_6H_5CH_2CH_2CH_2CH_2CH$), 1.52 ($C_6H_5CH_2CH_2CH_2CH_2CH$), 1.25 ($C_6H_5CH_2CH_2CH_2CH_2CH$), 1.20 ($COOCH_2CH_3$). ^{13}C NMR: 175.3 ($COOCH_2CH_3$), 156.5 ($C_6H_5CH_2OCO$), 142.6−126.4 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_6H_5CH_2CH_2CH_2CH_2CH$), 67.45 ($C_6H_5CH_2OCO$), 61.4 ($COOCH_2CH_3$), 52.2/50.9* ($C_6H_5CH_2CHP$), 39.7 ($C_6H_5CH_2CH_2CH_2CH_2CH$), 35.8 ($C_6H_5CH_2CH_2CH_2CH_2CH$), 34.0 ($C_6H_5CH_2CH_2CH_2CH_2CH$),31.2($C_6H_5CH_2CH_2CH_2CH_2CH$),26.4($C_6H_5CH_2CH_2CH_2CH_2CH$), 29.3/28.43* (PO_2CH_2), 14.2 ($COOCH_2CH_3$). ^{31}P NMR: 53.1/52.3* (PO_2CH_2).

9i. ^{1}H NMR: δ 7.16−7.27 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$), 5.46 (N*H*), 4.98 ($C_6H_5CH_2O$), 4.20 ($COOCH_2CH_3$), 4.26 ($C_6H_5CH_2CHP$), 3.28/2.85 ($C_6H_5CH_2CHP$), 2.81 ($CH_3(CH_2)_5CH_2CH$), 2.23/1.75 (PO_2CH_2), 1.63/1.50 ($CH_3CH_2CH_2CH_2CH_2CH_2CH$), 1.20 ($CH_3(CH_2)_5CH_2CH$), 1.23 ($COOCH_2CH_3$), 0.83 ($CH_3(CH_2)_5CH_2CH$). ^{13}C NMR: 175.37 ($COOCH_2CH_3$), 156.4 ($C_6H_5CH_2OCO$), 137.6−127.5 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$), 67.23 ($C_6H_5CH_2OCO$), 61.2 ($COOCH_2CH_3$), 52.8/51.25* ($C_6H_5CH_2CHP$), 39.31 ($CH_3(CH_2)_5CH_2CH$), 31.97/29.3/26.65/22.81 ($CH_3(CH_2)_5CH_2CH$), 14.7 ($COOCH_2CH_3$) 14.5 ($CH_3(CH_2)_5CH_2CH$), 29.73/28.45* (PO_2CH_2). ^{31}P NMR: 54.4/53.9* (PO_2CH_2).

9j. ^{1}H NMR: δ 7.36−7.16 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_6H_5CH_2OCH_2CH$), 5.48 (N*H*), 4.97 ($C_6H_5CH_2O$), 4.15 ($COOCH_2CH_3$), 4.45 ($C_6H_5CH_2OCH_2CH$), 4.30 ($C_6H_5CH_2CHP$), 3.65 ($C_6H_5CH_2OCH_2CH$), 3.28/2.85 ($C_6H_5CH_2CHP$), 3.12 ($C_6H_5CH_2OCH_2CH$), 2.27/2.0 (PO_2CH_2), 1.22 ($COOCH_2CH_3$). ^{13}C NMR: 173.2 ($COOCH_2CH_3$), 156.4 ($C_6H_5CH_2OCO$), 138.1−127.2 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_6H_5CH_2OCH_2CH$), 77.42 ($C_6H_5CH_2OCH_2CH$), 73.44 ($C_6H_5CH_2OCH_2CH$), 67.47 ($C_6H_5CH_2OCO$), 61.6 ($COOCH_2CH_3$), 52.65/50.9* ($C_6H_5CH_2CHP$), 39.93 ($C_6H_5CH_2OCH_2CH$), 26.4/24.9* (PO_2CH_2), 13.94 ($COOCH_2CH_3$). ^{31}P NMR: 53.7/53.59 (PO_2CH_2).

9k. ^{1}H NMR: δ 7.36−7.18 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $CH_3OC_6H_5CH_2SCH_2CH$), 5.41 (N*H*), 4.98 ($C_6H_5CH_2O$), 4.15 ($COOCH_2CH_3$), 4.30 ($C_6H_5CH_2CHP$), 3.65 ($CH_3OC_6H_5CH_2SCH_2CH$), 2.75/2.50 ($CH_3OC_6H_5CH_2SCH_2CH$), 3.0 ($CH_3OC_6H_5CH_2SCH_2CH$), 2.25/2.02 (PO_2CH_2), 1.35 ($CH_3OC_6H_5CH_2SCH_2CH$), 1.22 ($COOCH_2CH_3$). ^{13}C NMR: 173.6 ($COOCH_2CH_3$), 159 ($C_6H_5CH_2OCO$), 130.4−127.2 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $CH_3OC_6H_5CH_2SCH_2CH$), 67.8 ($C_6H_5CH_2OCO$), 61.7 ($COOCH_2CH_3$), 52.1/50.8* ($C_6H_5CH_2CHP$), 39.6 ($CH_3OC_6H_5CH_2SCH_2CH$), 35.6 ($CH_3OC_6H_5CH_2SCH_2CH$), 35.0 ($CH_3OC_6H_5CH_2SCH_2CH$), 29.1/27.9* (PO_2CH_2), 14.5 ($COOCH_2CH_3$). ^{31}P NMR: 54.07/53.76* (PO_2CH_2).

9l. ^{1}H NMR: δ 7.0−7.80 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_{10}H_7$), 5.28 (N*H*), 4.88 ($C_6H_5CH_2O$), 4.02 ($COOCH_2CH_3$), 4.12 ($C_6H_5CH_2CHP$), 3.20/2.74 ($C_6H_5CH_2CHP$), 3.18 ($C_{10}H_7CH_2CH$), 3.06/2.99 ($C_{10}H_7CH_2CH$), 2.18/1.85 (PO_2CH_2), 1.03 ($COOCH_2CH_3$). ^{13}C NMR: 177.6 (**C**$OOCH_2CH_3$), 158.0 ($C_6H_5CH_2OCO$), 132.8−126.1 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_{10}H_7$), 67.8 ($C_6H_5CH_2OCO$), 62.1 ($COOCH_2CH_3$), 53.5/51.63* ($C_6H_5CH_2CHP$), 41.6 ($C_{10}H_7CH_2CH$), 40.34 ($C_{10}H_7CH_2CH$), 34.45 ($C_6H_5CH_2CHP$), 29.15/28.22* (PO_2CH_2),14.13 ($COOCH_2CH_3$). ^{31}P NMR: 54.40/53.88* (P (PO_2-CH_2)).

9m. ^{1}H NMR: δ 7.0−7.80 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_{10}H_7$), 5.60 (N*H*), 5.01 ($C_6H_5CH_2O$), 4.02 ($COOCH_2CH_3$), 4.32 ($C_6H_5CH_2CHP$), 3.27/2.92 ($C_6H_5CH_2CHP$), 2.98 ($C_{10}H_7CH_2CH_2CH$), 2.85/2.73 ($C_{10}H_7CH_2CH_2CH$), 2.65 ($C_{10}H_7CH_2CH_2CH$), 2.18/2.05 (PO_2CH_2), 1.28 ($COOCH_2CH_3$). ^{13}C NMR: 177.6 ($COOCH_2CH_3$), 158.0 ($C_6H_5CH_2OCO$), 132.8−126.1 ($C_6H_5CH_2O$, $C_6H_5CH_2CHP$, $C_{10}H_7$), 68.4 ($COOCH_2CH_3$), 62.6 ($COOCH_2CH_3$), 54.1/52.0* ($C_6H_5CH_2CHP$), 39.98 ($C_{10}H_7CH_2CH_2CH$), 36.5 ($C_{10}H_7CH_2CH_2CH$), 37.2 ($C_{10}H_7CH_2CH_2CH$), 34.3 ($C_6H_5CH_2CHP$), 29.1/28.3* (PO_2CH_2),14.5 ($COOCH_2CH_3$)). ^{31}P NMR: 54.08 (PO_2CH_2).

General Procedure for the Preparation of Phosphinic

Table 8

compds **10**, **11**	R_2	R_3	yield, % (for **10**)	yield, % (for **11**)	R_f of **10**[a]	R_f of **11**[b]
a	H	CH_2-Ph	95	80	0.70	0.53[a]
b	H	CH_2-CH_2-Ph	97	81	0.72	0.59[a]
c	H	CH_2-CH_2-CH_2-Ph	97	85	0.73	0.21
d	H	CH_2-O-CH_2-Ph	93	90	0.77	0.42[a]
e	H	CH_2-S-CH_2-Ph	72	92	0.72	0.50[a]
f	Ph	CH_2-CH_2-Ph	89	95	0.61	0.42
g	Ph	CH_2-CH_2-CH_2-Ph	75	97	0.66	0.42
h	Ph	CH_2-CH_2-CH_2-CH_2-Ph	95	95	0.63	0.44
i	Ph	CH_2-$(CH_2)_5$-CH_3	96	78	0.78	0.56
j	Ph	CH_2-O-CH_2-Ph	91	80	0.71	0.26
k	Ph	CH_2-S-CH_2-Ph-OMe	93	94	0.66	0.46
l	Ph	2-CH_2-naphthyl	91	92	0.53	0.38
m	Ph	2-$(CH_2)_2$-naphthyl	95	86	0.57	0.35

[a] Hex/AcOEt/AcOH (3:3:0.2). [b] $CHCl_3$/MeOH (9.5:0.5).

Synthons Type 10 (Scheme 3). The type **9** synthon (1 mmol) and 1-adamanthylbromide (1.2 mmol) were dissolved in chloroform (10 mL). This reaction mixture was refluxed. Silver oxide (2 mmol) was then added in five equal portions, over 50 min. This solution was refluxed for an additional 30 min. After removal of solvents, the residue was treated with diethyl ether and filtered through Celite. The filtrates were concentrated. The residue was purified by column chromatography using chloroform/2-propanol (9.8:0.2) as eluent. The compounds type **10** were obtained in yields reported in Table 8.

General Procedure for the Preparation of Phosphinic Synthons Type 11 (Scheme 3). One milliliter of 4 N NaOH was added dropwise to a stirred solution of type **10** compounds (1 mmol) in methanol (5.5 mL). The reaction mixture was stirred for 18 h. The solvent was then removed, and the residue was diluted with water and acidified with 0.5 N HCl in an ice−water bath. The aqueous phase was extracted with ethyl acetate (2 × 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give compounds type **11** as white solids in yields reported in Table 8.

Procedure for the Preparation of Phosphinic Synthons Type 13. To a solution of methanol (7 mL), containing compound **11g** (0.64 g 1.0 mmol) and ammonium formate (0.19 g, 3.0 mmol), was added 10% Pd/C (0.15 g). After 12 min at room temperature, the catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. Methylene chloride was added to the residue, and the solution was evaporated to dryness. This procedure was repeated twice. The resulting residue was dissolved in 20% Na_2CO_3 (2 mL). This reaction mixture was concentrated in vacuo until half of the volume was removed, and then water (1 mL) and dioxane (2 mL) were added. The mixture was ice-cooled, and a solution of Fmoc−Cl (0.31 g, 1.20 mmol) in dioxane (2 mL) was added dropwise over a period of 1 h. The solution was stirred for 2 h at 4 °C and 4 h at room temperature, and the reaction mixture was then diluted with water (15 mL), cooled in an ice−water bath, and acidified to pH 2 with 2 N HCl. The solid product which precipitated was quickly taken up in diethyl ether, and the organic layer was rinsed with water, dried over Na_2SO_4, and evaporated to dryness to give the crude product **13g,** which was purified by silica gel column chromatography using chloroform/methanol (9.5:0.5) as eluent. The pure product **13g** was obtained in 52% (0.38 g) yield.

Procedure for the Preparation of Compound Type 14. This phosphinic peptide was obtained following the protocol described for the synthesis of compounds type **12**.

General Procedure for the Preparation of Compounds 17−34. The phosphinic pseudo-peptides were prepared by conventional solid-phase peptide synthesis, on a Rink amide resin, using a Fmoc strategy. Typically, 3 equiv of a Fmoc amino acid, 3 equiv of HBTU, and 5 equiv of diisopropylethylamine (DIEA) in *N*-methylpyrrolidone (NMP) were added to the resin, and the coupling reaction was allowed to proceed for 30 min. The Fmoc *N*-protection group was removed with a 30% solution of piperidine in NMP. The coupling of the phosphinic synthons (compound type **11**) was performed using the coupling conditions described above, except that only 1.5 equiv of the phosphinic synthon was used and that the reaction was allowed to proceed for 60 min. Cleavage of the peptides from the resin, together with the cleavage of the side chain protection groups, was performed using a solution of TFA in dichloromethane containing 5% triisopropylsilane and 5% water.

Table 9

compd	HPLC gradients	calcd mass	ES-MS (M + H)
17	1	590.23	590.5
18	1	604.25	604.2
19	3	618.26	618.7
20	2	620.24	620.6
21	1	636.22	636.5
22	1	590.23	590.5
23	2	632.28	632.6
24	3	680.28	680.7
25	5	694.29	694.8
26	4	708.31	708.9
27	4	696.27	696.7
28	7	742.26	742.8
29	6	716.28	716.8
30	6	730.29	730.9
31	6	674.32	674.8
32	2	579.25	579.6
33	4	694.29	694.7
34	6	760.26	761.7
35	2	602.27	602.7
36	3	708.31	709
37	2	693.31	693.8
38	4	727.27	728.2
39	2	743.26	744.2
40	3	703.29	703.9
41	7	771.21	772.7
42	4	715.29	715.8
43	8	800.19	801.8
44	4	765.33	765.8
45	5	807.38	808

General Procedure for the Preparation of Compounds 35−45. These phosphinic peptides were prepared as described above, using phosphinic synthons type **13**. The Fmoc group of compound type **14** was removed using 20% piperidine/NMP solution. Compounds **40−43** were prepared by coupling indole-2-carboxylic acid, 4,6-dichloro indole-2-carboxylic acid, 2-quinoline carboxylic acid, and 6−8 dichloro-coumarine-3-carboxylic acid to the free amino-phosphinic peptide compound type **15**, bound to the resin. These acids were incorporated by multiple couplings, using 3 equiv of the acid to be coupled with 3 equiv of HBTU and 10 equiv of DIEA. Compounds **35** and **36** were prepared by coupling acetyl and benzyloxyacetyl chlorides to the free amino-phosphinic peptide compound **15**. Solutions (0.5 M) of chlorides and DIEA in dichloromethane were used, in 25-fold excess. Compounds **37−39** were prepared from compound **15** by a two-step acylation−alkylation procedure using bromoacetic bromide. The conditions used to incorporate the bromide derivative were similar to those described for chlorides coupling. Aniline derivatives (50 equiv) were alkylated using a solution of DIEA in DMSO. Cleavage of the peptides from the resin, together with the cleavage of the side chain protection groups, was performed as described above.

Purification and Characterization of Compounds 17−45. Purification of the diastereoisomeric forms of the phosphinic peptides was achieved by reverse-phase HPLC, using different types of gradients (Table 9). Typically, four main HPLC peaks were observed for all phosphinic peptides prepared. Mass spectroscopy analysis of these four peaks showed that they corresponded to the different diastereoisomeric forms of these phosphinic peptides. Correctness of the phosphinic peptide structure was checked by ES-MS. The data reported in Tables 1−4 correspond to the most potent diastereoisomer of the phosphinic compounds.

Enzymes. Recombinant human full-length pro-MMP-1, pro-MMP-2, pro-MMP-9, and pro-MMP-8 were expressed in NSO myeloma cells and purified as described before.[42,43] The proenzymes were activated immediately prior to use. Pro-MMP-1

was treated with trypsin (10 μg/mL) for 25 min at 25 °C, followed by bovine pancreatic trypsin inhibitor (100 μg/mL). Pro-MMP-2, pro-MMP-9, and Pro-MMP-8 were activated with 4-aminophenylmercuric acetate.[44] Catalytic domains of human MMP-14 and MMP-7 and mouse MMP-11, expressed in *Escherichia coli* BL21 DE3 pLysS cells, were obtained and purified as described before.[45]

Enzyme Assays. Enzyme inhibition assays were carried out in 50 mM Tris/HCl buffer, pH 6.8, 10 mM $CaCl_2$ at 25 °C. With the exception of MMP-11, all the assays were performed in black 96-well plates. Progress curves were monitored by following the increase in fluorescence at 400 nm ($\lambda_{ex} = 340$ nm), induced by the cleavage of the Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 fluorogenic substrate by MMPs.[46] The conditions of a typical experiment were 200 μL of buffer, 0.2−5 nM MMP, and 6 μM Mca-substrate. Inhibitors were dissolved at various concentrations in buffer containing the MMP. The reaction was then immediately initiated by addition of 1−2 μL of a stock solution of substrate in DMSO. Under these conditions, steady-state rates of hydrolysis were observed over a period of 50 min. Data were collected for 30 min.

In the specific case of MMP-11, assays were performed in quartz cuvettes, with mixing by a magnetic stirrer, using the Mca-Pro-Leu-Ala-Cys(OMeBn)-Trp-Ala-Arg-Dpa-NH_2 substrate, as described before.[19]

For most inhibitors, no sign of slow binding inhibition was evident from the progress curves. For those inhibitors where time-dependence of inhibition was observed, inhibitor and enzyme were preincubated for at least 1 h before addition of the substrate.

For some highly hydrophobic compounds, binding of the inhibitor to the 96-well plates was detected, biasing the determination of the K_i value. In this case, these compounds were studied in quartz cells.

Determination of the K_i Values. For each MMP, initial rate measurements in the absence of inhibitor were made for eight different substrate concentrations. From these data, K_m values were determined using the graphical direct linear plot method.[47] A correction factor for each substrate concentration, determined experimentally as described before,[19] was used to correct the fluorescence observed at high substrate concentrations for the inner filter effect. The K_m values determined for MMP-1, MMP-2, MMP-7, MMP-8, MMP-9, MMP-11, and MMP-14 were, respectively, 100, 49, 67, 61, 14, 0.2, and 21 μM.

For each inhibitor, percentages of inhibition were determined in triplicate experiments at five concentrations. Inhibitor concentrations were selected in order to observe a 20−80% range of inhibition. K_i values were determined using the method proposed by Horovitz and Levitski.[48] This approach explicitly takes into account the effect of the substrate, enzyme, and inhibitor concentrations and applies to the situation of both standard and tight-binding inhibition.

Equipment and Instrumentation. Assays were performed in black flat-bottomed 96-well plates (nonbinding-surface plates 999999, Corning Costar France.). With these plates, normal progress curves were observed for all MMPs, avoiding the requirement for prior BSA coating of the plates to prevent abnormal kinetics. Fluorescence signals were monitored using a Biolumin 960 photon-counter spectrophotometer (Molecular Dynamics), equipped with a temperature device control and a plate shaker. For assays performed in quartz cells, fluorescence measurements were performed with a Perkin-Elmer LS 50 spectrophotometer.

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