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α-Biphenylsulfonylamino 2-methylpropyl phosphonates: Enantioselective synthesis and selective inhibition of MMPs

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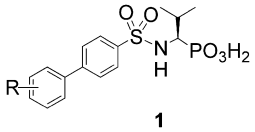
Abstract—(*R*)-α-Biphenylsulfonylamino 2-methylpropyl phosphonates attain nM potency against several MMPs and are the most effective inhibitors based on phosphonate as zinc binding group. Since their preparation by direct N-acylation of expensive, enantiopure, α-aminophosphonic acids proceeds in low yields, we devised and evaluated a stereoselective and straightforward method of synthesis that avoids the unfavourable step of N-acylation. The key intermediate (*R*)-4-bromophenylsulfonylamino 2-methylpropyl phosphonate **9** was obtained by highly stereoselective addition of dibenzylphosphite to the enantiopure (*S*)-*N*-isobutylidene-*p*-bromobenzenesulfinamide **3**, followed by oxidation with *m*-CPBA. Suzuki coupling of **9** with the desired arylboronic acids, gave the expected biphenylsulfonylamino derivatives in satisfactory yields. Liberation of the phosphonic group by hydrogenolysis led to the desired (*R*)-α-biphenylsulfonylamino 2-methylpropyl phosphonates **14a**–**i**. Screening of the new compounds on MMP-1, -2, -3, -7, -8, -9, -13 and -14 showed IC_{50} in the range of nM in most cases.

1. Introduction

Matrix metalloproteinases (MMPs) are zinc endopeptidases that can degrade virtually all the constituents of the extracellular matrix (ECM).[1] While they intervene in various physiological processes, chronic over-regulation of MMP activity results in excessive degradation of ECM components and has been implicated in numerous pathological conditions.[2] A great variety of synthetic, low molecular weight, MMP inhibitors (MPIs) are therefore studied for the development of innovative chemotherapeutics in several fields where effective drugs are not yet available,[3–5] and some of them are presently in advanced clinical trials.[4,6] Their structures include a peptide or a peptidomimetic moiety that is preferably accommodated in the S′ region of the active site, and a zinc binding group (ZBG) able to coordinate the catalytic zinc ion.

Hydroxamates[7] are the most popular MPIs owing to the exceptional strength of their ZBG, although metabolic instability may compromise their oral bioavailability. While hydroxamate optimization[4] points to improvement of selectivity and metabolic stability, other inhibitors based on different zinc binding functions, such as carboxylate, phosphonate and thiolate,[8] are currently investigated, and we also have been studying phosphonate MPIs for a long time.[9]



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Pursuing our programme in this subject, and stimulated by basic contributions discussing SAR versus several MMPs,[10] we focused our attention on α-biphenylsulfonylamino 2-methylpropyl phosphonates **1**, analogues of known carboxylate[11] and hydroxamate[12,13] MPIs.

Examples reported in a patent[14] and our own findings (K_i in the nM range for MMP-2, MMP-3, MMP-8

Keywords: MMP phosphonate inhibitors; Enantiopure sulfinimine; Dibenzyl phosphite stereoselective addition; Suzuki coupling.
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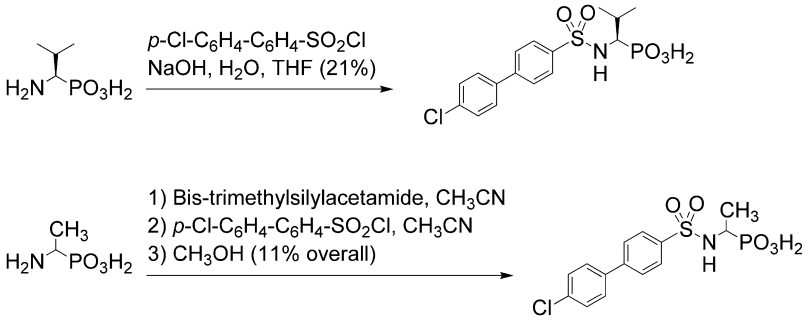
and aggrecanase) proved that the α-biphenylsulfonylamino substitution gives rise to the most potent MPIs based on phosphonate as ZBG. While the search for new analogues was abandoned, due to the ample coverage of the patent, we turned our attention to a stereoselective method of synthesis and to the elucidation of their mode of binding[9] in the active site of MMP-8.

Biphenylsulfonylamino 2-methylpropyl phosphonates were previously prepared[14] by direct N-acylation of the appropriate α-aminophosphonic acid (Scheme 1). Yields were rather low, owing probably to the steric hindrance around the amino group. Moreover, the *R*- and *S*-enantiomers of the α-aminophosphonic acids, when available, are expensive. We have therefore devised a new stereoselective and straightforward approach that avoids the unfavourable step of N-acylation. The method, previously used to prepare the α-biphenylsulfonylamino 2-methylpropyl phosphonates (*R*) and (*S*)-**14a**,[9] has been further improved, in this paper, and evaluated for the preparation of a series of the analogues **14b–i** and **16a**, **b**, only one of which (**14e**) was previously reported.[14]

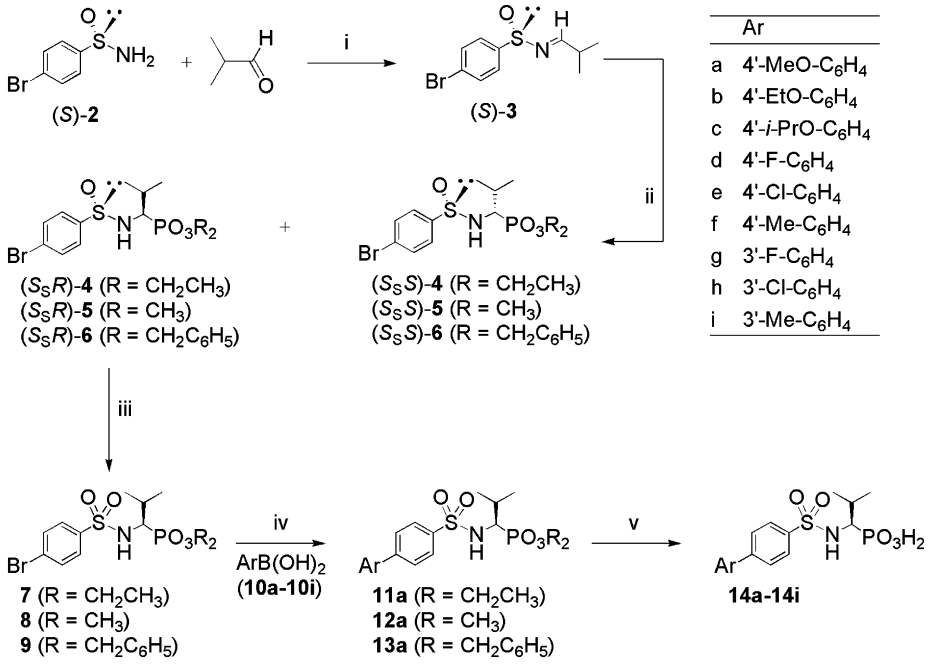
2. Chemistry

Addition of lithium dialkyl phosphites to enantiopure and configurationally restricted sulfinimines occurs with excellent diastereoselectivity and the resulting *N*-sulfinyl-α-amino phosphonates can be readily converted into the enriched (*R*)- and (*S*)-α-amino phosphonic acids.[15] Further studies[16] confirmed the effectiveness of this method, clarified[17] that preferred adducts are (S_S, *R*)-*N*-sulfinyl-α-amino phosphonates and brought out the proposal[18] of a unified asymmetric induction model to explain the stereoselective outcome of the addition.

Adaptation of this excellent diastereoselective method (Scheme 2) to prepare the biphenylsulfonylamino 2-methylpropyl phosphonates (*R*)- and (*S*)-**14a** for cocrystallisation in complexes with MMP-8[9] was previously outlined. Enantiopure (*S*)-*p*-bromobenzenesulfinamide **2**, readily obtained from the (1*R*,2*S*,5*R*)-menthyl (*S*)-*p*-bromobenzenesulfinate,[19] can be converted into the (*S*)-*p*-bromobenzenesulfinimine **3** by treatment with *i*-butyraldehyde in the presence of



Scheme 1.



Scheme 2.

Table 1. Addition of lithium dialkyl phosphites to the enantiopure sulfinimine (*S*)-**3**[a]

Reagent	Product	Yield (%)	$S_S,R/S_S,S$
$(EtO)_2POLi$	**4**[9]	84	91/9[b]
$(MeO)_2POLi$	**5**	83	90:10[c]
$(BnO)_2POLi$	**6**	86	95.5/4.5

[a] All reactions were carried out for 3 h, in THF at −78 °C.
[b] By integration of ^{31}P NMR resonances in $CDCl_3$ at 25.12 and 25.25 ppm.
[c] By integration of ^{31}P NMR resonances in $CDCl_3$ at 27.42 and 27.58 ppm.

5 equiv of $Ti(OEt)_4$.[20] Addition of lithium diethyl phosphite at −78 °C to the *N*-isobutylidene-(*S*)-*p*-bromobenzenesulfinimine **3**, according to the procedure of Lefebvre,[15] afforded a mixture of the two diastereoisomers (S_S,R)-**4** and (S_S,S)-**4** in a 91:9 ratio (Table 1). The major adduct (S_S,R)-**4** could be further enriched by silica-gel chromatography to improve the enantiomeric purity (99.8%) of the final phosphonate **14a**. Oxidation of the (S_S,R)-*N*-*p*-bromobenzene sulfinamide **4** with *m*-CPBA gave the corresponding *N*-*p*-bromobenzene sulfonamide (*R*)-**7** in essentially quantitative yield. This intermediate was converted into the diethyl α-biphenylsulfonylamino 2-methylpropyl phosphonates (*R*)-**11a** by coupling with 4-methoxyphenylboronic acid in the presence of $Pd(PPh_3)_4$ and aqueous sodium carbonate, and finally converted into the free phosphonates **14a** by acid hydrolysis.

Since the Suzuki–Miyaura reaction[21] is one of the most efficient methods for creation of a C–C bond between aromatics and a large range of the required arylboronic acids are commercially available, we decided to evaluate the effectiveness of this method for the preparation of a small series (**14a–i**) of α-biphenylsulfonylamino (*R*)-2-methylpropyl phosphonates (Scheme 2).

While diastereoselectivity of lithium diethyl phosphite addition was satisfactory and yields were good, the rather vigorous conditions (6 M HCl in H_2O/acetic acid under reflux) required for hydrolysis of the phosphonate diethyl ester caused an extensive hydrolysis of the sulfonylamino group, and an unsatisfactory recovery (50%) of the expected phosphonate (*R*)-**14a**. With the aim to further improve the overall yields of this method, addition of both lithium dimethyl phosphite or dibenzyl phosphite in the first step of the process was tried since the final dimethyl phosphonates require milder conditions for their hydrolysis[22] and dibenzyl phosphonates undergo facile and quantitative hydrogenolysis.[23]

Addition of lithium dimethyl phosphite (Scheme 2) to the enantiopure (*S*)-*N*-isobutylidene-*p*-bromobenzenesulfinimine **3** and the following oxidation of the adduct **5** to the dimethylphosphonate **8** occurred with yield and stereoselectivity similar to that of the diethyl analogue (Table 1). The Suzuki reaction in toluene under reflux, in the presence of aqueous sodium carbonate, however, caused extensive hydrolysis of the dimethyl to monomethyl phosphonate, poor yields of the arylation reaction and poor recovery of the expected biphenyl derivative **12a** (Table 2). When coupling was carried out in the presence of anhydrous sodium carbonate, yield was only slightly increased since sodium carbonate, in its behaviour as a base, releases a water molecule and dimethyl phosphonates are particularly sensitive to base catalyzed hydrolysis.

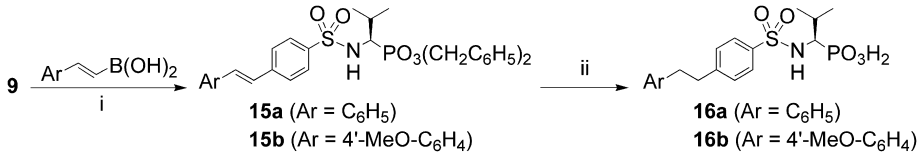
Table 2. Arylation of *p*-bromophenylsulfonylamino phosphonates **7–9**[a]

Ester	Boronic acid	Base[b]	Product	Yield (%)
7	**10a**	A	**11a**	86
8	**10a**	A	**12a**	6
8	**10a**	B	**12a**	15
9	**10a**	A	**13a**	5
9	**10a**	B	**13a**	45
9	**10a**	C	**13a**	73
9	**10a–i**	C	**13a–i**	43–73

[a] All reactions were carried out in toluene under reflux by using $Pd(PPh_3)_4$ as catalyst (3 mol%) and boronic acids **10a–i** (1.2 equiv).
[b] A, aqueous Na_2CO_3; B, anhydrous Na_2CO_3; C, anhydrous K_3PO_4.

Addition of lithium dibenzyl phosphite to the enantiopure (*S*)-*N*-isobutylidene-*p*-bromobenzene-sulfinimine **3** occurred in high yields and with an even higher diastereoselectivity (Table 1). The major diastereoisomer (S_S,R)-**6** could be easily purified by silica-gel chromatography and highly enriched (99.8% de) by crystallisation from EtOAc/*n*-hexane. After satisfactory oxidation with *m*-CPBA, however, the dibenzyl phosphonate **9** showed to be too sensitive to basic catalyzed hydrolysis in the Suzuki coupling. In the presence of either aqueous or anhydrous sodium carbonate, unsatisfactory recovery of the expected biphenylsulfonylamino dibenzyl phosphonate **13a** was obtained (Table 2). When anhydrous potassium triphosphate was used in place of sodium carbonate, the dibenzylester hydrolysis was notably repressed and recovery of the dibenzyl phosphonate was satisfactory. The free α-biphenylsulfonylamino (*R*)-2-methylpropyl phosphonic acid **14a** was obtained by Pd catalyzed hydrogenolysis and isolated as the cyclohexylamine salt in practically quantitative yields. The enantiomeric purity of the phosphonate (99.9% ee) was monitored by HPLC analysis on a Chiralpack AD column, after conversion into the corresponding methylester by treatment with diazomethane and guarantees that no racemization intervenes in the Suzuki coupling and hydrogenolysis step. To confirm the usefulness of this process, the α-biphenylsulfonylamino dibenzyl phosphonates **13b–i** were also prepared according to Scheme 2 and successfully converted into the phosphonates **14b–i**.

Since the Suzuki reaction also included coupling with vinylboronic acids, the two arylsulfonylamino dibenzyl phosphonates **15a** and **15b** (Scheme 3) were successfully prepared, starting from two, commercially available, 2-arylvinylboronic acids. The expected α-arylsulfonylamino phosphonic acids, where the two aromatics are connected by means of an ethylenic spacer, would have been potentially interesting as selective MPIs, in accordance with previous studies on a carboxylate analogue.[10a] Liberation of the phosphonic group by Pd



Scheme 3.

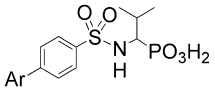
catalyzed hydrogenolysis led, however, to the saturated analogues **16a** and **16b**.

3. Biological evaluation and structure–activity relationships

Since the extension of the present method of synthesis to a series of α-biphenylsulfonylamino 2-methylpropyl phosphonates led to a small library of new MPIs, their in vitro potencies were determined by fluorimetric assay. To achieve a wide selectivity profile, in view of possible clinical applications,[6b] the screening was extended to MMP-1, -2, -3, -7, -8, -9, -13 and -14 (Table 3). The following results, in terms of potency and selectivity, may be underlined.

The 4′-methoxybiphenylsulfonylamino-2-methylpropyl phosphonate (*R*)-**14a** is 750- and 580-fold more potent than the opposite enantiomer (*S*)-**14a**[9] against MMP-2

Table 3. Inhibition data of 2-methylpropyl phosphonates **14a**–**i** and **16a**, **b** against MMPs[a]



Compound	Ar	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-13	MMP-14
(*S*)-**14a**	MeO	5%[b]	1100	16%[b]	17%[b]	810	40%[b]	68%[b]	63%[b]
(*R*)-**14a**	MeO	150	1.5	52	460	1.4	8.0	2.6	79
(*R*)-**14b**	EtO	450	0.39	12	450	0.37	0.56	1.1	11
(*R*)-**14c**	*i*-PrO	600	1.8	36	2000	1.50	24	4.2	130
(*R*)-**14d**	F	160	20	150	1400	1.1	59	13	32
(*R*)-**14e**	Cl	77	8.7	28	1700	0.81	21	5.3	47
(*R*)-**14f**	Me	98	2.3	45	150	0.39	4.1	3.3	11
(*R*)-**14g**	F	590	53	1200	20,000	2.4	170	63	180
(*R*)-**14h**	Cl	320	30	440	2900	1.3	160	24	75
(*R*)-**14i**	Me	320	24	230	1100	0.4	64	15	26
(*R*)-**16a**	$(CH_2)_2$	33%[b]	980	34%[b]	50,000	260	7100	820	6000
(*R*)-**16b**	MeO-$(CH_2)_2$	9%[b]	790	31%[b]	130,000	110	680	480	40,000

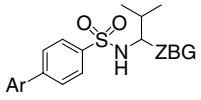
[a] IC_{50} (nM); errors in the range of 5–10% of the reported value (from three different assays).
[b] % Inhibition at 100 μM.

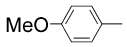
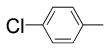
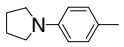
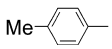
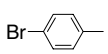
and MMP-8, respectively. Preference of MMP-8 for the *R*-enantiomer **14a** has been discussed in detail on the basis of the crystal complexes of (*R*)-**14a** and (*S*)-**14a** with MMP-8.[9] The even larger potency of the *R*-relative to the *S*-enantiomer, observed for all the other tested MMPs, can be related to the strong similarities in the active sites of this family of enzymes. Similar results (Table 4) were found for the examples previously reported [(*R*)-**14e**, (*S*)-**14e** and (*R*)-**14l**] in the aforementioned patent.[14]

All the (*R*)-α-biphenylsulfonylamino 2-methylpropyl phosphonates **14a–i** confirm to be excellent MPIs. They exhibit IC_{50} values in the nM range against all the tested enzymes and some of them attain sub-nM potency against MMP-2 and MMP-8. The IC_{50} values (2–20 nM) against MMP-2 of the 4′-substituted (*R*)-α-biphenylsulfonylamino phosphonates (*R*)-**14a–f** are very similar to those of previously studied carboxylate analogues[13] (*S*)-**17a**,**d**,**f**,**m** (Table 4). The (*R*)-α-biphenylsulfonylamino hydroxamate (*R*)-**18m** (Table 4), in addition, is more potent than the carboxylate analogue (*S*)-**17m**, and closely approaches potencies of phosphonate analogues versus the tested enzymes.

While carboxylates retain nM potency only versus MMP-2, -3 and -13 and exhibit micromolar potency versus MMP-1, -7 and -9, the majority of corresponding phosphonates **14a–f** retain nM potency also versus

Table 4. Inhibition of selected examples of phosphonates **14** and analogous carboxylates (**17**) and hydroxamates (**18**) against MMPs[a]



Ar	Compound	ZBG	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-13	MMP-14
	(*R*)-**14a**	PO_3H_2	150	1.5	52	460	1.4	8.0	2.6	79
	(*S*)-**17a**[b]	COOH	1500	3	8	7200	ND[c]	2200	6	ND
	(*R*)-**14d**	PO_3H_2	160	20	150	1400	1.1	59	13	32
	(*S*)-**17d**[b]	COOH	4200	39	10	4800	ND	64,000	43	ND
	(*R*)-**14e**	PO_3H_2	77	8.7	28	1700	0.81	21	5.3	47
	(*R*)-**14e**[d]	PO_3H_2	ND	ND	6	ND	1	ND	ND	ND
	(*S*)-**14e**[d]	PO_3H_2	ND	ND	10,000	ND	1000	ND	ND	ND
	(*S*)-**17e**[b]	COOH	6500	11	9	7500	ND	16,000	48	ND
	(*R*)-**14l**[d]	PO_3H_2	ND	ND	5	ND	2	ND	ND	ND
	(*R*)-**14f**	PO_3H_2	98	2.3	45	150	0.39	4.1	3.3	11
	(*S*)-**17f**[b]	COOH	2200	2	3	4500	ND	3900	11	ND
	(*S*)-**17m**[b]	COOH	6000	4	7	7200	NT	7900	8	NT
	(*R*)-**18m**[b]	CONHOH	110	1	5	140	ND	18	2	ND

[a] IC_{50} (nM).
[b] Ref. 13.
[c] Not determined.
[d] Ref. 14.

MMP-1, -8 and -9, and decrease their potency to micromolar level only versus MMP-7. As previously observed for carboxylates,[13] the decrease in potency of phosphonates against MMP-1 and -7 can be attributed to the restriction of the S1′ pocket of these enzymes, where an arginine or a tyrosine residue defining the bottom of the pocket hinders accommodation of the distal ring of the biphenyl moiety of the inhibitor.[24,25] Flexibility of the protein in this area in some cases allows an induced fit, upon ligand binding, that moves the occluding residue and favours accommodation of large P1′ groups.[26,27] Phosphonates **14a**–**i** retain nM potency versus MMP-9 and are some 1000-fold more potent (Table 4) than the corresponding carboxylates.[20] This large difference in selectivity between carboxylate and phosphonate analogues versus MMP-9 was unexpected. It can probably be explained by assuming that only phosphonate, rather than carboxylate, can be coordinated as ZBG in such a way as to allow proper orientation of the biphenyl moiety for easy accommodation in the narrow S1′ pocket[25] of MMP-9.

Replacement of MeO by EtO in 4′ position improves selectivity towards MMP-1 and increases potency against most of the tested MMPs, attaining IC_{50} in the range 0.37–1.1 nM for MMP-2, -8, -9 and -13. When R = *i*-Pr, a small decrease in potency against these MMPs is observed, but selectivity against MMP-1, MMP-7 and MMP-14 is improved. Docking studies clearly show that 4′-ethoxy group increases hydrophobic interactions with Leu193 side chain (MMP-8 numbering), a conserved residue in all the MMPs of the present study, except MMP-1 and -7. Further increase of hydrophobic interactions (*i*-PrO in place of EtO group) causes an even greater increase of steric hindrance (Fig. 1) and results in a decrease of the potency.

The shift of an F or Cl substituent from the 4′ to the 3′ position generally causes a decrease in binding affinities for all the observed cases. When a methyl group in 3′ is involved a sub-nM IC_{50} against MMP-8 is retained and selectivity versus the remaining MMPs is notably increased. Binding affinities of the two α-arylsulfonylamino phosphonates **16a** and **16b**, where the phenyl rings are connected by means of a CH_2–CH_2 spacer, are generally strongly decreased owing to the high flexibility and conformational mobility of the P1′ hydrophobic group. IC_{50} in the range of nM are however retained versus MMP-2 and -8.

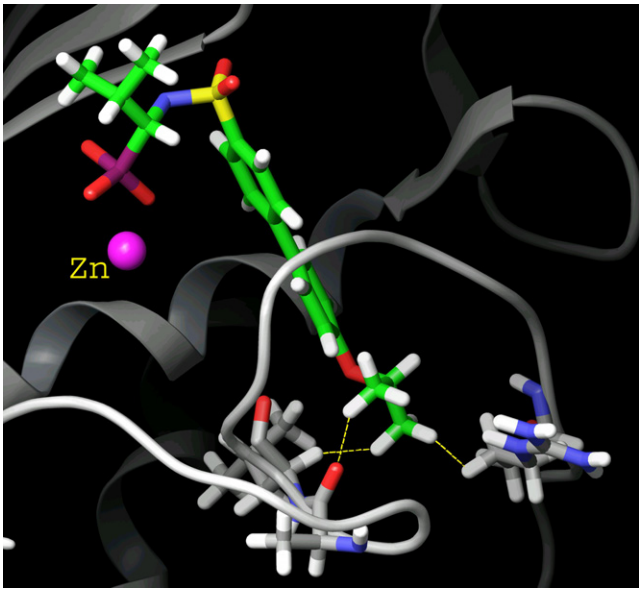


Figure 1.

4. Conclusion

The efficient stereoselective synthesis of the key intermediate (S_S,*R*)-**6** allows easy access into a series of variously substituted (*R*)-α-biphenylsulfonylamino 2-methylpropyl phosphonates through the Suzuki coupling with arylboronic acids, followed by Pd catalyzed hydrogenolysis. Facile diastereomeric enrichment of the key intermediate by column chromatography and crystallisation guarantees very high levels of enantiomeric purity of the final products.

Evaluation of the inhibiting activities of (*R*)- and (*S*)-**14a** against MMP-1, -2, -3, -7, -8, -9, -13 and -14 showed IC_{50} in the range of nM in most cases only for the *R*-enantiomer. The most powerful analogues studied in this paper contain an alkoxy substitution in 4′ and attain IC_{50} in the range 0.37–1.1 nM for MMP-2, -8, -9 and -13. These examples, in addition to some 3′ substituted analogues, show that appropriate structural variations on the biphenyl distal ring can strongly affect potency and selectivity.

5. Experimental

5.1. Chemistry

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 digital polarimeter at 20 °C; concentrations are expressed as g/100 mL. HPLC analyses were performed with a Perkin-Elmer HPLC chromatograph equipped with a Rheodyne 7725i model injector, a 785A model UV/Vis detector, a series 200 model pump and NCI 900 model interface. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Varian VXR 300 spectrometer, operating at 300, 75 and 121 MHz, respectively. Chemical shifts are expressed in δ (ppm) values relative to an internal standard (TMS for proton and carbon; H_3PO_4 for phosphorus); coupling constants (*J*) are given in Hz. ^{13}C and ^{31}P NMR spectra are fully proton decoupled. Elemental microanalyses of C, H and N were performed on a Carlo Erba mod. 1106 analyzer and were within ±0.4% of the calculated values.

5.1.1. (*S*)-*N*-(2-Methylpropylidene)-*p*-bromobenzenesulfinimine (3). Isobutyraldehyde (2.19 mL, 24.0 mmol) and titanium(IV) ethoxide (29.8 mL, 120 mmol) were added to a solution of the sulfinamide[19] **2** (5.29 g,

24.0 mmol) in CH_2Cl_2 (370 mL), under N_2. The reaction mixture was refluxed for 3 h and quenched at 0 °C by addition of H_2O (270 mL). After filtration through a short pad of Celite, the organic phase was secured and the aqueous solution was further extracted with CH_2Cl_2 (2× 100 mL). The pooled organic phases were dried (Na_2SO_4) and concentrated to leave the crude product **3**[17] (6.02 g; 91%) as a yellow oil, that was employed without further purification.

5.1.2. Dibenzyl (S_S,*R*)-*N*-(*p*-bromobenzenesulfinyl)-2-amino-2-methylpropylphosphonate [(S_S,*R*)-6]. To a solution of dibenzyl phosphite (12.6 g, 48.0 mmol) in THF (360 mL), contained in a 500 mL two-necked round-bottommed flask equipped with a magnetic stirring bar and rubber septum, a solution (1.0 M in THF) of lithium bis(trimethylsilyl)amide (48 mL, 48.0 mmol) was slowly added under nitrogen, at −78 °C. After stirring for 30 min, the resulting mixture was transferred *via cannula* into a 1 L two-necked round-bottommed flask equipped with a magnetic stirring bar, rubber septum and nitrogen inlet, containing a solution of sulfinimine **3** (6.02 g, 22.0 mmol) in THF (230 mL), cooled at −78 °C. After 3 h at −78 °C, the reaction mixture was quenched with saturated NH_4Cl (250 mL) solution, the organic layer was secured and the aqueous phase was further extracted with EtOAc (3× 150 mL). The pooled organic phases were dried, concentrated, and fractionated by silica gel chromatography (3% *iso*-propanol in $CHCl_3$), to give a 95.5/4.5 mixture of (S_S,*R*)-**6** and (S_S,*S*)-**6** (10.15 g, 86%). Relative abundances of the two diastereoisomers were determined by integration of the HPLC chromatograms: Luna C18 column (4.6 × 250 mm); 75:25 MeOH/H_2O containing 1% TFA at a flow rate of 1 mL/min; peak detection at 280 nm; retention times 9.9 and 12.0 min, respectively.

Crystallisation from EtOAc/*n*-hexane of the 95.5/4.5 mixture afforded 6.4 g (54%) of the prevailing (S_S,*R*)-**6** diastereoisomer as white crystals: mp 88.0–88.5 °C; $[\alpha]_D^{20}$ + 21.2° (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.98 (d, 3H, *J* = 6.9 Hz), 1.07 (dd, 3H, *J* = 6.9 and 1.2 Hz), 2.21–2.33 (m, 1H), 3.46 (ddd, 1H, *J* = 20.1, 9.6 and 2.7 Hz), 4.58 (t, 1H, *J* = 9.6 Hz), 4.82–5.12 (m, 4H), 7.27–7.36 (m, 10H), 7.54–7.56 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 18.9 (d, *J* = 6.0 Hz), 19.8 (d, *J* = 7.4 Hz), 28.3, 53.4 (d, *J* = 152.0 Hz), 68.9 (d, *J* = 6.9 Hz), 69.1 (d, *J* = 6.5 Hz), 128.1, 128.4, 128.8 (d, *J* = 3.0 Hz), 128.9 (d, *J* = 3.0 Hz), 131.6, 132.6, 133.2, 135.6 (d, *J* = 4.3 Hz), 135.6 (d, *J* = 5.7 Hz), 138.1, 143.6. Relative abundance (HPLC) of (S_S,*R*)-**6** relative to (S_S,*S*)-**6**: 99.8/0.2.

5.1.3. Dibenzyl (*R*)-*N*-(*p*-bromobenzenesulfonyl)-2-amino-2-methylpropylphosphonate (9). To a solution of (S_S,*R*)-**6** (6.4 g, 11.9 mmol) in CH_2Cl_2 (160 mL), *m*-CPBA (4.1 g, 23.9 mmol) was added portionwise, at 0 °C, under stirring. After 0.5 h, the reaction mixture was sequentially washed with saturated solutions of $Na_2S_2O_3$ (2× 200 mL), $NaHCO_3$ (2× 200 mL) and brine. The organic phase was dried and concentrated in vacuo to give 6.39 g (97%) of the pure phosphonate **9** as a white solid: mp 97.5–98.2 °C (EtOAc/*n*-hexane); $[\alpha]_D^{20}$ − 11.8° (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.93–0.98 (m, 6H), 2.11–2.23 (m, 1H), 3.73 (ddd, 1H, *J* = 21.0, 9.9 and 3.3 Hz), 4.63–4.88 (m, 4H), 5.11 (dd, 1H *J* = 10.2 and 4.8 Hz), 7.18–7.22 (m, 4H), 7.31–7.33 (m, 6H), 7.46–7.49 (m, 2H), 7.65–7.68 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 17.8 (d, *J* = 2.8 Hz), 20.6 (d, *J* = 13.1 Hz), 29.7 (d, *J* = 4.8 Hz), 56.2 (d, *J* = 151.4 Hz), 68.1 (d, *J* = 7.1 Hz); 68.3 (d, *J* = 7.1 Hz), 127.6, 128.4, 128.8, 128.8, 128.9, 132.3, 135.8 (d, *J* = 5.7 Hz), 135.9 (*J* = 5.4 Hz), 140.6. Enantiomeric excess 99.8 determined by integration of the HPLC chromatograms: Chiralpack AD column (4.6×250 mm); *iso*-propanol/*n*-hexane (1:1) containing 0.025% TFA, at a flow rate of 0.5 mL/min; peak detection at 280 nm; retention times 16.5 and 22.3 min for (*R*)- and (*S*)-**9**, respectively.

5.1.4. Dimethyl (*R*)-*N*-(*p*-bromobenzenesulfonyl)-2-amino-2-methylpropylphosphonate (8). Dimethyl phosphite (0.46 mL, 5.0 mmol) and sulfinimine **3** (685 mg, 2.5 mmol) were reacted according to the procedure reported for preparation of (S_S,*R*)-**6** to give a crude mixture of (S_S,*R*)-**5** and (S_S,*S*)-**5** stereoisomers (846 mg, 83%; 90:10 by integration of ^{31}P NMR resonances in $CDCl_3$ at 27.42 and 27.58 ppm). After enrichment by silica gel chromatography (3% *iso*-propanol in $CHCl_3$), the (S_S,*R*)-**5** stereoisomer was oxidized according to the procedure employed for preparation of **9**, to give 614 mg of the pure compound **8** as a white solid: mp 98.0–99.0 °C; $[\alpha]_D^{20}$ − 11.8 (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 3H, *J* = 6.3 Hz), 0.96 (d, 3H, *J* = 6.9 Hz), 2.14–2.22 (m, 1H), 3.45 (d, 3H, *J* = 7.5 Hz), 3.50 (d, 3H, *J* = 7.5 Hz),3.57 (ddd, 1H, *J* = 19.2, 9.9 and 2.0 Hz), 5.14–5.19 (m, 1H), 7.64 (d, 2H, *J* = 8.1 Hz), 7.74 (d, 2H, *J* = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 17.6 (d, *J* = 2.8 Hz), 20.4 (d, *J* = 13.2 Hz), 29.8 (d, *J* = 4.8 Hz), 56.7 (d, *J* = 152.0 Hz), 127.8, 128.4, 132.8, 141.6.

5.1.5. Synthesis of phosphonate dibenzylesters 13a–i and 15a, b (General Procedure A). A solution of **9** (1.0 mmol) and the appropriate boronic acid (1.2 mmol) in toluene (10 mL) was treated with tetrakis(triphenylphosphine)palladium(0) (22 mg) and K_3PO_4 (2.0 mmol), under nitrogen. After being refluxed for 18–48 h (TLC monitoring for disappearance of **9**), the reaction mixture was diluted with EtOAc (15 mL), added with 1 M HCl (15 mL) under stirring and filtered through a short pad of Celite. The organic layer was separated and further washed with $NaHCO_3$ saturated solution (20 mL) and brine, dried and concentrated in vacuo. The resulting yellow oil was purified by chromatography on silica gel (40% EtOAc/*n*-*n*-hexane) and crystallisation.

5.1.6. Hydrogenolysis of phosphonate dibenzylesters 13a–i and 15a, b (General Procedure B). A solution of the phosphonate dibenzylester (1.0 mmol) in 1:1 CH_2Cl_2/MeOH (40 mL) was stirred under hydrogen, in the presence of 5% Pd/C (100 mg), for 24 h. The reacting mixture was filtered and evaporated under reduced pressure to give the crude phosphonic acid as a white solid that was dissolved in CH_3OH and added with a small excess of cyclohexylamine. Evaporation of the solvent and recrystallisation from CH_3OH/Et_2O gave the pure cyclohexylamine salts **14a**–**i** and **16a**, **b**.

5.2. MMP inhibition assays[28]

Recombinant human progelatinase A (pro-MMP-2) and B (pro-MMP-9) from transfected mouse myeloma cells were supplied by Prof Gillian Murphy (Department of Oncology, University of Cambridge, UK); pro-MMP-1, pro-MMP-3, pro-MMP-7, pro-MMP-8, pro-MMP-13 and pro-MMP-14 were purchased from Calbiochem. Proenzymes were activated immediately prior to use with *p*-aminophenylmercuric acetate (APMA 2 mM for 1 h at 37 °C for MMP-2, MMP-1 and MMP-8; 1 mM for 1 h at 37 °C for MMP-9 and MMP-7; 1 mM for 0.5 h at 37 °C for MMP-13). Pro-MMP-14 and pro-MMP-3 were activated with trypsin 5 μg/mL for 15 min at 37 °C followed by soybean trypsin inhibitor (SBTI) 23 μg/mL for pro-MMP-14 and 62 μg/mL for pro-MMP-3.

For assay measurements, the inhibitor stock solutions (DMSO, 100 mM) were further diluted, at seven different concentrations (0.01 nM–300 μM) for each MMP in the fluorimetric assay buffer (FAB: Tris 50 mM, pH 7.5, NaCl 150 mM, $CaCl_2$ 10 mM, Brij 35 0.05% and DMSO 1%). Activated enzyme (final concentration 2.9 nM for MMP-2, 2.7 nM for MMP-9, 2.4 nM for MMP-7, 1 nM for MMP-14 and MMP-3, 1.5 nM for MMP-8, 0.66 nM for MMP-13 and 0.20 nM for MMP-1) and inhibitor solutions were incubated in the assay buffer for 4 h at 25 °C. After the addition of 200 μM solution of the fluorogenic substrate Mca-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH2 (Sigma) for MMP-3 and Mca-Lys-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Bachem) for all the other enzymes in DMSO (final concentration 2 μM), the hydrolysis was monitored every 15 s for 20 min recording the increase in fluorescence (λ_{ex} = 325 nm, λ_{em} = 400 nm) using a Molecular Device Spectramax Gemini XS plate reader. The assays were performed in triplicate in a total volume of 200 μL per well in 96-well microtitre plates (Corning, black, NBS). Control wells lack inhibitor. The MMP inhibition activity was expressed in relative fluorescent units (RFU). Percent of inhibition was calculated from control reactions without the inhibitor. IC_{50} was determined using the formula: $V_i/V_o = 1/(1 + [I]/IC_{50})$, where V_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration [I] and V_o is the initial velocity in the absence of the inhibitor. Results were analyzed using SoftMax Pro software and GraFit software.[29]

5.3. Molecular modelling

All calculations were performed on a notebook ASUS A6770KLH, equipped with an AMD Turion MT32 processor and a RedHat Enterprise Desktop 4 OS. Crystal structures of MMP-2 (PDB code 1qib), MMP-3 (PDB code 1caq) and MMP-8 (PDB code 1z) were used as the basis of the docking experiments. The GLIDE[30] protein preparation protocol was used to remove all crystallographic water molecules and add hydrogen atoms using the predicted protonation states of the amino acid residues at pH 7.4. The crystal structures were then minimized to optimize hydrogen atoms position.

The sulfonamide ligand **14a** was used to define the active site box and to set up grid calculation. The prepared crystal structures were used for the docking campaign with GLIDE that was successful in reproducing the (*R*)-**14a** crystal structure binding mode. All ligands of the present study were manually built in Maestro[31], exploiting the Build function and minimized with MMFFs force field as implemented in the MacroModel software package,[32] using a distance-dependent dielectric constant of 4.0, with Polak-Ribier conjugate gradient as minimizer and a threshold value of 0.01 kJ/(Å mol) as the convergence criterion. Minimized structures were the input for docking calculations into each enzyme. GLIDE was used with Extra Precision docking protocol default settings, enlarging the energy window filtering higher level poses to 5 kcal/mol. The top 10 docked poses for each docked structure were analyzed.

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Supplementary data

^1H and ^{13}C NMR spectral data and physical constants for compounds **13a**–**i**, **14a**–**i**, **15a**, **b** and **16a**, **b** are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.10.047.

References and notes

1. Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, J. H. *Chem. Rev.* **1999**, *99*, 2735.
2. *Matrix Metalloproteinases and TIMPs*; Woessner, J. P., Nagase, H., Eds.; Oxford University Press: New York, NY, USA, 2000.
3. Puerta, D. T.; Cohen, S. M. *Curr. Top. Med. Chem.* **2004**, *4*, 1551.
4. Matter, H.; Schudok, M. *Curr. Opin. Drug Discovery* **2004**, *7*, 513.
5. Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. *Curr. Med. Chem.* **2004**, *11*, 2911.
6. (a) Abbenante, G.; Fairlie, D. P. *Med. Chem.* **2005**, *1*, 71; (b) Overall, C. M.; Kleifeld, O. *Br. J. Cancer* **2006**, *94*, 1355.
7. Muri, E. M. F.; Nieto, M. J.; Sindelar, R. D.; Williamson, J. S. *Curr. Med. Chem.* **2002**, *9*, 1631.
8. Breuer, E.; Frant, J.; Reich, R. *Expert Opin. Ther. Patents* **2005**, *15*, 253.
9. Pochetti, G.; Gavuzzo, E.; Campestre, C.; Agamennone, M.; Tortorella, P.; Consalvi, V.; Gallina, C.; Hiller, O.; Tschesche, H.; Tucker, P. A.; Mazza, F. *J. Med. Chem.* **2006**, *49*, 923, and references cited therein.
10. (a) Matter, H.; Schwab, W.; Barbier, D.; Billen, G.; Haase, B.; Neises, B.; Schudok, M.; Thorwart, W.;

Schreuder, H.; Brachvogel, V.; Lonze, P.; Weithmann, K. U. *J. Med. Chem.* **1999**, *42*, 1908; (b) Matter, H.; Schwab, W. *J. Med. Chem.* **1999**, *42*, 4506.
11. Watanabe, F.; Tsuzuki, H.; Ohtani, M. (Shionogi & Co. Ltd. Japan), 1999, WO 9727174.
12. Robinson, R. P. (Pfizer Products Inc. USA), 1999, EP 895988 A1.
13. O'Brien, P. M.; Ortwine, D. F.; Pavlovsky, A. G.; Picard, J. A.; Sliskovic, D. R.; Roth, B. D.; Dyer, R. D.; Johnson, L. L.; Man, C. F.; Hallak, H. *J. Med. Chem.* **2000**, *43*, 156.
14. Schudok, M.; Schwab, W.; Zoller, G.; Bartnik, E.; Buttner, F.; Weithmann, K. U. (Aventis Pharma Deutschland Gmbh), 2001, US 6,235,727 B1, and 2002, US 6,500,811 B2.
15. Lefebvre, I. M.; Evans, S. A., Jr. *J. Org. Chem.* **1997**, *62*, 7532.
16. Mikolajczyk, M.; Lyzwa, P.; Drabowicz, J. *Tetrahedron: Asymmetry* **1997**, *8*, 3991.
17. Lefebvre, I. M.; Evans, S. A. *Phosphorus, Sulfur Silicon Relat. Elem.* **1999**, 397.
18. Davis, F. A.; Lee, S.; Yan, H.; Titus, D. D. *Org. Lett.* **2001**, *3*, 1757.
19. Capozzi, M. A.; Cardellicchio, C.; Naso, F.; Spina, G.; Tortorella, P. *J. Org. Chem.* **2001**, *66*, 5933.
20. Davis, F. A.; Zhang, Y.; Andemichael, Y.; Fang, T.; Fanelli, D. L.; Zhang, H. *J. Org. Chem.* **1999**, *64*, 1403.
21. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
22. Christov, V. C.; Aladinova, V. M.; Prodanov, B. *Phosphorus, Sulfur Silicon Relat. Elem.* **1999**, *155*, 67.
23. Page, P. C. B.; McKenzie, M. J.; Gallagher, J. A. *J. Org. Chem.* **2001**, *66*, 3704.
24. Pavlovsky, A.; Ye, Q. Z.; Ortwine, D.; Humblet, C.; Purchase, C.; White, A.; Roth, B.; Johnson, L.; Hupe, D.; Williams, M.; Dhanaraj, V.; Blundell, T. *Protein Sci.* **1999**, *8*, 1455.
25. Dhanaraj, V.; Ye, Q. Z.; Johnson, L. L.; Hupe, D. J.; Ortwine, D. F.; Dunbar, J. B., Jr.; Rubin, J. R.; Pavlovsky, A.; Humblet, C.; Blundell, T. L. *Structure* **1996**, *4*, 375.
26. Lovejoy, B.; Welch, A. R.; Carr, S.; Luong, C.; Broka, C.; Hendricks, R. T.; Campbell, J. A.; Walker, K.; Martin, R.; Van Wart, H.; Browner, M. *Struct. Biol.* **1999**, *6*, 217.
27. Moy, F. J.; Chanda, P. K.; Chen, J.; Cosmi, S.; Edris, W.; Levin, J. I.; Rush, T. S.; Wilhelm, J.; Powers, R. *J. Am. Chem. Soc.* **2002**, *124*, 12659.
28. (a) Murphy, G.; Crabbe, T. *Methods Enzymol.* **1995**, *248*, 470; (b) Tuccinardi, T.; Martinelli, A.; Nuti, E.; Carelli, P.; Balzano, F.; Uccello-Barretta, G.; Murphy, G.; Rossello, A. *Bioorg. Med. Chem.* **2006**, *14*, 4260.
29. (a) SoftMax Pro 4.7.1 by Molecular Device; (b) GraFit version 4 by Erithecus Software.
30. GLIDE; Schrödinger, LLC: Portland, OR 97204.
31. MAESTRO; Schrödinger, LLC: Portland, OR 97204.
32. MACROMODEL, version 7.2; Schrödinger, LLC: Portland, OR 97204.