Diastereoselective Solution and Multipin-Based Combinatorial Array Synthesis of a Novel Class of Potent Phosphinic Metalloprotease Inhibitors

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Abstract: The solution-phase synthesis and resolution of new phosphinopeptidic building blocks containing a triple bond was realized in high yields and optical purities (units **3a–d**). The absolute configuration of the target compounds was unambiguously established by NMR studies. A post-assembly diversification strategy of these blocks was developed through 1,3-dipolar cycloaddition of a variety of in situ prepared nitrile oxides. This strategy led to the rapid and efficient diastereoselective preparation of a novel class of isoxazole-containing phosphinic peptides (peptides **5a–i**). Solid-phase version of this strategy was efficiently achieved on multipin solid technology, by developing a new protocol for the coupling of P-unprotected dipeptidic blocks with solid supported amino acids in a quantitative and diastereoselective manner. Optimization of dipolar cycloadditions onto pin-embodied phosphinic peptides allowed the convenient preparation of this new class of pseudopeptides. The crude phosphinic peptides (**9a–k**) were obtained in high yields and purity as determined by RP-HPLC. Inhibition assays of some of these peptides revealed that they behave as very potent inhibitors of MMPs, outmatching previously reported phosphinic peptides, in terms of potency (K_i in the range of few nM).

Keywords: combinatorial chemistry · cycloaddition · inhibitors · metalloproteins · solid-phase synthesis

Introduction

Phosphinic peptides incorporating in their sequence the motif -X^{1}aaΨ[P(O)(OH)CH$_2$]X^{2}aa- are well recognized peptide isosters and powerful inhibitors of many classes of enzymes, especially the zinc-proteases (Figure 1).[1–3] Matrix metalloproteases (MMPs),[4] bacterial collagenases,[2c, 5] enkephalin-degrading enzymes,[6] other zinc metalloproteases,[1a, 7] HIV-protease[8] and ligases[9] are a few examples of such enzymes inhibited by this class of pseudopeptidic inhibitors.

Unlike hydroxamic, carboxylalkyl or thiol-containing inhibitors, which have been extensively developed to prepare potent inhibitors of zinc-metalloproteases, phosphinic peptides are devoid of toxicity, but more importantly these compounds are stable in vivo,[3b, 10] allowing their use in vivo at rather low concentration.[11a,b] Moreover, phosphinic peptide chemistry offers the possibility to develop inhibitors able to interact with both the prime and unprimed side of the active site cleft, thus allowing optimization of the inhibitor selectivity by diversification of the P and P′ positions of the inhibitor (see for example RXP407 where the P_2 residue is critical for selectivity, Figure 1).[4b, 10, 12]

Recently, the phosphinic peptide RXP03[4b] (Figure 1), bearing an unusual long side chain at the P_1' position, was reported as a highly potent inhibitor of different MMPs.[3b, 11a,c]

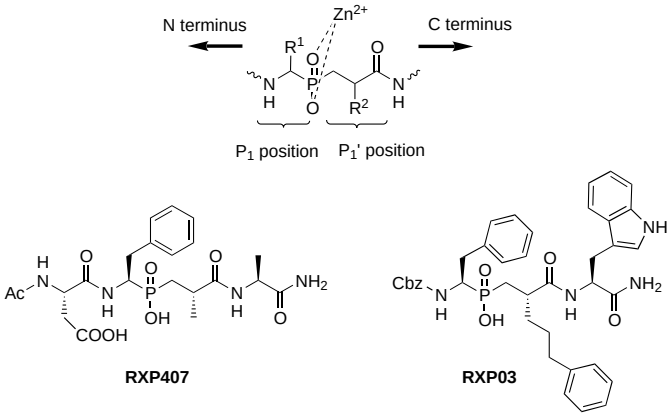

Figure 1. Standard representation of phosphinic peptides. P_1 and P_1' positions are the segments of the peptide situated at the left and right-hand site of the phosphinic moiety respectively (above). The chemical structures of the two phosphinic inhibitors RXP407 and RXP03 (below).

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The resolution of the crystal structure of stromelysin-3 (MMP-11) complexed to RXP03[13] provided the basis for the development of new phosphinic compounds with improved properties in terms of selectivity and potency.

Despite the interesting properties of phosphinic peptides, the difficulties often encountered in producing the appropriate starting materials or lack of reactivity have greatly limited the extensive use of this class of inhibitors.[9a, b, 14] Elongation of phosphinic pseudodipeptidic units, which are obtained by a Michael-type addition of silyl activated N-protected aminophosphinic acids to suitably substituted acrylates,[15] constitutes the classical approach to prepare phosphinic (poly)peptides.[4] Such an approach usually consists of several synthetic steps and is absolutely dependent on the availability of the starting materials. In addition, another drawback of this strategy is that the chemical features of the P_1 and P_1' positions (Figure 1) are inherently fixed, thus allowing diversity introduction only in the positions framing the dipeptidic block. Therefore, this is not a convenient strategy to rapidly prepare large series of widely diversified phosphinic peptide libraries.

Within the framework of our ongoing research concerning the syntheses of novel polyfunctionalized phosphinic-based inhibitors, we became interested in the development of reliable and versatile alternative strategies that would provide rapid access to the target molecules. Toward this end, we speculated that the utilization of a modular strategy, which would allow the desired diversification of a phosphinic pseudopeptidic template at the very final step of the synthesis, would be extremely useful. Such an approach is bound to give a boost to the development of novel phosphinic inhibitors by either parallel synthesis or combinatorial chemistry, as preliminary studies from our laboratory have shown.[16]

A literature search revealed that most of the reported phosphinic peptides generally involve amino acids such as phenylalanine, alanine, glycine, valine that lack side-chain functionality. However, we were interested in preparing side-chain functionalized pseudopeptides, and especially peptides comprising a heterocyclic core, since they have not yet been reported. Taking into consideration the promising results exhibited by RXP03 (see above), we chose to focus our attention on the development of analogues of this inhibitor, with particular emphasis on the P_1' position. Indeed, it is well known that this position is one of the key side chains for the control of inhibitor potency and selectivity as far as MMPs are concerned.[3b, 4b]

Regarding the nature of the heterocyclic core to be incorporated in the peptides, the choice was not straightforward since no reports to similar compounds could be found. Although many heterocyclic compounds have interesting biological activities, an overview of the literature stimulated our interest in the isoxazole ring. Isoxazole moieties (3,5-disubsituted isoxazoles illustrated in Figure 2) have been extensively utilized not only as precursors to versatile intermediates for the synthesis of a variety of complex natural products[17] but also as scaffolds for peptidomimetics,[18] and core structures in medicinal chemistry, where they represent a class of unique pharmacophores that are constituents of diverse therapeutic agents.[19] Moreover, there exist several naturally occurring isoxazoles with important pharmacological activity (e.g. Muscimol, 4-hydroxyisoxazole, Figure 2).[20]

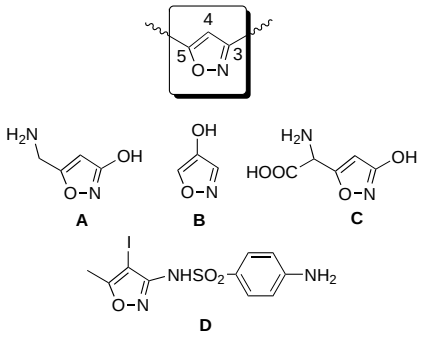


Figure 2. General structure of 3,5-disubstituted isoxazole core (top). Examples of naturally occurring and synthetic isoxazoles of pharmaceutical interest. **A**: Muscimol;[20] **B**: 4-hydroxyisoxazole;[20] **C**: ibotenic acid;[19d] **D**: selective endothelin receptor A (ET_A) antagonist.[19f]

Another key feature of the isoxazole ring that drew our attention is its ionophoric properties. Generally, isoxazoles coordinate to metals mainly through the nitrogen lone pair.[21] Taking into account that our program is concerned with zinc-metalloenzymes, which, in addition to zinc, may contain other cations (e.g. Ca^{2+}), this isoxazole Lewis basicity may prove extremely useful. Moreover, the enhanced hydrogen bonding acceptor capability of the ring was envisioned to increase the contact points of the inhibitors with the enzymes (Figure 3).

The above arguments render the isoxazole core a privileged structure, which is well worthy of introduction into the field of phosphinic pseudopeptides. Determined to design and develop a novel approach to the synthesis of phosphinic peptides, we wish to report herein our efforts toward this end. The general structure of the target molecules is illustrated in Figure 3. Useful precursors for the introduction of the isoxazole ring into the P_1' position of these peptides were

Abstract in Greek:

Η σύνθεση σε διάλυμα και ο διαχωρισμός των διαστερεοϊσομερών μορφών νέων φωσφινοπεπτιδικών μορίων-κορμών που φέρουν τριπλό δεσμό επετεύχθη σε υψηλή απόδοση και οπτική καθαρότητα (μόρια **3a-d**). Η απόλυτη στερεοχημεία τους καθορίστηκε με NMR μελέτες. Εφαρμογή 1,3-διπολικής προσθήκης μιας ποικιλίας in situ παρασκευασμένων οξειδίων νιτριλίου οδήγησε σε μια νέα στρατηγική διαφοροποίησης αυτών των μορίων-κορμών. Αυτή η προσέγγιση επέτρεψε τη γρήγορη και αποδοτική διαστερεοεκλεκτική σύνθεση μιας νέας τάξης ισοξαζολο-φωσφινικών πεπτιδίων (πεπτίδια **5a-i**). Επιτυχής εφαρμογή της στρατηγικής αυτής σε στερεά φάση επετεύχθη αφού αναπτύχθηκε ένα νέο πρωτόκολλο για τη σύζευξη μη υδροξυφωσφίνυλο-προστατευμένων διπεπτιδικών μονάδων με αμινοξέα ήδη προσδεμένα σε στερεό υπόστρωμα ποσοτικά και διαστερεοεκλεκτικά. Ως στερεό υπόστρωμα επελέγη η τεχνολογία **multipin**. Βελτιστοποίηση των διπολικών κυκλοπροσθηκών σε φωσφινικά πεπτίδια προσδεμένα σε pin επέτρεψε την αποδοτική παρασκευή αυτής της νέας τάξης ψευδοπεπτιδίων. Τα προκύπτοντα πεπτίδια (**9a-k**) ελήφθησαν σε υψηλή απόδοση και καθαρότητα, όπως διαπιστώθηκε με χρήση RP-HPLC. Μελέτη της αναστολτικής δράσης μερικών από αυτές τις ενώσεις έδειξε ότι είναι πολύ ισχυροί αναστολείς των ματριξινών, πιο ισχυροί ακόμα και από ήδη δημοσιευμένους φωσφινικούς αναστολείς (τιμές K_i λίγων nM).

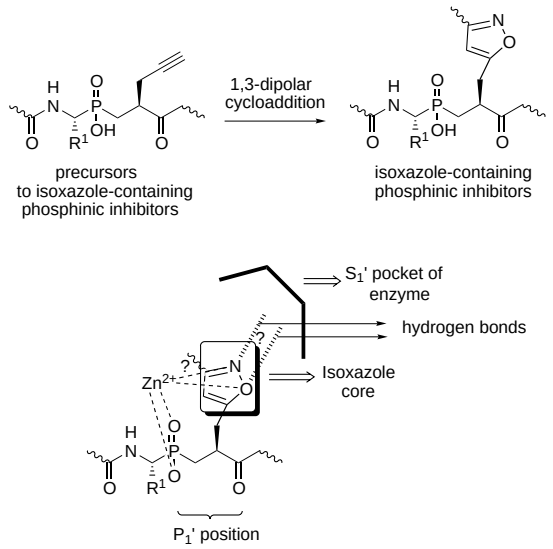


Figure 3. General structure of the phosphinic precursors and isoxazole-containing peptidic inhibitors obtained by 1,3-dipolar cycloaddition (above). Presumable interaction of these inhibitors with the active site of zinc-metalloproteases through hydrogen bonding and/or coordination of the isoxazole ring to the zinc cation (below).

prepared, containing a triple bond in this position (Scheme 1). Construction of the isoxazole ring would be accomplished by a [3 + 2] cycloaddition of nitrile oxides to the alkyne moiety, since the former are reported to add regioselectively to terminal triple bonds giving exclusively 3,5-disubstituted isoxazoles.[22]

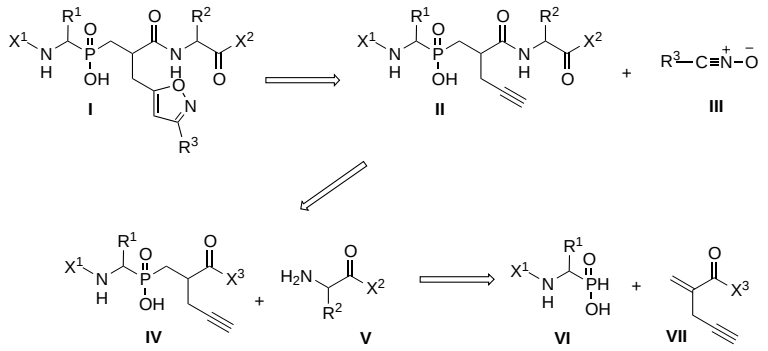
Results and Discussion

Solution-phase synthesis: The retrosynthetic analysis of the target pseudopeptide **I** is described in Scheme 1. The tripeptidic scaffolds **II**, containing a triple bond at P_1' position, could serve as good substrates for nitrile oxide **III** cycloadditions (DCR_S), allowing rapid access to the novel class of isoxazole phosphinic peptides. This approach is consistent with our initial goal and would allow the *direct* and *straightforward diversification* of a tripeptidic unit at the final step of the synthesis in one reaction step. Compounds **II** could be synthesized by coupling of the pseudodipeptidic units **IV** with amino acids **V**. Finally, phosphinic dipeptides **IV** could be obtained by Michael addition of silyl-activated versions of aminophosphinic acids **VI** to acrylic derivatives **VII**. An alternative route might rely on the prior assembly of isoxazole-containing dipeptide units derived from **IV** and subsequent coupling with amino acids; nevertheless, such an approach would involve more synthetic steps, and preliminary experiments showed that it is less efficient, suffering from by-product formation.

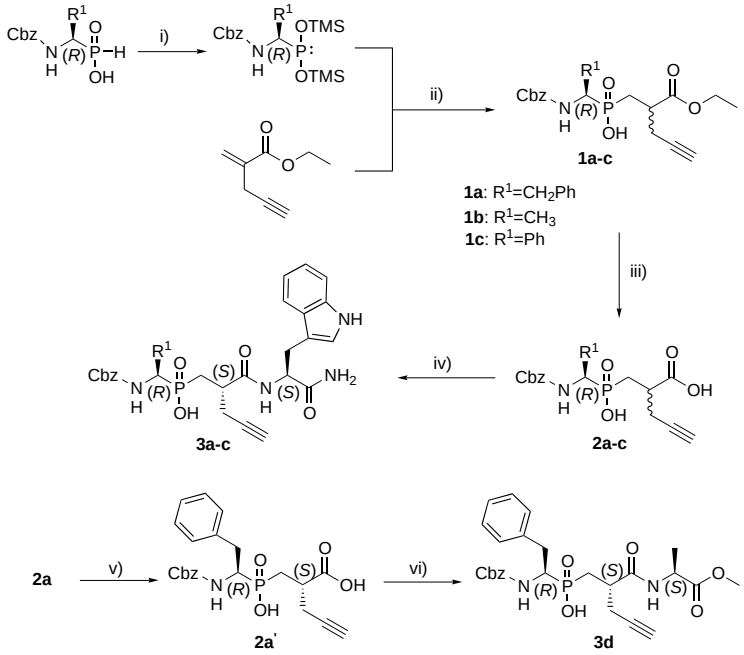
The key-step in the strategy for the development of the pseudopeptidic isoxazole-containing phosphinic inhibitors is the construction of tripeptidic units **II** (Scheme 1) or **3** (Scheme 2).

This synthesis commenced with the Michael addition of silyl-activated α-amino phosphinic acids[15] to ethyl α-propargylic acrylate.[23] Optically pure (*R*) Cbz N-protected amino phosphinic acids[24] (L configuration) were chosen based on enzymatic preferences, allowing thus the control of stereochemistry at the P_1 position of the final inhibitors. The use of optically pure aminophosphinic acids had a great impact on the stereochemistry of the newly generated stereogenic center of the dipeptidic unit **1** (Scheme 2). Two methods were applied to prepare **1a–c** (Scheme 2): HMDS activation at elevated temperatures,[15a] in the presence of 1.3 equivalent of acrylic derivative and subsequent quenching of the mixture of proposed diastereomeric enolates (formed during the Michael addition, Figure 4) with dry ethanol at $-10\,^{\circ}C$ for 1 h,[25] afforded the dipeptidic blocks **1** as a mixture of diastereomers in a 3.5:1 ratio (Table 1, entries **1a** (footnote a) and **1c**). Activation with TMSCl/DIPEA[36a] at low temperatures and quenching of the mixture under the above described conditions improved the diastereomeric ratio slightly (4.5:1, **1a**, footnote b). The ratio of the two diastereomers was easily determined by ^{31}P NMR spectroscopy. RP-HPLC could not be used since no separation of the dipeptidic diastereomers could be achieved at this stage. In the case of alanine phosphinic analogue (**1b**) the ratio of diastereomers (3.5:1) was lower than that obtained for the phenylalanine analogue, presumably due to the smaller size of the methyl group. It should be noted that use of bulky or chiral nonracemic alcohols such as *tert*-butanol or (*R*)-α-amino phenyl glycinol to quench the reaction mixture did not improve the obtained ratios.

Saponification of the ethyl ester of **1** afforded the diacids **2a–c**. Alkaline removal of the ethyl ester of **1** was carefully carried out, since large excess of hydroxide anions (≈10 equiv) and long reaction times (≈10 h) caused partial racemization at the P_1' position (≈3–4%). Accordingly, controlled saponification (5–6 equiv NaOH, TLC monitoring 6–8 h) allowed minimization of this undesired result. The major diastereomer **2a′** (*R*,*S* configuration) of diacid mixture **2a** could be easily obtained by simple recrystallization from ethyl acetate. ^{31}P NMR spectroscopy proved to be a very con-



Scheme 1. Retrosynthetic analysis of the isoxazole-containing tripeptidic inhibitors **I**.



Scheme 2. Diastereoselective preparation of the tripeptidic units **3**, suitable for 1,3-dipolar cycloaddition with nitrile oxides. i) HMDS (5 equiv), 110 °C, 1 h (activation); or TMSCl (4.5 equiv), DIPEA (4.5 equiv), 0 °C → RT, 3 h (activation); ii) 100 °C, 3 h (Michael addition), then dry EtOH, −10 °C → RT; or 0 °C → RT, 48 h (Michael addition), then abs. EtOH, −10 °C → RT; iii) 0.5 M $NaOH_{aq}$/EtOH (5 equiv), 0 °C → RT, 6–8 h, then 1 M HCl to pH ≈1; iv) HCl·$HTrpNH_2$ (1 equiv), EDC·HCl (4 equiv), HOBt (1 equiv), DIPEA (3 equiv), CH_2Cl_2, 1.5–2 h, crystallization in AcOEt or CH_2Cl_2; v) recrystallization in AcOEt; vi) HCl·HAlaOMe (1 equiv), EDC·HCl (4 equiv), HOBt (1 equiv), DIPEA (3 equiv), CH_2Cl_2, 1.5–2 h.

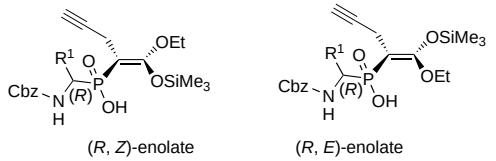


Figure 4. Proposed diastereomeric enolates formed during the Michael-type addition of silyl phosphonites to acrylic derivatives.

Table 1. Methods, yields and ratio of diastereomers of the dipeptidic units of type **1**.

Entry	R^1	Yield [%]	Ratio (*R*,*S*)/(*R*,*R*)[c]
1a[a]	CH_2Ph	96[a]	3.5.1
1a[b]	CH_2Ph	89[b]	4.5:1
1b	Me	92[b]	3.5:1
1c	Ph	88[a]	3.5:1

[a] HMDS-method. [b] TMSCl/DIPEA-method. [c] Based on ^{31}P NMR; for determination of the absolute configuration see Scheme 3.

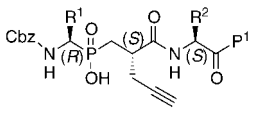
venient means for monitoring recemization during the ethyl ester hydrolysis or the progress of attempted resolution of the dipeptidic units **2**. For diacids **2b**,**c** such a resolution could not be accomplished. Nevertheless, this result did not impact the progress of our project, since tripeptide mixtures **3a–c** were easily resolved by fractional crystallization.

The determination of the absolute configuration of the new stereogenic center was accomplished after coupling of the dipeptides **2** with (*S*)-tryptophan amide to afford the tripeptides **3a–c** (Table 2). Although the diastereomeric mixtures **3** could be easily separated into its components by RP-HPLC, we were able to succeed in a more practical process and achieved our initial goal (isolation of *RSS* diastereomer) by means of simple crystallization from appropriate solvent. This property of tripeptides of the general formula Cbz(*R*)XaaΨ[P(O)(OH)CH_2]CH(propargyl)-(*S*)$TrpNH_2$ was successfully exploited to prepare peptides with different side chains at P_1 position in very good yields and excellent optical purities (≈97% *de*) (Table 2).

Furthermore, the resolved (*R*,*S*)-diastereomer **2a′** turned out to be a valuable tool for many reasons. It could be easily coupled with amino acids to afford an array of propargyl-containing tripeptidic blocks diversified at P_2' position (e.g. entry **3d**, Table 2). To the best of our knowledge, only very few examples of diastereomeric phosphinic dipeptides have been successfully resolved at this stage.[6] We utilized this advance in our studies regarding racemization at the P_1' position throughout our project, since we were not aware of any other relevant studies. Notably when **2a′** was coupled with alanine methyl ester to afford tripeptide block **3d**, no racemization was observed under these conditions (EDC·HCl, HOBt, short reaction time) according to HPLC analysis (Scheme 2, Table 2).

Before we embarked on the DCRs to these precursors, it was essential to unambiguously establish the absolute configuration of the newly formed stereogenic carbon (P_1' position). A literature search regarding RP-HPLC analysis of phosphinic pseudotripeptides, containing an (*S*)-amino acid (P_2' position) linked to P_1' position of the phosphinic dipeptidic unit by a classical amide bond and therefore existing as mixture of two diastereomers (e.g. *R*, *R*/*S*, *S*), indicated that the isomer with the shorter retention time is the one

Table 2. Tripeptides of type **3**.



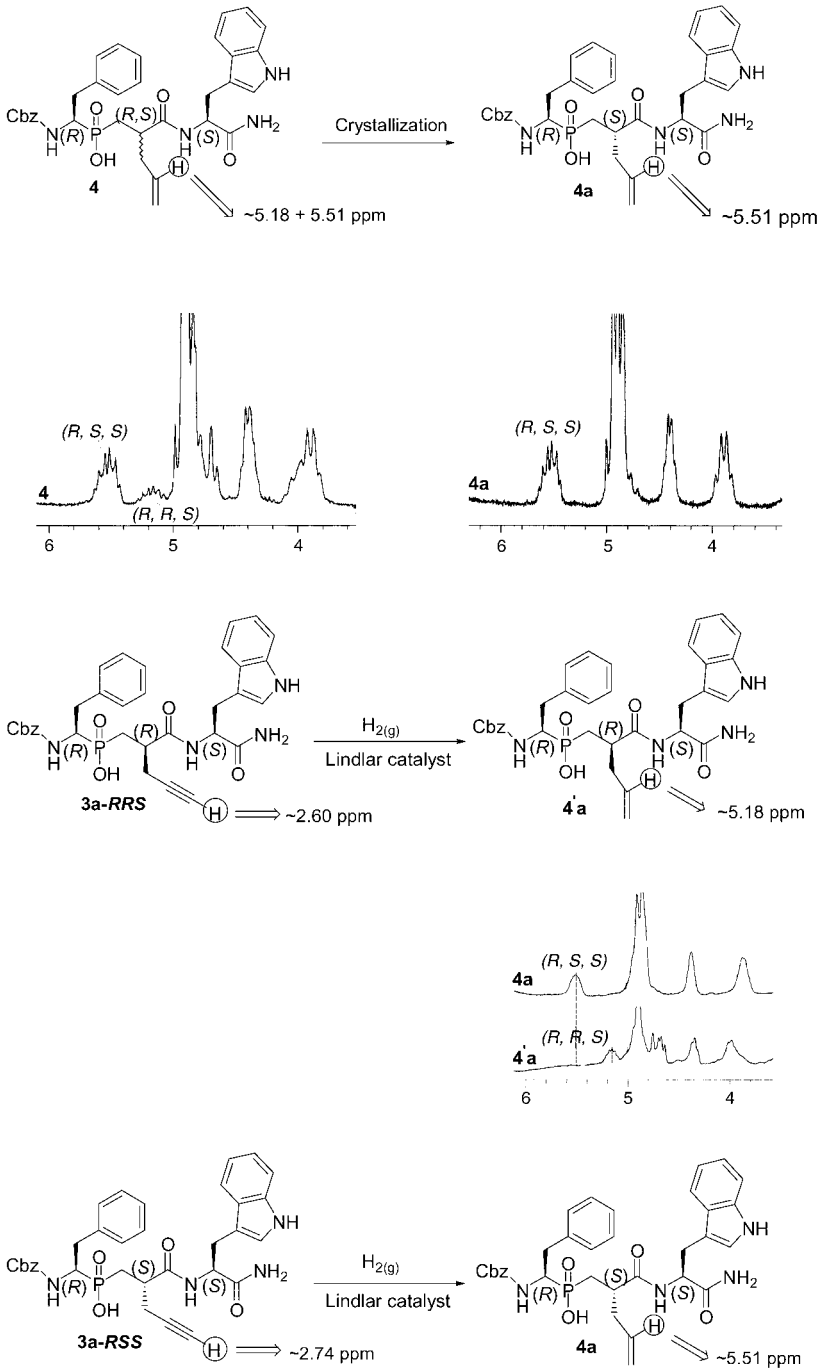
Entry	R^1	R^2	P^1	Yield [%]
3a	CH_2Ph	3-indolylmethyl[a,b]	NH_2	79
3b	Me	3-indolylmethyl[a,c]	NH_2	73
3c	Ph	3-indolylmethyl[a,b]	NH_2	82
3d	CH_2Ph	Me	OMe	79

[a] Combined yields of (*R*,*S*,*S*) and (*R*,*R*,*S*) diastereomers, starting from the appropriate dipeptide as mixture of diastereomers. [b] Recrystallization in AcOEt (≈98% *de*). [c] Recrystallization in CH_2Cl_2 (97% *de*).

possessing the (*S*) absolute configuration at the P_1' position.[4e, 6, 25, 26] In our case, given the (*R*) and (*S*) configuration of the amino phosphinic acid and tryptophan, respectively, we observed two tripeptidic diastereomers, with the firstly eluted in RP-HPLC formed in much higher percentage than the other one. Based on the above mentioned literature results, we surmised that the first fraction is the (*R*,*S*,*S*) diastereomer, while the second is the (*R*,*R*,*S*) one.

To unambiguously establish this hypothesis, we sought to obtain further evidence. To this end, we applied the ^{1}H NMR method of Chen et al.[6b] for the two diastereomers of phosphinic tripeptide **3a**, isolated by means of simple crystallisation [besides the insoluble isomer of this tripeptide mixture (assumed to be the (*R*,*S*,*S*) diastereomer judging from HPLC analysis), the soluble isomer (assumed as the (*R*,*R*,*S*)) was also recovered from the filtrate, at least 95% (HPLC analysis) and in high optical purity (≈93% *de*)].

According to Chen's method, in a dipeptidic unit containing one aromatic residue, the relative absolute configuration of each amino acid could be deduced from the chemical shifts of the nonaromatic side chain protons: a shielding of these protons is observed when the two amino acids have opposite absolute configuration (*R*,*S* or *S*,*R*) as compared to those having identical configuration (*R*,*R* or *S*,*S*). In our case, the existing tryptophan residue at P_2' position of the tripeptides rendered this method highly relevant. ^{1}H NMR analysis of the two diastereomers clearly showed, in accordance with the published method, deshielding of the propargylic proton in the "(*R*,*S*,*S*)" isomer by ≈0.14 ppm, as compared to the propargylic proton of the "(*R*,*R*,*S*)" isomer (2.74 vs 2.60 ppm respectively confirmed by HMQC experiments, Scheme 3). This result confirmed the assumed absolute configuration of the P_1' position.



Scheme 3. Correlation between characteristic chemical shifts of propargyl and allyl side chain (P_1' position) protons and absolute configuration of these chains in phosphinic tripeptides precursors to DCRs. The ^{1}H NMR spectra were acquired using the crude hydrogenated products.

In the course of our program aiming at the development of novel heterocyclic phosphinic peptides, it occurred to us that our results could be ultimately confirmed by applying the same method to the analogue of tripeptide **3a**, bearing an allylic instead of a propargylic side chain (**4a**, Scheme 3). Isolation of such a tripeptide was achieved in the same fashion as for tripeptide **3a**. Crystallisation from ethyl acetate afforded the desired (*R*,*S*,*S*) diastereomer (**4a**, Scheme 3), as proven by ^{1}H NMR analysis. Indeed, ^{1}H NMR analysis of the mixture of the two diastereomers of **4** (a mixture with a 3.5:1 unoptimised ratio by HPLC analysis) showed two multiples, due to the single allylic proton at ≈5.2 and 5.5 ppm, respectively, in a 3.5:1 ratio. Moreover, in the ^{1}H NMR

spectrum of the pure assumed (*R*,*S*,*S*) diastereomer (**4a**), only the peak of the most deshielded proton (5.5 ppm) was retained, leading to the conclusion that the isolated isomer indeed possessed the (*R*,*S*,*S*) configuration.

With this pure diastereomer in hand, we were ready to reconfirm our results for the propargyl-containing tripeptide **3a**. We thought that hydrogenation of the triple bond of any diastereomer of **3a**, in the presence of Lindlar catalyst,[27] could afford the corresponding tripeptides **4a** or **4a′**. This proved to be the case, thus allowing a direct comparison and evaluation of the two diastereomeric tripeptides. This conversion is depicted in Scheme 3, where the (*R*,*S*,*S*) propargylic diastereomer **3a** afforded, after controlled hydrogenation, the corresponding allylic diastereomer **4a** (85% product, 10% starting tripeptide, 5% fully hydrogenated tripeptide after 4 h of hydrogenation, HPLC analysis), still suitable for NMR analysis. The same motif was observed when the (*R*,*R*,*S*) diastereomer was subjected to hydrogenation (product **4a′**). It should be noted that assessment of the ^{1}H NMR spectrum is much more convenient for the allylic derivatives, since their protons are greatly deshielded as compared to propargylic protons. Comparison of the spectra of these compounds demonstrated unequivocally their configurational identity.

Having developed reliable methods for the preparation of a wide range of optically pure propargylic phosphinic tripeptides, we were in position to study their reactivity toward 1,3-dipolar cycloadditions (DCRs) with nitrile oxides. Although such additions onto activated and nonactivated triple bonds have been extensively studied and applied to incorporate the isoxazole ring into numerous organic compounds ranging from natural products to amino acids analogues and low molecular weight non peptide bioactive compounds, they have been only sparingly used in peptidic modulation.[28]

The chemistry of phosphinic pseudopeptides is subject to many restrictions compared with classical peptides, mainly due to the hydroxyphosphinyl moiety. Without precedent for DCRs in the phoshopeptide field, the evaluation of different isoxazole ring-constructing formation methods was essential (Figure 5). The isolated, nonactivated nature of the triple bond in our peptidic precursors was expected to render them fairly good substrates for DCRs.[29]

At the outset, the Mukaiyama method[30] (primary nitroalkanes/phenyl isocyanate/Et_3N), though extensively applied in DCRs, was deemed incompatible with the unprotected hydroxyphosphinyl functionality; in addition the troublesome removal of the by-product diphenylurea further rendered this method problematic. Next, we turned our attention to the phase transfer catalyzed (PTC) hypochloride method by Lee[31] that consists in the use of (common) bleach ($NaOCl_{aq}$) in CH_2Cl_2/cat. Et_3N. Although this convenient one-pot procedure worked very well for simple dipeptides (e.g. **1a**, data not shown), it failed in the case of the tryptophan-containing tripeptidic blocks **3a–c**. Specifically, upon addition of bleach into the tripeptide solution, oxidative destruction of the indole ring was observed leading to a mixture of unidentified products. Unexpectedly, in the case of tripeptide **3d**, this method failed again for a different reason; HPLC analysis of the isolated mixture indicated extensive removal of the methyl ester of alanine residue, traces of starting material and some product. It is likely that under the alkaline conditions of this method the sensitive methyl ester was saponified; this unexpected hydrolysis might be related to the position of the ester inside the peptidic sequence (P_2' position), since ethyl ester removal, when present at P_1' position (e.g. **1a**), was not observed at all under the same conditions.

The Huisgen method was the third option chosen for evaluation. While the two above mentioned methods are one-step procedures, the Huisgen method can be performed as either one- or two-step procedure depending on the nature of the oxime used and the volition of the chemist.[32] This versatile method consists of prior conversion of an oxime into its hydroximinoyl chloride by action of *N*-chlorosuccinimide (NCS), isolation of this chloride, and subsequent slow generation of the corresponding nitrile oxide by means of base-induced dehydrochlorination in the presence of a dipolarophile (triple bond in this work).

Indeed, applying a one-pot Huisgen procedure we were able to perform DCRs of a wide range of nitrile oxides onto the phosphinic tripeptidic dipolarophiles **3a–d**. Chlorination of oximes was performed using NCS in chloroform, pyridine as catalyst, and gentle heating (method A, Scheme 4). The chlorination time for benzaldoximes is dependent on the nature of the ring substitution and for strongly deactivated oximes longer times (≈3 h) and heating are necessary to achieve chlorination. The same method was successfully

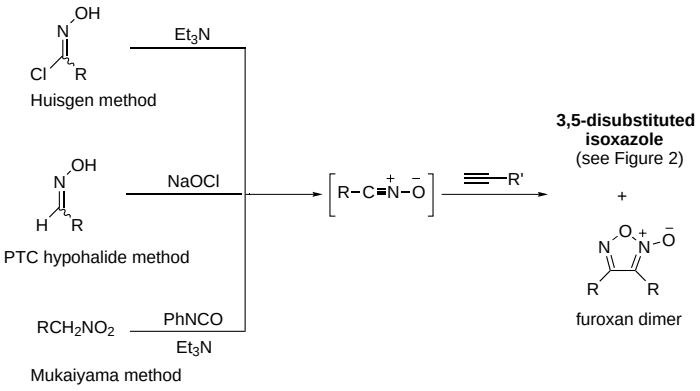
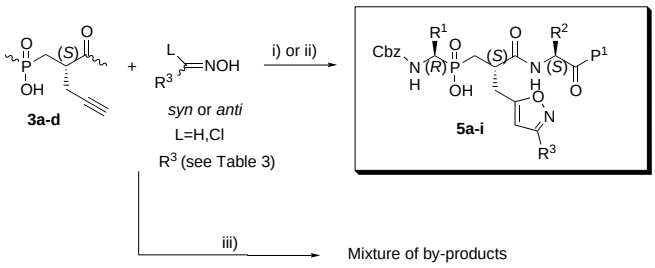


Figure 5. Methods of nitrile oxide generation.



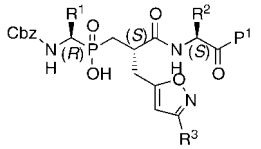
Scheme 4. Methods for the preparation of the isoxazole-containing phosphinic pseudopeptides of type **5**. i) Method A (one-flask procedure): L = H, oxime (6 equiv) in $CHCl_3$, pyridine(cat.), NCS (6 equiv), 45 °C, 3–4 h, then **3a–d**, Et_3N (7 equiv, portionwise), 45 °C, 48–60 h. ii) Method B (two-step procedure): L = Cl, oxime (6 equiv) in DMF, NCS (6 equiv), 45 °C, 40 min, isolation of the hydroximinoyl chloride, then **3a**, Et_3N (7 equiv, portionwise), RT, 48 h. iii) $NaOCl_{aq}$, CH_2Cl_2, Et_3N (cat.), RT, 12 h.

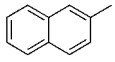
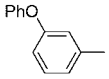
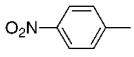
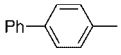
applied for chlorination of naphthaldoxime or heterocyclic oximes such as 2-furyl aldoxime. It is worth mentioning that the NCS chlorination of one isomer (*syn* or *anti*, stereochemistry undetermined) of the oximes was very rapid, while several hours of reaction were necessary to complete the chlorination of the other isomer. When the chlorination was over, a one-flask procedure was followed adding the tripeptidic dipolarophile **3a–d** and Et_3N. We found that we could, conveniently, perform the DCRs at ≈45 °C, thus raising the yield of the target molecules (**5**) by about 10% (Table 3).

For alkyl aldoximes thisx synthesis gave unsatisfactory results, which led us to modify the procedure. The best course of action proved to be a two-step Huisgen method.[33] Thus, the alkyl aldoxime was chlorinated in DMF for a short time (≈40 min) and the alkyl hydroximinoyl chloride was isolated by extraction. Once more, the in situ base-generated highly unstable alkyl nitrile oxide species was trapped by the dipolarophile at RT, leading to the smooth preparation of the isoxazole-containing tripeptide in satisfactory yield (Scheme 4, method B, Table 3, entry **5d**, see also [b] in this Table).

By inspection of Table 3, it becomes apparent that our approach to the synthesis of this novel heterocyclic class of phosphinic pseudopeptides is both versatile and promising. Amongst its merits, we should include for sure the ability of diversification of the tripeptidic blocks at the final stage, without the need for any hydroxyphosphinyl moiety protection. Moreover, it is applicable and high yielding for benzaldoxime or benzaldoximes with electron-donating substituents and naphthaldoxime (76–92%). For heterocyclic aldoximes such as 2-furyl aldoxime, benzaldoximes with electron-withdrawing substituents and alkyl aldoximes still worked well, giving rise to the target molecules in moderate to good yields (55–64%). In these cases, the starting tripeptide could easily be isolated from the reaction mixture (simple extraction) and resubjected to DCRs with the same nitrile oxide, allowing thus the preparation of the desired tripeptide in higher yield (e.g. Table 3, entry **5f**). According to ESMS analysis of all final compounds (**5a–i**) no ring-chlorinated material was detected. Furthermore, it must be underscored that the addition of all nitrile oxides to the terminal triple bonds was completely regioselective, affording only the desired 3,5-disubstituted isoxazoles[22] (NMR analysis); in addition, the integrity of the stereogenic carbons of the starting tripeptides was not affected under the alkaline conditions applied as concluded by HPLC analysis. In our project some model tripeptides (Table 3, entries **5a**,**e**,**i**) were prepared as mixture of epimers at P_1' position, in order to reevaluate the inhibitory potency of the individual diastereomers after HPLC separation.

Table 3. Isoxazole-containing tripeptides of type **5**.



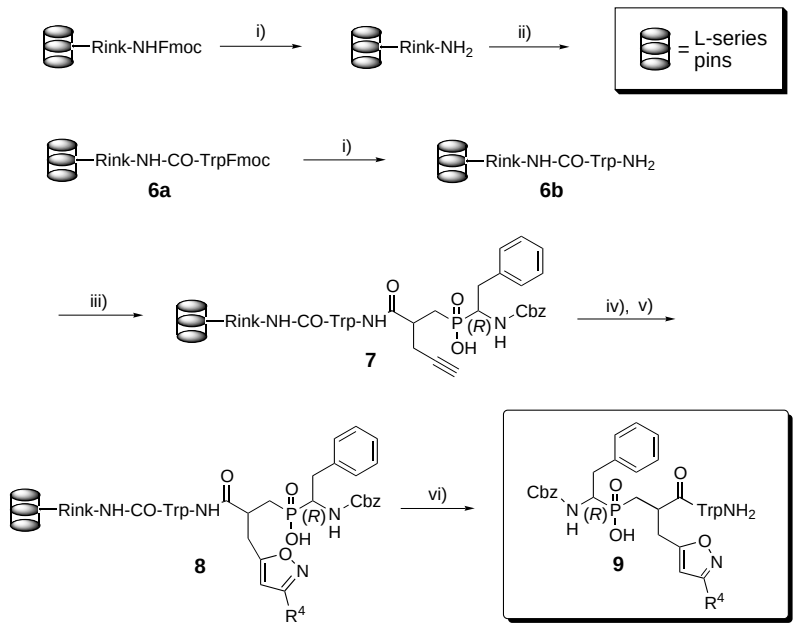
Entry	R^1	R^2	R^3	P^1	Yield [%]
5a	CH_2Ph	3-indolylmethyl		NH_2	86[a]
5b	CH_2Ph	3-indolylmethyl		NH_2	78
5c	CH_2Ph	3-indolylmethyl		NH_2	76
5d	CH_2Ph	3-indolylmethyl		NH_2	55[b]
5e	CH_2Ph	Me		OMe	81[a]
5f	CH_2Ph	Me		OMe	58 (87)[c]
5g	CH_3	3-indolylmethyl		NH_2	91
5h	Ph	3-indolylmethyl		NH_2	92
5i	Ph	3-indolylmethyl		NH_2	64[a]

[a] Prepared as mixture of two diastereomers at P_1' position; yield refers to the mixture of isomers. [b] Prepared by method B (see text and Experimental Section). The moderate yield is, in part, due to the relatively high solubility of the product in the organic solution used to precipitate and purify. [c] In parenthesis: overall yield after a second 1,3-dipolar addition to the isolated starting tripeptide.

Solid-phase synthesis: Solid-phase synthesis has been the major tool for combinatorial chemistry with apparent advantages over solution-phase chemistry. The application of solid-phase organic synthesis (SPOS) in the preparation of small compound libraries and peptides is of great significance in the discovery and development of new drug compounds.[34] Synthesis of phosphinic peptides has been successfully accomplished on the solid phase as well, using either parallel or combinatorial strategies. Such an approach has led to the rapid preparation and identification of highly potent and selective inhibitors of various zinc-metalloproteases.[3b, 4a,c, 7b, c, 12, 15a, 35] These synthetic routes rely mainly on the use of a building block approach.[36] The past few years have also witnessed explosive developments in the solid-phase synthesis of heterocyclic derivatives.[37] The isoxazole ring undoubtedly belongs to the class of compounds whose solid-phase construction has been extensively studied.[34, 37, 38]

Following the same trend, we were interested in developing conditions, which would allow construction of the final isoxazole-containing phosphinic inhibitors on the solid support. Were such an approach successfully developed, it would prove to be very convenient for the rapid construction of the target molecules in quantities sufficient for biological evaluation. Moreover, during a solid-phase synthesis of isoxazoles, one can with advantage use a large excess of nitrile oxides to drive the DCRs to completion, without being concerned about the removal of this excess from the target compounds. On the contrary, solution-phase synthesis of isoxazoles is often problematic due to the high propensity of nitrile oxides to undergo dimerization to furoxan N-oxide, which renders both isolation and purification of the desired compound difficult.[22] In addition, development of a solid-phase version of our strategy for the preparation of this novel class of phosphinic peptides would set the first example of post-modification in this field.

To date, solid-phase synthesis of phosphinic peptides has been realized mainly through the building block approach and in all cases, the used blocks lack of any side-chain functionality.[36] Another issue often addressed concerns the necessity of prior protection of the hydroxyphosphinyl moiety.[15a] Most synthetic schemes rely on the use of P-protected precursors; however, coupling of phosphinic dipeptidic blocks bearing an unprotected hydroxyphosphinyl moiety to solid-phase support has very briefly reported only once.[39] The use of P-unprotected dipeptidic blocks offers a few advantages such as fewer synthetic steps and easier analysis of the intermediates, but on the other hand it involves by-product formation because of the hydroxyphosphinyl moiety activation under the reaction conditions.[16] In our case, due to the existence of a triple bond not all protection schemes present orthogonality (e.g. hydrogenation of Cbz group would lead to simultaneous reduction of the alkyne moiety). Moreover, introduction of the adamantyl group into the phosphinic moiety[15a] proved a little troublesome, in addition to the potential steric hindrance that would exert to the subsequent DCRs. Therefore, we decided to develop a method that would allow the use of a P-unprotected dipeptidic block such as **2a** (Scheme 1).



Scheme 5. Solid-phase synthesis of the isoxazole-containing phosphinic tripeptide array of type **9**: i) 20% pip/DMF; ii) FmocTrpOH, DIC, HOBt, CH_2Cl_2/DMF; iii) **2a** or **2a′**, EDC·HCl, HOBt, DIPEA, CH_2Cl_2, RT, 2 h; iv) $R^4CH{=}NOH$ (oxime), NCS, Py (cat.), $CHCl_3$, 45 °C; v) Et_3N, 45 °C; vi) TFA/H_2O/TIS (95:2.5:2.5).

Another key-feature of our solid-phase synthetic effort is the use—for first time in the phosphinic peptide field—of Mimotopes SynPhase PS Lanterns (L-series "pins").[40] Use of multipin technology is emerging as a viable alternative to conventional resin solid-phase synthesis and is gaining in significance owing to its many significant fundamental advantages over resins.[28, 41] Given our desire to obtain C-terminal amides, we chose pins functionalized with a Rink amide linker and set out to develop and evaluate strategies for the preparation of a few-member combinatorial array of isoxazole-containing phosphinic tripeptides of the general type **9** (Scheme 5).

Our synthetic efforts (Scheme 5) commenced with coupling of Fmoc-protected tryptophan with pin-supported Rink amide linker under standard conditions to afford the intermediate **6a**. Piperidine-mediated removal of Fmoc protection delivered **6b**. At this point we had to establish a method for coupling of the P-unprotected dipeptidic block **2a** with amine **6b**. The previously described method[39] for a similar coupling was not thoroughly documented and described the simple building block [Z(*R*)PheΨ[(P(O)(OH)CH_2]GlyOH], and therefore, if employed, would require optimization. Although our solution coupling method has never been applied on the solid phase, we were very much interested in testing and evaluating its efficiency under solid-phase conditions. Special care had to be taken because the use of pins imply a standard, low loading (14 μmol per pin) and conditions applied in solution or on resin experiments needed careful evaluation to avoid both large excesses of reagents and overly dilute reaction solutions. In addition, the existence of a free hydroxyphosphinyl moiety called for further limitations.

Preliminary experiments using EDC/HOBt-mediated coupling started by using the block **2a** (not resolved at P_1' position). Use of one equivalent of dipeptidic block in several solvent systems (e.g. DMF, DMF/CH_2Cl_2, CH_2Cl_2/THF) proved completely unsuccessful, delivering the intermediate **7** in unacceptable yields (≈50%, RP-HPLC analysis). The best result was achieved using CH_2Cl_2 as solvent, probably due to high swelling of pins.[41b] Therefore, all subsequent experiments were carried out in this convenient solvent. *Extensive experimentation* revealed that the best course of action was double coupling using: 1.5 equivalent (per pin) of **2a**, four equivalents of EDC·HCl, 1.5 equivalent of HOBt, 2.5 equivalents of DIPEA in CH_2Cl_2 (0.25 mL per pin) for 3 h. This protocol allowed the smooth synthesis of **7** quantitatively and in excellent purity. Parallel studies showed that the DIC coupling reagent could perform such couplings but it gave lower yields (ca. 20%) than the EDC/HOBt system. In addition, when the coupling time was extended to 24 h partial racemization occurred.

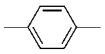
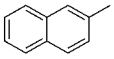
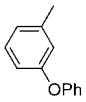
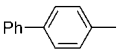
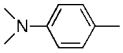
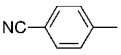
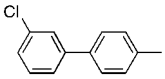
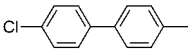
To examine this racemization problem during the EDC/HOBt-mediated coupling, we used the optically pure dipeptidic block **2a′**. Unfortunately, coupling of **2a′** for 24 h gave rise to an unexpectedly high racemization percentage (≈17%). We were not aware of any studies (concerning P_1' position) that could provide us with relevant data. Thereby, reevaluation of our established protocol had to be considered in order to minimize racemization in the case that optically pure blocks were used. Fortunately, shorter reaction times proved a very good remedy for this predicament. Thus, the

standard protocol was still applicable, but two couplings for *exactly* 2 h were necessary to obtain the tripeptidic block **7** in high yield and high optical purity. Given the fact that the starting dipeptidic block had an optical purity ≈98% (see below), the estimated racemization by application of this modified protocol was about 1%. This result was considered acceptable given the oligomeric nature of phosphinic peptides (only one phosphinic block incorporated in the peptidic sequence). PyBOP-mediated[39] coupling of **2a′** did not offer any additional advantage.

The mechanism of coupling of phosphinic dipeptides with amino acids has not been studied and reported, but it may be assumed that it involves the prior formation of a relatively hindered, and therefore more susceptible to racemization than a classical HOBt C-active ester, mixed cyclic anhydride intermediate, which is later on attacked by the amino component.[9a] The fact that no racemization at all was observed under solution- versus solid-phase condition couplings (see above) may be related to some special features of the microenvironment of the resin (e.g. more difficult attack of the pin-attached amine onto the presumed cyclic mixed anhydride, extended reaction time for formation of such an anhydride etc.). The hypothesis of the cyclic anhydride versus classical C-active ester formation is supported by the fact that performance of the same coupling (EDC/HOBt protocol) in the presence of copper(II) chloride[42] did not cut down on recemization as it has been reported, but actually decreased the yield and increased the extent of racemization (≈7%). This was presumably the result of difficulty/delay in the formation of the mixed anhydride due to potential coordination of copper to the phosphinyl moiety.[43]

Having assembled the tripeptidic unit **7** on solid support as both optically pure and mixture of epimers at P_1' position, we focused our efforts on studying the incorporation of the isoxazole core into these precursors. To this end, a one-pot Huisgen method (see above) was applied. Optimization of DCRs of nitrile oxides onto **7** was easily and rapidly made by using multipin technology. The model used in these studies was compound **9a** (see below, R^4 = *p*-methyl phenyl, Table 4). Several parameters were studied such as concentration of nitrile oxide, temperature, reaction time. Our results are illustrated in Figure 6. From this Figure it is obvious that the best set of conditions for conducting DCRs is the use of a 0.56 M chloroform solution (namely 10 equivalents per pin) of in situ generated nitrile oxide and gentle heating (45 °C) for 24 h. Prolonged reaction times (e.g. 48 h) did not improve the yield, probably due to dimerization of nitrile oxides after a certain point; therefore all subsequent experiments were conducted for 24 h.

Table 4. Characteristics of the members of the isoxazole-phosphinic peptide combinatorial array **9a–k**, prepared by DCRs on pins.

Entry	R^4	Yield[a] [%]	Purity[b] [%]	t_R value[c] [min][d]	M_W	ES-MS $[M-H]^-$
9a		82	87	27.4/28.8	747.2	746.3
9b		69	89	28.9	783.2	782.3
9c		66	70	30.7/31.9	825.3	824.2
9d		89	81	30.5/31.9	809.3	808.3
9e		93 (54)[e]	71 (95)[e]	21.4/22.1	776.3	775.3
9f		78	89	26.1/27.1	793.3	792.3
9g		55[f]	61[f]	25.5/26.1	758.3	757.2
9h		81	82	32.5/34.1	843.3	842.3
9i		73	80	33.6/35.3	843.3	842.2
9j		77	83	26.0	733.3	732.2
9k		65	81	26.9	811.2	810.1

[a] Based on the initial loading of pins (14 μmol per pin). [b] Concluded by combining area of product HPLC peaks (254 and/or 280 nm detection). [c] Each HPLC analysis was carried out with the gradient described at the experimental section, general procedures. [d] The double time value corresponds to the two diastereomers of the product; in the case of optically pure inhibitor only one diastereomer was detected. [e] In parentheses yield and purity after post-cleavage solution extraction. [f] Low yield and purity due to uncompleted DCR; the starting tripeptide was also recovered in the TFA-cleaved mixture.

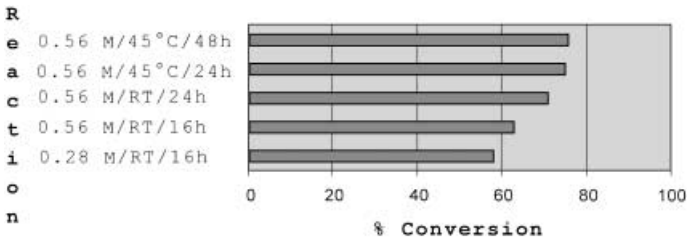
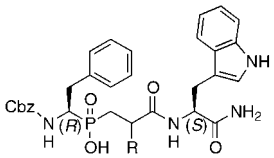
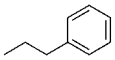
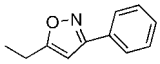
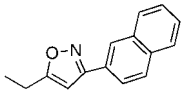
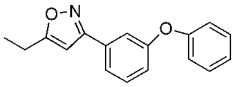


Figure 6. Optimization of nitrile oxide DCRs onto the pin-supported dipolarophilic tripeptidic unit **7**. Concentration at this histogram (left) is that of nitrile oxide generated in situ from the corresponding hydroxyiminoyl chloride. % Conversion was concluded by combining area of product HPLC peaks.

Application of this optimized set of conditions allowed the facile preparation of the desired combinatorial array of type **9**. HPLC monitoring of the progress of the DCRs indicated that three to four repetitions of the addition were necessary in order to achieve a high enough percentage of conversion and purity of the target molecules. This course of action afforded the peptides **9** in very good yields and purities after TFA-effected cleavage from the solid support, as shown in Table 4. The representative analytical HPLC chromatogram of the crude tripeptide **9b** (Figure 7), clearly indicates the high purity of the product. It is worth mentioning that when optically pure tripeptides were used as precursors to DCRs, no racemization was observed. In the case of strongly deactivated benzaldoximes such as **9g** (*p*-CN phenyl, Table 4), the addition was not driven to completion even after four repetitions. Nevertheless, the desired peptide could be isolated by post-cleavage (solution) extraction or semipreparative HPLC. All peptides of type **9** were identified by direct comparison with solution phase produced peptides (e.g. **9b**,**j**) as well as by electrospray mass spectrometry (Table 4).

Table 5. K_i Values of some isoxazole-containing phosphinic peptides and RXP03 toward MMP-13 and MMP-14.



Compound	R	Diastereomer	K_i [nM] MMP-13	K_i [nM] MMP-14
RXP03		*R*,*S*,*S*	16	90
		R,*R*,*S*	32	386
5a		*R*,*S*,*S*	1.6	2.5
		R,*R*,*S*	160	330
5b		*R*,*S*,*S*	14	20
5c		*R*,*S*,*S*	28	5100

Biological Evaluation

At preliminary stage, some of the developed isoxazole-containing phosphinic peptides were evaluated for their in vitro activities to inhibit two MMPs: collagenase-3 (MMP-13), which is over-expressed in breast carcinomas[44] and membrane type-1 matrix metalloprotease (MT1-MMP, MMP-14), a primary target to block pro-MMP-2 activation.[45] Our results are summarized in Table 5.

Comparison of the K_i values of Table 5 clearly indicates that isoxazole-containing phosphinic peptides are more potent MMP inhibitors than alkyl-aryl analogues, such as RXP03. For example, compound **5a**-(*R*,*S*,*S*) is 36-fold more potent toward MMP-14 than RXP03-(*R*,*S*,*S*); this indicates the advantages of this novel class of phosphinic peptides. The high potency displayed by compound **5a**-(*R*,*S*,*S*) demonstrates that the stereochemistry and the electronic properties of the isoxazole ring are very well suited to interact with S_1' pocket of MMPs. This compound is one of the most potent phosphinic inhibitors of MMP-14, well compared with reported hydroxamic inhibitors such as Marimastat ($K_i = 1.8$ nM toward MMP-14).[12] Moreover, the fact that the compound **5a**-*R*,*S*,*S* is at least 100-fold more potent than **5a**-*R*,*R*,*S*, reveals that the stereochemistry of the P_1' side chain is critical for potency in this series of isoxazole compounds. This critical role of the P_1' stereochemistry was no previously observed with compound such as RXP03. This difference is probably related to the presence of the isoxazole ring that involves higher conformational restriction than aryl alkyl side chains. In this respect, as shown by compound **5c**, further substitution of the isoxazole-attached ring may represent a strategy to control the selectivity of the inhibitors. Indeed, compound **5c** is 180-fold more potent toward MMP-13 than MMP-14. This issue is particularly important, as there is a high demand for compounds able to block only a subset of MMPs.[45–47] Substitution of this kind, using the approach reported in this study, will give access to various compounds with improved potency and selectivity toward the MMPs. Full evaluation of all prepared compounds is in progress and will be reported soon.

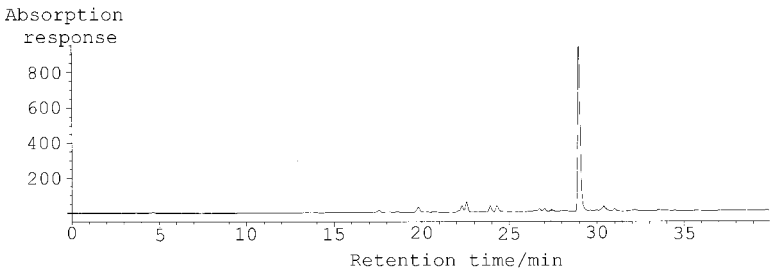


Figure 7. Analytical RP-HPLC chromatogram of the crude phosphinic peptide **9b** (one diastereomer).

Conclusion

The efficient synthesis and resolution of phosphinic peptides containing a propargylic side chain at P_1' position was described. Their absolute configuration was established using NMR studies. These pseudopeptidic blocks were studied

for their reactivity toward 1,3-dipolar cycloaddition reactions (DCRs) with nitrile oxides. To this end, different methods of cycloadditions were evaluated, leading to the successful development of a novel class of phosphinic peptides, incorporating an isoxazole ring at P_1' position, in high yields and optical purity. Extension of this strategy on solid-phase support was achieved using multipin technology. Development of a new racemization-free protocol for coupling of P-unprotected dipeptidic blocks with solid-phase-embodied amino acids and subsequent optimization of DCRs onto the produced peptides allowed the preparation of a few-member combinatorial array of isoxazole-containing phosphinic peptides. The crude pseudopeptides obtained from the solid-phase synthesis were of high purity according to RP-HPLC analysis. Preliminary inhibition assays of these new heterocyclic pseudopeptides demonstrated that they are very potent inhibitors of MMPs.

Current work in our laboratory is aiming at the development of libraries of this class of phosphinic peptides, widely diversified around the heterocyclic core and the P_2' position. Full report of inhibitory properties of these compounds toward MMPs and other Zn-metalloproteases will be reported in a forthcoming paper.

Experimental Section

Abbreviations: ACE-I: angiotensin converting enzyme I, AcOEt: ethyl acetate, DCR(s): 1,3-dipolar cycloaddition reaction(s), DIC: *N*,*N*′-diisopropylcarbodiimide, DIPEA: *N*,*N*-diisopropylethylamine, DMF: *N*,*N*-dimethyl formamide, EDC·HCl: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, Et_3N: triethylamine, HMDS: 1,1,1,3,3,3-hexamethyldisilazane, HOBt: *N*-hydroxy benzotriazole, MMPs: matrix metalloproteases, NCS: *N*-chloro succinimide, Py: pyridine, PyBOP: benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, RP-HPLC: reversed phase high performance liquid chromatography, TFA: trifluoroacetic acid, THF: tetrahydrofuran, TIS: triisopropylsilane, TMSCl: trimethylsilyl cloride, Ψ: symbol for peptide surrogates.

General procedures: Most anhydrous solvents were obtained by storing analytical quality solvents over 4 Å activated molecular sieves. DMF and THF were fractionally distilled (over P_2O_5 and CaH_2 respectively) and stored over 4 Å activated molecular sieves. Synthetic starting materials were purchased from Aldrich, Fluka, NovaBiochem or Bachem and were used without further purification. SynPhase PS Lanterns (L-series pins) and Multipin stems were purchased from Mimotopes, Paris, France. All aminophosphinic acids[24] and acrylic derivatives[23] were prepared according to previously described methods. Oximes used in this work were prepared from the corresponding aldehydes under standard procedures.[32b, 48] Preparation and full characterization of compound **4a** will be reported elsewhere. Chemical reactions, washings and cleavages involving pins were performed in chemically resistant glass tubes. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on Varian spectrometers (200 MHz Mercury or 300 MHz Gemini models). Assignment of the ^1H NMR signals were achieved using COSY and, in some cases only, HMQC experiments. ^{13}C and ^{31}P NMR spectra are fully proton decoupled. All spectra are referenced (δ scale in ppm) using the residual solvent as an internal standard (e.g. for [D_6]DMSO; $\delta = 2.50$, ^1H and $\delta = 39.50$, ^{13}C). ^{31}P NMR chemical shifts are reported in ppm downfield from 85% H_3PO_4 (external standard). Column chromatography was performed on silica gel (E. Merck, 70–230 mesh). TLC analyses were performed on silica gel plates (E. Merck silica gel 60 F_{254}) and components were visualized by the following methods: UV light absorbance, and/or charring after spraying with a solution of NH_4HSO_4, and/or ninhydrin spraying and heating. Melting points (measured on a Electrothermal apparatus) are uncorrected. Electron spray mass spectrometry (ESMS) was performed on a Micromass Platform II instrument (Atheris Laboratories, Geneva, Switzerland). RP-HPLC analyses were performed on a Hewlett Packard 1100 model (C_{18}-Cromasil-RP, 5 μm, UV/Vis detector, flow: 0.5 mL min^{-1}, 254 and/or 280 nm detection), with the gradient: 0 min: 0% B buffer, 10 min: 35% B buffer, 35 min: 100% B buffer, 40 min: 35% B buffer (A buffer: 90% H_2O with 0.1% TFA, 10% CH_3CN; B buffer: 10% H_2O with 0.09% TFA, 90% CH_3CN). Enzyme assays and K_i values determination were performed as previously described.[4b]

General procedures for Michael addition reaction

HMDS method: A mixture of Cbz N-protected α-aminophosphinic acid (1 mmol) and HMDS (5 mmol) was heated at 110 °C for 1 h under Ar, then ethyl α-propargyl acrylate (1.3 mmol) was added dropwise. The resulting mixture was stirred at 100–105 °C for additional 3 h and slowly cooled to RT. Then, the mixture was further cooled to −10 °C and dry ethanol was added portionwise, still under Ar. The cooled mixture was stirred at this temperature for 1 h and was left to reach RT. The solvent was removed under vacuum and the residue was purified by column chromatography (chloroform/methanol/acetic acid 7:0.3:0.3).

TMSCl/DIPEA method: DIPEA (4.5 mmol) and TMSCl (4.5 mmol) were added under Ar to an ice-cold suspension of Cbz N-protected α-aminophosphinic acid (1 mmol) in dry CH_2Cl_2 (7 mL per mmol). The mixture was stirred for 2 h at RT. Then, the solution was cooled to 0 °C, and ethyl α-propargyl acrylate (1.3 mmol) was added dropwise. The mixture was stirred at RT for 36 h under Ar. Then, it was cooled to −10 °C and dry ethanol was added portionwise, still under Ar. The cooled mixture was stirred at this temperature for 1 h and was left to reach RT. The solvents were removed under vacuum and the residue was purified by column chromatography (chloroform/methanol/acetic acid 7:0.3:0.3).

(1*R*)-1-{[(Benzyloxy)carbonyl]amino}-2-phenylethyl[2-(ethoxycarbonyl)-pent-4-ynyl]phosphinic acid (1a): M.p. 113–115 °C; $R_f = 0.68$ (chloroform/methanol/acetic acid 7:0.5:0.5). ^1H NMR (200 MHz, [D_6]DMSO/1% CF_3COOD): $\delta = 1.26$ (t, $^3J(HH) = 6.8$ Hz, 3H; CH_2CH_3), 1.78–2.18 (m, 2H; PCH_2), 2.38–3.18 (m, 6H; PCH_2CH, CHHPh, CH*H*Ph, $CH_2C{\equiv}CH$, $C{\equiv}CH$), 3.81–4.19 (m and q, $^3J(HH) = 6.8$ Hz, 3H; CH_2CH_3, PC*H*), 4.97 (s, 2H; $PhCH_2O$), 7.06–7.38 (m, 10H; Ar), 7.72 (d, $^3J(HH) = 9.7$ Hz, 1H; N*H*); ^{13}C NMR (50 MHz, [D_6]DMSO/1% CF_3COOD): $\delta = 14.1$, 21.8, 26.9 (d, $J(PC) = 88.4$ Hz), 32.6, 38.0, 52.2 (d, $J(PC) = 104.3$ Hz), 60.5, 65.2, 73.2 (d, $J(PC) = 15.5$ Hz), 80.9, 113.4, 126.2, 127.2, 128.2, 129.2, 137.2, 138.4 (d, $J(PC) = 14$ Hz), 156.1 (d, $J(PC) = 3.8$ Hz), 172.9 (d, $J(PC) = 10.8$ Hz); ^{31}P NMR (81 MHz, [D_6]DMSO/1% CF_3COOD): $\delta = 45.0$, 45.3, two diastereomers: (*R*,*S*)/(*R*,*R*) = 3.5/1 (**1a**, see Table 1), 4.5:1 (**1a**, see Table 1); elemental analysis calcd (%) for $C_{24}H_{28}NO_6P$: C 63.01, H 6.17, N 3.06; found: C 62.71, H 5.92, N 2.81; ES-MS: *m*/*z*: calcd for $C_{24}H_{27}NO_6P$: 456.2; found: 456.3 $[M-H]^-$.

(1*R*)-1-{[(Benzyloxy)carbonyl]amino}ethyl[2-(ethoxycarbonyl)pent-4-ynyl]phosphinic acid (1b): M.p. 95–97 °C; $R_f = 0.38$ (chloroform/methanol/acetic acid 7:0.5:0.5). ^1H NMR (200 MHz, [D_6]DMSO/1% CF_3COOD): $\delta = 1.22$ (dd, $^3J(HH) = 7.1$ Hz, $^3J(PH) = 15.8$ Hz, 3H; $CHCH_3$), 1.27 (t, $^3J(HH) = 6.8$ Hz, 3H; CH_2CH_3), 1.68–1.93 (m, 1H; PC*H*H), 1.95–2.20 (m, 1H; PCH*H*), 2.47–2.62 (m, 2H; $CH_2C{\equiv}CH$), 2.72–2.96 (m, 2H; PCH_2CH, $C{\equiv}CH$), 3.62–3.95 (m, 1H; PC*H*), 3.99–4.18 (q, $^3J(HH) = 6.8$ Hz, 2H; CH_2CH_3), 5.02 (s, 2H; $PhCH_2O$), 7.23–7.39 (m, 5H; Ar), 7.61 (d, $^3J(HH) = 9.6$ Hz, 1H; N*H*); ^{13}C NMR (50 MHz, [D_6]DMSO/1% CF_3COOD): $\delta = 13.8$, 14.1, 21.4, 26.2 (d, $J(PC) = 87.9$ Hz), 37.7, 45.9 (d, $J(PC) = 105.4$ Hz), 60.3, 65.6, 72.7, 81.1, 112.3, 118.0, 127.7, 127.8, 128.4, 137.2, 156.3 (d, $J(PC) = 3.9$ Hz), 172.7 (d, $J(PC) = 10.5$ Hz); ^{31}P NMR (81 MHz, [D_6]DMSO/1% CF_3COOD): $\delta = 45.8$, 46.0, two diastereomers (*R*,*S*)/(*R*,*R*) = 3.5/1; elemental analysis calcd (%) for $C_{18}H_{24}NO_6P$: C 56.69, H 6.34, N 3.67; found: C 56.51, H 6.28, N 3.52.

(*R*)-{[(benzyloxy)carbonyl]amino}phenylmethyl[2-(ethoxycarbonyl)pent-4-ynyl] phosphinic acid (1c): M.p. 98–100 °C; $R_f = 0.55$ (chloroform/methanol/acetic acid 7:0.5:0.5); ^1H NMR (200 MHz, [D_6]DMSO/1% CF_3COOD): $\delta = 1.21$ (t, $^3J(HH) = 6.9$ Hz, 3H; CH_2CH_3), 1.65–1.94 (m, 1H; PC*H*H), 1.96–2.22 (m, 1H; PCH*H*), 2.42–2.65 (m, 2H; $CH_2C{\equiv}CH$, [D_6]DMSO overlapping), 2.70–2.95 (m, 2H; PCH_2CH, $C{\equiv}CH$), 3.99–4.20 (q, $^3J(HH) = 6.8$ Hz, 2H; CH_2CH_3), 4.83–5.12 (m, 3H; $PhCH_2O$, PC*H*), 7.17–7.58 (m, 10H; Ar), 7.82 (d, $^3J(HH) = 9.6$ Hz, 1H; N*H*); ^{13}C NMR (50 MHz, [D_6]DMSO/1% CF_3COOD): $\delta = 14.1$, 21.7 (d, $J(PC) = 7.6$ Hz), 26.9 (d, $J(PC) = 92.1$ Hz), 54.8 (d, $J(PC) = 100.2$ Hz), 60.4, 65.5, 73.1, 80.8, 113.3, 126.2, 127.1, 128.5, 129.2, 137.3, 138.4 (d, $J(PC) = 14$ Hz), 155.3 (d,

J(PC) = 3.8 Hz), 172.7 (d, J(PC) = 11.3 Hz); ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 42.1, 42.4, two diastereomers (*R*,*S*)/(*R*,*R*) = 3.5/1; elemental analysis calcd (%) for $C_{23}H_{26}NO_6P$: C 62.30, H 5.91, N 3.16; found: C 62.22, H 6.18, N 3.33.

General procedure for removal of ethyl ester: Dipeptides **1a–c** (1 mmol) were dissolved in ethanol (9 mL per mmol), and the solution was cooled to 0 °C. 1M NaOH (5–6 mmol) was added portionwise and the mixture was stirred at RT for 6–8 h. After acidification with 2M HCl, ethanol was evaporated, diluted with water, and extracted with AcOEt. The organic phase was washed with water and brine, dried over Na_2SO_4 and evaporated under vacuum. Precipitation from a mixture of diethyl ether/light petroleum (1:4) afforded the desired diacids as white solids.

(2*S*)-2-{[((1*R*)-1-{[(benzyloxy)carbonyl]amino}-2-phenylethyl)(hydroxy)-phosphoryl] methyl}pent-4-ynoic acid (2a′): Recrystallization of the precipitated diacid from AcOEt (30 mL per mmol) afforded the pure diastereomer, yield: 96% (for saponification), (70% based on the pure diastereomer); M.p. 176–178 °C; R_f = 0.21 (chloroform/methanol/acetic acid 7:0.5:0.5); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.76–2.18 (m, 2H; PCH_2), 2.38–2.97 (m, 5H; PCH$_2$C*H*, C*H*HPh, CH_2C≡CH, C≡C*H*), 2.99–3.17 (m, 1H; CH*H*Ph), 3.81–4.05 (m, 1H; PC*H*), 4.96 (s, 2H; PhCH_2O), 7.05–7.41 (m, 10H; Ar), 7.76 (d, ^3J(HH) = 9.7 Hz, 1H; N*H*); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 21.6, 26.8 (d, J(PC) = 88.8 Hz), 32.6, 38.2, 52.4 (d, J(PC) = 104.6 Hz), 65.2, 73.4, 80.7, 113.4, 126.3, 127.2, 128.2, 129.3, 137.2, 138.3 (d, J(PC) = 13.8 Hz), 157.2 (d, J(PC) = 3.8 Hz), 174.1 (d, J(PC) = 10.6 Hz); ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 45.7 (45.3 for **2a**); elemental analysis calcd (%) for $C_{22}H_{24}NO_6P$: C 61.54, H 5.63, N 3.26; found: C 61.67, H 5.48, N 3.43.

2-{[((1*R*)-1-{[(benzyloxy)carbonyl]amino}ethyl)(hydroxy)phosphoryl]methyl}pent-4-ynoic acid (2b): Yield: 92%; M.p. 149–151 °C; R_f = 0.32 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.22 (dd, ^3J(HH) = 7.1 Hz, ^3J(PH) = 14.1 Hz, 3H; CHCH_3), 1.70–1.92 (m, 1H; PC*H*H), 1.96–2.20 (m, 1H; PCH*H*), 2.47–2.62 (m, 2H; CH_2C≡CH), 2.75–2.96 (m, 2H; PCH$_2$C*H*, C≡C*H*), 3.65–3.96 (m, 1H; PC*H*), 5.03 (s, 2H; PhCH_2O), 7.21–7.40 (m, 5H; Ar), 7.61 (d, ^3J(HH) = 9.7 Hz, 1H; N*H*); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 13.8, 21.5, 26.2 (d, J(PC) = 87.2 Hz), 37.7, 45.7 (d, J(PC) = 105.2 Hz), 65.5, 72.8, 81.3, 112.3, 118.1, 127.7, 127.8, 128.4, 137.0, 158.4 (d, J(PC) = 3.8 Hz), 174.2 (d, J(PC) = 10.3 Hz); ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 46.3, 46.9, two diastereomers (*R*,*S*)/(*R*,*R*) = 3.5/1; elemental analysis calcd (%) for $C_{16}H_{20}NO_6P$: C 54.39, H 5.71, N 3.96; found: C 54.51, H 5.88, N 3.83.

2-{[[(*R*)-{[(benzyloxy)carbonyl]amino}(phenyl)methyl](hydroxy)phosphoryl]methyl} pent-4-ynoic acid (2c): Yield: 95%; M.p. 163–165 °C; R_f = 0.38 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.63–1.95 (m, 1H; PC*H*H), 1.98–2.23 (m, 1H; PCH*H*), 2.24–2.96 (m, 4H; CH_2C≡CH, [D$_6$]DMSO overlapping, PCH$_2$C*H*, C≡C*H*), 4.84–5.11 (m, 3H; PhCH_2O, PC*H*), 7.15–7.55 (m, 10H; Ar), 8.23 (d, ^3J(HH) = 9.7 Hz, 1H; N*H*); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 21.6, 26.8 (d, J(PC) = 94.3 Hz), 54.9 (d, J(PC) = 101.3 Hz), 65.5, 73.1, 80.8, 113.4, 126.2, 127.1, 128.6, 129.2, 137.3, 138.5 (d, J(PC) = 14 Hz), 156.7 (d, J(PC) = 3.9 Hz), 174.2 (d, J(PC) = 11.1 Hz); ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 42.1, 42.4, two diastereomers (*R*,*S*)/(*R*,*R*) = 3.5/1; elemental analysis calcd (%) for $C_{21}H_{22}NO_6P$: C 60.72, H 5.34, N 3.37; found: C 60.41, H 5.21, N 3.11.

General procedure for coupling of diacids 2a–c with tryptophan amide (3a–c): To a suspension of **2a-c** (1 mmol) in CH_2Cl_2 (20 mL per mmol) DIPEA (3 mmol), hydrochloric L-tryptophan amide (1 mmol), HOBt (1 mmol) and EDC·HCl (4 mmol) were added. The mixture was stirred for 1.5 h at RT. Then, it was diluted with CH_2Cl_2 and 1M HCl was added to form two phases. Upon addition of mineral acid the desired diastereomer (*R*,*S*,*S*) was precipitated. The cloudy organic phase was washed carefully once more with HCl and was separated. It was concentrated under vacuum and the residue was re-dissolved in AcOEt and cooled to 0 °C for several hours. The white solid, which was formed, was filtered, washed with dilute HCl, water, and AcOEt. For compound **3b**, resolution was more effectively performed in CH_2Cl_2, following the same work-up. Precipitation of the concentrated organic filtrates from diethyl ether/light petroleum (2:1) afforded a diastereomeric mixture of the tripeptidic units essentially enriched in the (*R*,*R*,*S*) isomer (e.g. for **3a** *de* ≈93%, HPLC analysis).

(2*S*)-2-({[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}carbonyl)pent-4-ynyl ((1*R*)-1-{[(benzyloxy)carbonyl]amino}-2-phenylethyl)-phosphinic acid (3a): M.p. 237–238 °C; R_f = 0.41 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.78–2.13 (m, 2H; PCH_2), 2.23–2.58 (m, 2H; CH_2C≡CH,), 2.60–2.97 (m, 3H; PCH$_2$C*H*, C*H*HPh, C≡C*H*), 2.99–3.23 (m, 3H; CH*H*Ph, CH_2indolyl), 3.84–4.09 (m, 1H; PC*H*), 4.36–4.49 (m, 1H; C*H*CONH$_2$), 4.96 (s, 2H; PhCH_2O), 6.83–7.38 and 7.40–7.59 (m, 18H; Ar, indolyl, N*H*$_2$), 7.69 (d, ^3J(HH) = 9.7 Hz, 1H; N*H*CHP), 8.11 (d, ^3J(HH) = 9.7 Hz, 1H; N*H*CHCONH$_2$); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 22.2, 27.8 (d, J(PC) = 86.7 Hz), 32.7, 38.2, 52.4 (d, J(PC) = 108.4 Hz), 53.5, 65.2, 72.7 (d, J(PC) = 19.7 Hz), 81.9, 110.5, 111.2, 112.3, 118.2, 120.7, 123.6 (d, J(PC) = 15.2 Hz), 126.3, 126.9, 127.2, 127.4, 128.2, 128.9, 136.1, 138.3, 138.4 (d, J(PC) = 13.6 Hz), 156.1 (d, J(PC) = 3.8 Hz), 172.2 (d, J(PC) = 8.3 Hz), 173.4; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 45.9 (46.6 for (*R*,*R*,*S*) diastereomer). HPLC: t_R = 22.3 min (23.3 min for (*R*,*R*,*S*) diastereomer); elemental analysis calcd (%) for $C_{33}H_{35}N_4O_6P$: C 64.49, H 5.74, N 9.12; found: C 64.31, H 5.65, N 9.01; ES-MS: m/z: calcd for $C_{33}H_{34}N_4O_6P$: 613.2; found: 613.2 $[M-H]^-$.

(2*S*)-2-({[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}carbonyl)pent-4-ynyl ((1*R*)-1-{[(benzyloxy)carbonyl]amino}ethyl)phosphinic acid (3b): M.p. 181–183 °C; R_f = 0.25 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.20 (dd, ^3J(HH) = 7.3 Hz, ^3J(PH) = 14.3 Hz, 3H; CHCH_3), 1.68–2.05 (m, 2H; PCH_2), 2.22–2.44 (m, 2H; CH_2C≡CH), 2.63–2.93 (m, 2H; PCH$_2$C*H*, C≡C*H*), 2.96–3.28 (m, 2H; CH_2indolyl), 3.63–3.87 (m, 1H; PC*H*), 4.28–4.43 (m, 1H; C*H*CONH$_2$), 5.04 (s, 2H; PhCH_2O), 6.90–7.59 (m, 14H; Ar, indolyl, N*H*CHP, N*H*$_2$), 8.05 (d, ^3J(HH) = 7.6 Hz, 1H; N*H*CHCONH$_2$); ^{13}C NMR (70 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 14.0, 22.3 (d, J(PC) = 9.2 Hz), 27.1 (d, J(PC) = 89.4 Hz), 27.2, 46.0 (d, J(PC) = 103.2 Hz), 53.6, 65.6, 72.6, 81.9, 110.4, 111.2, 118.3 (d, J(PC) = 15.9 Hz), 120.8, 123.5, 127.4, 127.7, 127.8, 128.3, 136.1, 137.0, 155.8 (d, J(PC) = 4.8 Hz), 172.1 (d, J(PC) = 8.1 Hz), 173.3; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 46.6. HPLC: t_R = 18.5 min (19.7 min for (*R*,*R*,*S*) diastereomer); elemental analysis calcd (%) for $C_{27}H_{31}N_4O_6P$: C 60.22, H 5.80, N 10.40; found: C 60.33, H 5.89, N 10.21; ES-MS: m/z: calcd for $C_{27}H_{30}N_4O_6P$: 537.2; found: 537.4 $[M-H]^-$.

(2*S*)-2-({[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}carbonyl)pent-4-ynyl [(*R*)-{[(benzyloxy)carbonyl]amino}(phenyl)methyl]phosphinic acid (3c): M.p. 197–199 °C; R_f = 0.39 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.63–2.13 (m, 2H; PCH_2), 2.18–2.63 (m, 2H; CH_2C≡CH, [D$_6$]DMSO overlapping), 2.65–2.91 (m, 2H; PCH$_2$C*H*, C≡C*H*), 2.94–3.32 (m, 2H; CH_2indolyl), 4.27–4.58 (m, 1H; C*H*CONH$_2$), 4.82–5.22 (m, 3H; PhCH_2O, PC*H*), 6.81–7.85 (m, 18H; Ar, indolyl, N*H*$_2$), 8.07 (d, ^3J(HH) = 9.2 Hz, 1H; N*H*CHP), 8.21 (d, ^3J(HH) = 9.2 Hz, 1H; N*H*CHCONH$_2$); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 22.4, 27.2, 27.4 (d, J(PC) = 91.0 Hz), 53.7, 55.8 (d, J(PC) = 98.1 Hz), 65.9, 72.8, 81.8, 110.4, 111.3, 118.3 (d, J(PC) = 11.7 Hz), 120.8, 123.6, 127.7, 128.0, 128.4, 136.1 (d, J(PC) = 8.3 Hz), 136.9, 156.1 (d, J(PC) = 3.1 Hz), 172.1 (d, J(PC) = 7.2 Hz), 173.2; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 42.4. HPLC: t_R = 21.3 min (22.3 min for (*R*,*R*,*S*) diastereomer); elemental analysis calcd (%) for $C_{32}H_{33}N_4O_6P$: C 63.99, H 5.54, N 9.33; found: C 63.83, H 5.39, N 9.18; ES-MS: m/z: calcd for $C_{32}H_{32}N_4O_6P$: 599.2; found: 599.3 $[M-H]^-$.

(1*R*)-1-{[(Benzyloxy)carbonyl]amino}-2-phenylethyl[(2*S*)-2-({[(1*S*)-2-methoxy-1-methyl-2-oxoethyl]amino}carbonyl)pent-4-ynyl]phosphinic acid (3d): Preparation was performed according to the general procedure for compounds **3a–c**, using the pure dipeptidic unit **2a′**. After the acidic work-up, the organic phase was dried and concentrated, and the tripeptide was received after precipitation from diethyl ether/light petroleum (1:2). M.p. 182–184 °C; R_f = 0.39 (chloroform/methanol/acetic acid 7:0.5:0.5); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.22 (d, ^3J(HH) = 6.7 Hz, 3H; CHCH_3), 1.73–2.13 (m, 2H; PCH_2), 2.33–2.60 (m, 2H; CH_2C≡CH, [D$_6$]DMSO overlapping), 2.62–2.97 (m, 3H; PCH$_2$C*H*, C*H*HPh, C≡C*H*), 2.99–3.18 (m, 1H; CH*H*Ph), 3.59 (s, 3H; OCH_3), 3.79–4.08 (m, 1H; PC*H*), 4.18–4.38 (m, 1H; C*H*COOCH$_3$), 4.98 (s, 2H; PhCH_2O), 7.02–7.41 (m, 10H; Ar), 7.62 (d, ^3J(HH) = 9.6 Hz, 1H; N*H*CHP), 8.41 (d, ^3J(HH) = 9.6 Hz, 1H; N*H*CHCOO); ^{13}C NMR (70 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 16.9, 22.3, 27.3 (d, J(PC) = 87.6 Hz), 32.9, 38.1, 47.7, 51.7, 52.6 (d, J(PC) = 95.9 Hz), 65.1, 72.6, 81.7, 126.2, 127.0, 127.5, 128.1, 128.3, 128.9, 137.2, 138.7 (d, J(PC) = 13.6 Hz),

156.0, 172.5 (d, J(PC) = 7.1 Hz), 173.0; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 45.8; HPLC: t_R = 21.9 min; elemental analysis calcd (%) for $C_{26}H_{31}N_2O_7P$: C 60.69, H 6.07, N 5.44; found: C 60.33, H 5.89, N 5.24; ES-MS: m/z: calcd for $C_{26}H_{30}N_2O_7P$: 513.2; found: 513.5 $[M-H]^-$.

General procedures for DCRs to tripeptidic units 3-d (5a–i)

One-pot method A (for aryl-type aldoximes): The corresponding oxime (6 equiv) was dissolved in $CHCl_3$ (5 mL per mmol), and two drops of pyridine were added. Then, NCS (6 equiv) was added at RT and after 10 min the resulting mixture was stirred at 45 °C for 3–4 h. Tripeptides **3a–c** were after added (1 equiv), followed by slow addition of Et_3N (7 equiv) at the same temperature. The reaction mixture was stirred for 36–60 h at 45 °C. Then, it was diluted with AcOEt and washed with 1M HCl (×2), water, a 10% solution of NH_4HCO_3 (×2; 1 mL for 30 mL AcOEt), 1M HCl (×2) and brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. Precipitation from diethyl ether and washings with diethyl ether/light petroleum afforded the desired compounds (**5a–c**, **e–i**). Recovery of the starting tripeptide from the basic solution could be performed by acidification and extraction with AcOEt. This peptide can be re-subjected to DCRs (e.g. **5f**).

Two-step method B (for alkyl aldoximes e.g. product **5d**): The corresponding oxime (6 equiv) was dissolved in DMF (1 mL per mmol) and NCS (6.5 equiv) was added at RT. The resulting mixture was stirred at 45–50 °C for 40 min and then it was diluted with diethyl ether and extracted rapidly with water (×2). The organic phase was dried (Na_2SO_4) and concentrated and the crude hydroximinoyl chloride was used immediately in DCRs. Thus, this residue was dissolved in CH_2Cl_2 containing molecular sieves 4 Å, and the tripeptide **3a** was added (1 equiv), followed by slow addition of Et_3N (7 equiv) at RT. The reaction mixture was stirred for 48 h at RT. Then, it was diluted with AcOEt and washed with 1M HCl (×2), water, a 10% solution of NH_4HCO_3 (×2; 1 mL for 30 mL AcOEt), 1M HCl (×2) and brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. Precipitation from diethyl ether/light petroleum (0.5:9.5) and washings with this solvent mixture afforded the desired compound (**5d**). Recovery of the starting tripeptide from the basic solution could be once more performed by acidification and extraction with AcOEt.

3-{[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}-3-oxo-2-[(3-phenylisoxazol-5-yl)methyl]propyl((1*R*)-1-{[(benzyloxy)carbonyl]amino}-2-phenylethyl)phosphinic acid (5a): M.p. 165–169 °C; R_f = 0.47 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 1.62–2.21 (m, 2H; PCH_2), 2.52–2.82 (m, 2H; CHHPh, CHHindolyl), 2.84–3.39 (m, 5H; CH_2isoxazolyl, PCH$_2$CH, CHHPh, CHHindolyl), 3.82–4.17 (m, 1H; PCH), 4.33–4.57 (m, 1H; CHCONH$_2$), 4.92 (s, 2H; PhCH_2O), 6.39 (*R*,*R*,*S*) and 6.57 (*R*,*S*,*S*) (s, 1H; H of isoxazole ring), 6.62–7.92 (m, 24H; Ar, indolyl, NHCHP, NH_2), 8.23 (*R*,*S*,*S*) and 8.49 (*R*,*R*,*S*) (d, ^3J(HH) = 9.2 Hz, 1H; NHCHCONH$_2$); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 27.6, 29.6, 32.8, 52.4 (d, J(PC) = 105.3 Hz), 53.5, 65.2, 100.1, 100.6, 110.9 (d, J(PC) = 43.1 Hz), 111.6 (d, J(PC) = 63.6 Hz), 118.0, 118.3, 118.5, 120.9, 123.5, 126.2, 126.9, 127.0, 127.4, 127.5, 128.2, 128.5, 128.8, 129.0, 130.0, 136.1, 137.1, 138.3 (d, J(PC) = 14.0 Hz), 156.0, 161.7, 170.9, 171.6, 172.2 (d, J(PC) = 9.8 Hz), 173.5, 173.8; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 45.4 for (*R*,*S*,*S*) diastereomer, 46.2 for (*R*,*R*,*S*) diastereomer; HPLC: t_R = 26.0 min (*R*,*S*,*S*)/27.1 min (*R*,*R*,*S*); elemental analysis calcd (%) for $C_{40}H_{40}N_5O_7P$: C 65.48, H 5.49, N 9.54; found: C 65.22, H 5.33, N 9.29; ES-MS: m/z: calcd for $C_{40}H_{39}N_5O_7P$: 732.3; found: 732.5 $[M-H]^-$.

(2*S*)-3-{[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}-2-{[3-(2-naphthyl)isoxazol-5-yl]methyl}-3-oxopropyl((1*R*)-1-{[(benzyloxy)carbonyl]amino}-2-phenylethyl)phosphinic acid (5b): M.p. 208–210 °C; R_f = 0.60 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 1.68–2.22 (m, 2H; PCH_2), 2.52–2.83 (m, 2H; CHHPh, CHHindolyl), 2.85–3.39 (m, 5H; CH_2isoxazolyl, PCH$_2$CH, CHHPh, CHHindolyl), 3.82–4.16 (m, 1H; PCH), 4.37–4.61 (m, 1H; CHCONH$_2$), 4.94 (s, 2H; PhCH_2O), 6.78 (s, 1H; H of isoxazole ring), 6.82–8.43 (m, 27H; Ar, indolyl, NH, NH_2); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 27.6, 29.5, 32.9, 52.2 (d, J(PC) = 104.8 Hz), 53.5, 65.2, 100.3, 110.9 (d, J(PC) = 43.2 Hz), 118.3 (d, J(PC) = 11.3 Hz), 120.9, 123.7, 126.3, 126.4, 126.8, 127.0, 127.5, 127.8, 128.2, 128.5, 128.7, 129.0, 133.2 (d, J(PC) = 32.2 Hz), 136.1, 137.1, 138.3 (d, J(PC) = 14.0 Hz), 156.1, 161.8, 171.7, 172.2 (d, J(PC) = 9.8 Hz), 173.5; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 45.4; HPLC: t_R = 28.9 min; elemental analysis calcd (%) for $C_{44}H_{42}N_5O_7P$: C 67.42, H 5.40, N 8.94; found: C 67.09, H 5.22, N 8.79; ES-MS: m/z: calcd for $C_{44}H_{41}N_5O_7P$: 782.3; found: 782.3 $[M-H]^-$.

(2*S*)-3-{[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}-3-oxo-2-{[3-(3-phenoxyphenyl)isoxazol-5-yl]methyl}propyl((1*R*)-1-{[(benzyloxy)carbonyl]amino}-2-phenylethyl)phosphinic acid (5c): M.p. 215–218 °C; R_f = 0.64 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 1.68–2.21 (m, 2H; PCH_2), 2.52–2.85 (m, 2H; CHHPh, CHHindolyl), 2.83–3.37 (m, 5H; CH_2isoxazolyl, PCH$_2$CH, CHHPh, CHHindolyl), 3.82–4.16 (m, 1H; PCH), 4.37–4.61 (m, 1H; CHCONH$_2$), 4.96 (s, 2H; PhCH_2O), 6.76 (s, 1H; H of isoxazole ring), 6.85–8.58 (m, 29H; Ar, indolyl, NH, NH_2); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 27.6, 29.4, 32.9, 52.4 (d, J(PC) = 105.1 Hz), 53.4, 65.2, 100.2, 110.9 (d, J(PC) = 43.2 Hz), 118.4 (d, J(PC) = 11.2 Hz), 120.9, 123.7, 126.3, 126.6, 126.8, 127.1, 127.5, 127.8, 128.2, 128.5, 128.7, 129.0, 133.2 (d, J(PC) = 32.1 Hz), 136.1, 137.3, 138.4 (d, J(PC) = 14.0 Hz), 156.2, 156.8, 157.3, 161.7, 171.7, 172.3 (d, J(PC) = 9.7 Hz), 173.5; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 45.3; HPLC: t_R = 30.7 min; elemental analysis calcd (%) for $C_{46}H_{44}N_5O_8P$: C 66.90, H 5.37, N 8.48; found: C 66.55, H 5.18, N 8.36; ES-MS: m/z: calcd for $C_{46}H_{43}N_5O_8P$: 824.3; found: 824.7 $[M-H]^-$.

(2*S*)-3-{[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}-2-[(3-nonylisoxazol-5-yl)methyl]-3-oxopropyl((1*R*)-1-{[(benzyloxy)carbonyl]amino}-2-phenylethyl)phosphinic acid (5d): M.p. 76–80 °C; R_f = 0.91 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 0.84 (t, ^3J(HH) = 6.9 Hz, 3H; (CH$_2$)$_8$CH_3), 1.03–1.61 (m, 17H; CH$_2$(CH_2)$_7$CH$_3$, CH$_2$CH_2), 1.64–2.18 (m, 2H; PCH_2), 2.21–2.58 (m, 2H; CH_2(CH$_2$)$_7$CH$_3$, [D$_6$]DMSO overlapping), 2.59–2.83 (m, 2H; CHHPh, CHHindolyl), 2.83–3.39 (m, 5H; CH_2isoxazolyl, PCH$_2$CH, CHHPh, CHHindolyl, partially overlapped by the multiple peak at 2.59–2.83), 3.79–4.08 (m, 1H; PCH), 4.31–4.57 (m, 1H; CHCONH$_2$), 4.94 (s, 2H; PhCH_2O), 5.81 (s, 1H; H of isoxazole ring), 6.65–7.81 (m, 19H; Ar, indolyl, NHCHP, NH_2), 8.17 (d, ^3J(HH) = 9.2 Hz, 1H; NHCHCONH$_2$); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 14.0, 22.1, 25.3, 25.8, 26.2, 26.6, 27.0, 27.5, 27.6, 28.0, 28.7, 28.9, 31.3, 32.8, 52.5 (d, J(PC) = 105.3 Hz), 53.5, 65.2, 101.6, 110.5, 111.3, 112.3, 118.1, 118.3 (d, J(PC) = 11.0 Hz), 120.8, 123.5, 123.8, 126.2, 127.0, 127.4, 127.5, 128.2, 128.3, 129.0, 136.1, 137.2, 138.4 (d, J(PC) = 14.4 Hz), 156.1 (d, J(PC) = 3.4 Hz), 163.4, 170.1, 172.2 (d, J(PC) = 9.1 Hz), 173.4; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 45.4; HPLC: t_R = 35.8 min; elemental analysis calcd (%) for $C_{43}H_{54}N_5O_7P$: C 65.88, H 6.94, N 8.93; found: C 65.72, H 6.88, N 8.79; ES-MS: m/z: calcd for $C_{43}H_{53}N_5O_7P$: 782.4; found: 782.3 $[M-H]^-$.

(1*R*)-1-{[(Benzyloxy)carbonyl]amino}-2-phenylethyl{3-{[(1*S*)-2-methoxy-1-methyl-2-oxoethyl]amino}-3-oxo-2-[(3-phenylisoxazol-5-yl)methyl]propyl}phosphinic acid (5e): M.p. 145–148 °C; R_f = 0.41/0.30 (two diastereomers) (chloroform/methanol/acetic acid 7:1:0.5); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 1.18 (*R*,*R*,*S*) and 1.27 (*R*,*S*,*S*) (d, ^3J(HH) = 7.2 Hz, 3H; CHCH_3), 1.67–2.17 (m, 2H; PCH_2), 2.59–2.93 (m, 2H; CHHisoxazolyl, CHPHh), 2.97–3.46 (m, 3H; PCH$_2$CH, CHHPh, CHHisoxazolyl), 3.51 (s, 3H; OCH_3), 3.77–4.11 (m, 1H; PCH), 4.17–4.38 (m, 1H; CHCO$_2$CH$_3$), 4.84/4.98 (s, 2H; PhCH_2O), 6.75 (*R*,*R*,*S*) and 6.80 (*R*,*S*,*S*) (s, 1H; H of isoxazole ring), 6.87–7.88 (m, 16H; Ar, NHCHP), 8.41 (*R*,*R*,*S*) and 8.56 (*R*,*S*,*S*) (d, ^3J(HH) = 9.2 Hz, 1H; NHCHCOO); ^{13}C NMR (70 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 16.8, 22.3, 27.4 (d, J(PC) = 88.8 Hz), 32.9, 38.1, 47.6, 51.7, 52.8 (d, J(PC) = 96.1 Hz), 65.1, 100.2, 100.8, 126.2, 127.1, 127.3, 128.1, 128.3, 128.8, 137.2, 138.6 (d, J(PC) = 13.8 Hz), 156.2, 161.6, 170.8, 171.2, 172.5 (d, J(PC) = 7.1 Hz), 173.3; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 45.4 for (*R*,*S*,*S*) diastereomer, 45.8 for (*R*,*R*,*S*) diastereomer; HPLC: t_R = 28.3 min (*R*,*S*,*S*)/29.4 min (*R*,*R*,*S*); elemental analysis calcd (%) for $C_{33}H_{36}N_3O_8P$: C 62.55, H 5.73, N 6.63; found: C 62.67, H 5.88, N 6.72; ES-MS: m/z: calcd for $C_{33}H_{35}N_3O_8P$: 632.2; found: 632.4 $[M-H]^-$.

(1*R*)-1-{[(Benzyloxy)carbonyl]amino}-2-phenylethyl((2*S*)-3-{[(1*S*)-2-methoxy-1-methyl-2-oxoethyl]amino}-2-{[3-(4-nitrophenyl)isoxazol-5-yl]-methyl}-3-oxopropyl)phosphinic acid (5f): M.p. 219–221 °C; R_f = 0.44 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF_3COOD): δ = 1.26 (d, ^3J(HH) = 7.4 Hz, 3H; CHCH_3), 1.68–2.18 (m, 2H; PCH_2), 2.57–2.95 (m, 2H; CHHisoxazolyl, CHPHh), 2.99–3.38 (m, 3H; PCH$_2$CH, CHHPh, CHHisoxazolyl), 3.51 (s, 3H;

OCH_3), 3.77–4.08 (m, 1H; PCH), 4.14–4.36 (m, 1H; CHCO_2CH_3), 4.93 (s, 2H; PhCH_2O), 6.97 (s, 1H; H of isoxazole ring), 6.90–7.38, 7.62–7.7.75, 8.04–8.20, 8.33–8.44 (m, 15H; Ar, NHCHP), 8.58 (d, ^3J(HH) = 7.2 Hz, 1H; NHCHCOO); ^{13}C NMR (70 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 16.8, 22.3, 27.5 (d, J(PC) = 89.1 Hz), 32.9, 38.2, 47.3, 51.6, 52.8 (d, J(PC) = 96.2 Hz), 65.3, 100.4, 126.2, 127.2, 127.3, 128.2, 128.3, 128.7, 137.2, 138.4 (d, J(PC) = 13.9 Hz), 147.9, 156.2, 161.7, 171.2, 172.5 (d, J(PC) = 9.4 Hz), 173.3; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 45.2; HPLC: t_R = 27.1 min; elemental analysis calcd (%) for $C_{33}H_{35}N_4O_{10}P$: C 58.41, H 5.20, N 8.26; found: C 58.32, H 5.07, N 8.34; ES-MS: m/z: calcd for $C_{33}H_{34}N_4O_{10}P$: 677.2; found: 677.2 $[M-H]^-$.

(2*S*)-3-{[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}-2-[(3-[1,1′-biphenyl]-4-ylisoxazol-5-yl)methyl]-3-oxopropyl((1*R*)-1-{[(benzyloxy)-carbonyl]amino}ethyl)phosphinic acid (5g): Part of **5g** was recovered from the basic solution and purified by column chromatography (chloroform/methanol/acetic acid 7:0.8:0.5). Yield given in Table 3 is after combination with the isolated product by precipitation. M.p. 165–168 °C; R_f = 0.52 (chloroform/methanol/acetic acid 7:1:0.5); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.21 (dd, ^3J(HH) = 7.3 Hz, ^3J(PH) = 14.6 Hz, 3H; CHCH_3), 1.63–2.08 (m, 2H; PCH_2), 2.39–2.45 (m, 1H; CHHindolyl, [D$_6$]DMSO/overlapping), 2.89–3.23 (m, 4H; PCH$_2$CH, CHHindolyl, CH_2isoxazolyl), 3.64–3.83 (m, 1H; PCH), 4.37–4.56 (m, 1H; CHCONH$_2$), 5.01 (s, 2H; PhCH_2O), 6.59 (s, 1H; H of isoxazole ring), 6.83–7.89 (m, 28H; Ar, indolyl, NHCHP, NH_2), 8.26 (d, ^3J(HH) = 8.4 Hz, 1H; NHCHCONH$_2$); ^{13}C NMR (70 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 14.3, 22.3 (d, J(PC) = 9.4 Hz), 27.1 (d, J(PC) = 90.6 Hz), 27.2, 46.1 (d, J(PC) = 103.6 Hz), 53.5, 65.3, 100.2, 110.4, 111.3, 118.2 (d, J(PC) = 15.6 Hz), 120.8, 123.8, 126.3, 126.7, 127.4, 127.7, 127.8, 128.3, 128.5, 128.8, 129.0, 136.1, 137.0, 138.8, 139.1, 139.8, 140.2, 142.2, 155.9 (d, J(PC) = 4.6 Hz), 161.8, 171.6, 172.1 (d, J(PC) = 8.4 Hz), 173.3; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 46.2; HPLC: t_R = 27.3 min; elemental analysis calcd (%) for $C_{40}H_{40}N_5O_7P$: C 65.48, H 5.49, N 9.54; found: C 65.23, H 5.17, N 9.28; ES-MS: m/z: calcd for $C_{40}H_{39}N_5O_7P$: 732.3; found: 732.5 $[M-H]^-$.

(2*S*)-3-{[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}-2-{[3-(4-fluorophenyl)isoxazol-5-yl]methyl}-3-oxopropyl[(*R*)-{[(benzyloxy)carbonyl]amino}(phenyl)methyl]phosphinic acid (5h): M.p. 242–244 °C; R_f = 0.41 (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.62–2.18 (m, 2H; PCH_2), 2.23–3.36 (m, 5H; CH_2isoxazolyl, PCH$_2$CH, CH_2indolyl, [D$_6$]DMSO overlapping), 4.28–4.56 (m, 1H; CHCONH$_2$), 4.82–5.18 (m, 3H; PhCH_2O, PCH), 6.54 (s, 1H; H of isoxazole ring), 6.95–8.56 (m, 29H; Ar, indolyl, NH, NH_2); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 27.5, 27.9 (d, J(PC) = 91.1 Hz), 53.6, 55.8 (d, J(PC) = 98.1 Hz), 65.9, 100.2, 110.4, 111.4, 116.1 (d, J = 21.6 Hz), 118.3 (d, J = 11.8 Hz), 120.8, 123.6, 127.2, 127.8, 128.1, 128.8, 128.9, 136.1, 136.8, 156.2 (d, J(PC) = 3.8 Hz), 160.9, 163.1 (d, J(FC) = 247.3 Hz), 171.5, 172.2 (d, J(PC) = 7.6 Hz), 173.4; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 41.9; HPLC: t_R = 25.7 min; elemental analysis calcd (%) for $C_{39}H_{37}FN_5O_7P$: C 63.50, H 5.06, N 9.49; found: C 63.37, H 4.96, N 9.34; ES-MS: m/z: calcd for $C_{39}H_{36}FN_5O_7P$: 736.2; found: 736.3 $[M-H]^-$.

3-{[(1*S*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]amino}-2-{[3-(2-furyl)isoxazol-5-yl]methyl}-3-oxopropyl[(*R*)-{[(benzyloxy)carbonyl]amino}(phenyl)methyl]phosphinic acid (5i): M.p. 190–195 °C (decomp); R_f = 0.51/0.54 (two diastereomers) (chloroform/methanol/acetic acid 7:2:1); ^{1}H NMR (200 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 1.59–2.08 (m, 2H; PCH_2), 2.21–3.40 (m, 5H; CH_2isoxazolyl, PCH$_2$CH, CH_2indolyl, [D$_6$]DMSO overlapping), 4.25–4.56 (m, 1H; CHCONH$_2$), 4.86–5.20 (m, 3H; PhCH_2O, PCH), 6.35/6.45 (s, 1H; H of isoxazole ring), 6.65–8.45 (m, 23H; Ar, furyl, indolyl, NH, NH_2); ^{13}C NMR (50 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 27.3, 27.5 (d, J(PC) = 90.8 Hz), 53.7, 55.8 (d, J(PC) = 98.1 Hz), 65.4, 101.2, 102.3, 110.4, 111.3, 112.8, 114.2, 118.3 (d, J(PC) = 11.7 Hz), 118.6, 120.8, 123.6, 126.3, 126.7, 127.2, 127.5, 127.7, 128.1, 128.4, 136.1 (d, J(PC) = 8.5 Hz), 136.6, 137.2, 138.4 (d, J(PC) = 13.8 Hz), 144.4, 145.1, 148.6, 156.1 (d, J(PC) = 3.8 Hz), 162.0, 170.6, 171.3, 172.1 (d, J(PC) = 7.6 Hz), 173.2, 173.9; ^{31}P NMR (81 MHz, [D$_6$]DMSO/1% CF$_3$COOD): δ = 41.8, 42.4 (two diastereomers); HPLC: t_R = 23.3 min (*R*,*S*,*S*)/24.1 min (*R*,*R*,*S*); elemental analysis calcd (%) for $C_{37}H_{36}N_5O_8P$: C 62.62, H 5.11, N 9.87; found: C 62.33, H 4.86, N 9.56; ES-MS: m/z: calcd for $C_{37}H_{35}N_5O_8P$: 708.2; found: 708.4 $[M-H]^-$.

General procedures for preparation of tripeptides of type 9 on solid phase (pins)

Fmoc deprotection: Fmoc-Rink amide derivatized pins (L-series pins, loading: 14 μmol per pin) were treated with a 20% piperidine/DMF solution (0.5 mL per pin, 2 × 30 min, RT). Each time, the solvent was decanted, and the pins were washed with DMF (2 × 5 min, 1 mL per pin) and CH_2Cl_2 (2 × 5 min, 1 mL per pin) and dried in vacuo.

Fmoc-tryptophan coupling: The deprotected pins were treated with a preactivated (10 min) mixture of Fmoc-tryptophan (3 equiv per pin, 0.12 M), DIC (3 equiv per pin, 0.12 M) and HOBt (3 equiv per pin, 0.12 M) in a CH_2Cl_2/DMF (6:1 *v*/*v*) solution for 4–5 h at RT. Then, the solution was decanted and the pins were washed with CH_2Cl_2 (2 × 5 min, 1 mL per pin), DMF (2 × 5 min, 1 mL per pin), and CH_2Cl_2 (2 × 5 min, 1 mL per pin) and briefly air-dried to give **6a** (complete coupling was monitored by a negative Kaiser test). Fmoc-deprotection of **6a** was performed applying the above described deprotection method to afford **6b**.

Protocol for P-unprotected dipeptidic block (2a′) coupling: The dipeptidic block **2a′** (1.5 equiv per pin, 0.08 M) was added to pins in CH_2Cl_2 (0.25 mL per pin) containing DIPEA (1.5 equiv per pin). HOBt (1.5 equiv per pin, 0.08 M), EDC·HCl (4 equiv per pin, 0.22 M) and DIPEA (1 equiv per pin, 0.14 M overall concentration) were added sequentially to the pins solution. The reaction mixture was shaken from time to time and after 2 h the coupling was stopped by decanting the solution. The pins were washed with CH_2Cl_2 (2 × 5 min, 1 mL per pin), DMF (2 × 5 min, 1 mL per pin), and CH_2Cl_2 (2 × 5 min, 1 mL per pin) and briefly air-dried. The coupling was performed once more under the same conditions to afford **7** (Kaiser test and HPLC analysis were used to monitor the progress of the reaction and racemization). If racemization is not a problem (e.g. use of **2a**), the coupling can be performed only once, overnight.

General procedure for DCRs to tripeptides of type 7 (Following the optimized conditions described in Figure 6): The corresponding oxime (10 equiv per pin, 0.56 M) was dissolved in $CHCl_3$ (0.25 mL per pin), and one drop of pyridine was added (use of chemically resistant glass tubes of the desired dimensions). Then, NCS (10 equiv per pin, 0.56 M) was added at RT and after 10 min the resulting mixture was heated at 45 °C for 4 h, with shaking from time to time. Tripeptide **7** was after added (1 equiv per pin), followed by slow addition of Et_3N (11 equiv per pin) at the same temperature. The reaction mixture was left for 48 h at 45 °C, with shaking occasionally. The pins were washed with CH_2Cl_2 (2 × 5 min, 1 mL per pin), DMF (2 × 5 min, 1 mL per pin), MeOH (1 × 5 min, 1 mL per pin), DMSO (1 × 5 min, 1 mL per pin, 60 °C) and CH_2Cl_2 (2 × 5 min, 1 mL per pin) and briefly air-dried. DCRs were repeated twice (or three) more as above to afford **8**. HPLC analysis of cleaved products was used to monitor the progress of the reaction. The final isoxazole-containing phosphinic tripeptidic array of type **9** was obtained after treatment of **8** with a mixture of TFA/H_2O/TIS (95:2.5:2.5, 0.5 mL per pin) for 1 h at RT. After acidic cleavage and removal of the pins from the reaction mixture, the volatiles were removed by an argon stream. The oily residue was precipitated with cold diethyl ether and the mixture was centrifuged. The organic phase was carefully decanted and the residue was re-subjected to the same treatment once more. The off-white solid (product **9**) was analyzed by analytical RP-HPLC and ES-MS (see Table 4). Further purification and separation of diastereomers, if necessary, was performed by semipreparative RP-HPLC.

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