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Synthesis of Enantiomeric Aminoalkylcarbamoylphosphonates and Their Evaluation as Dual-Action Anticancer MMP and Carbonic Anhydrase Inhibitors

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ABSTRACT: *Carbamoylphosphonates (CPOs) have recently been identified as extracellular in vivo active inhibitors of the cancer and metastasis-promoting extracellular enzymes, carbonic anhydrases (CAs) IX and XII, and matrix metalloproteinase-2 (MMP-2). This article describes the stereoselective synthesis and the evaluation of the biological properties of a pair of enantiomeric aminoalkylcarbamoylphosphonates, which have been enantioselectively synthesized from* L*-serine, using functional group-transformations. The structures of the enantiomeric products have been determined by X-ray crystallography. The enantiomeric purities of compounds have been confirmed by chiral shift reagent NMR experiments and by circular dichroism. In vitro evaluation of the CPOs synthesized revealed that they possess micromolar IC_{50} inhibitory action against CAIX and CAXII. The two enantiomers as well as the racemic or optically inactive ones do not differ by any significant extent from previously reported CPOs' CA inhibitory values. On the other hand, MMPs inhibitory activities are rather weak; only MMP-2 showed a notable IC_{50} value.* © 2014 Wiley Periodicals, Inc. Heteroatom Chem. 00:1–13, 2015; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.21256

INTRODUCTION

For a brief introduction of the enzymes playing roles in this paper, it will suffice to mention that carbonic anhydrases (CAs) are known as an enzyme family catalyzing the interconversion of carbon dioxide and bicarbonate maintaining the required pH in biological fluids and in many physiological and pathological processes [1]. Among the more recently discovered members, there are two membrane-bound isozymes CAIX and CAXII, having extracellularly exposed active sites. These isoforms are expressed by tumor cells under hypoxic conditions, regulating extracellular pH and promoting tumor cell survival and invasion in hypoxic microenvironments [2]. Using various breast cancer cell lines [3], it has been recently demonstrated that CAIX is essential for the survival of tumor cells under the hypoxic condition

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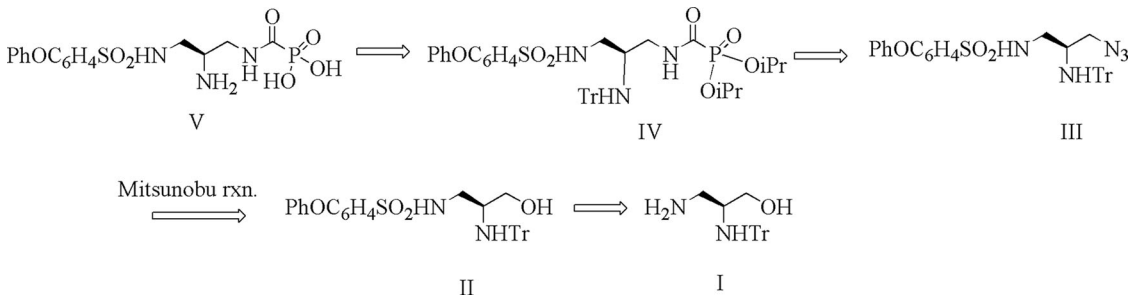


SCHEME 1 Retro synthetic strategy for the synthesis of a chiral CPO.

of breast tumors, and its activity contributes to metastasis formation and could serve as a specific biomarker for such tumors. Inhibition of CAIX and CAXII in tumors results in fewer "cancer stem cells," induction of tumor cell death, regression of tumors, and importantly dramatic inability to metastasize. It is also necessary to mention that in contrast to CAI and CAII that had been long known as clinically useful inhibitors, the membrane-bound, virulently active cancer-promoting enzymes, CAIX and CAXII, discovered in 1992 [4], have no clinically applicable inhibitors, similarly to the better known class of matrix metalloproteinases (MMPs).

The family of MMPs, discovered decades ago, does not need that much introduction in view of the worldwide (unsuccessful) activity to discover useful inhibitors [5], not counting our series of recently published carbamoylphosphonate (CPO) MMP inhibitors [6–10] and one article of CPO CAs [10].

These two kinds of enzymes are capable of together promoting cancer proliferation and metastasis dissemination. Furthermore, applying CPOs to cancer cells under hypoxic conditions resulted in the dose-dependent release of lactate dehydrogenase, confirming the direct interaction of the CPOs with CAIX and XII, thereby promoting cellular damage [10]. We have also demonstrated that the ionized CPO acids are unable to cross cell membranes, and thus they are limited to interact with the extracellular domains of isozymes CAIX and CAXII and matrix metalloproteinase-2 (MMP-2) [10].

This article describes the stereoselective synthesis and the biological properties of a pair of enantiomeric aminoalkylcarbamoylphosphonates, and their structure activity relationships regarding the two enzymes mentioned above.

RESULTS

Synthesis

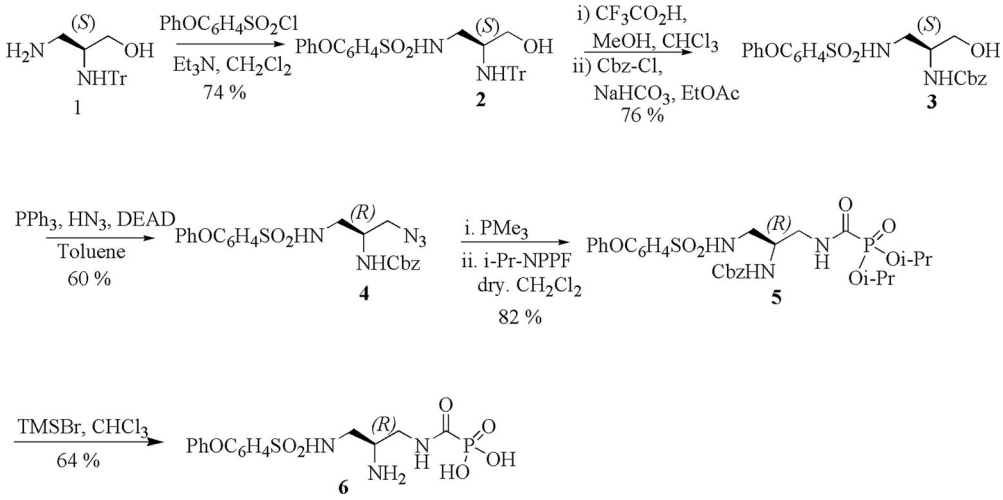
In a previous paper, we reported that the presence of a basic amino group in the carbon chain of the molecule of a CPO may enhance its binding to the enzyme [11]; therefore, we decided to synthesize two enantiomers of a three carbon chain CPO, having an NH_2 group in the middle of the chain to evaluate the effect of chirality on MMP and CA activity. The syntheses of the two enantiomers have been based on the single enantiomer L-serine as a starting material for both. The plan of the synthesis of one enantiomer is shown as the retro-synthetic strategy in Scheme 1). In addition, we also synthesized the racemic molecule as well.

According to the retro synthetic strategy (Scheme 1, carbamoylphosphonic acid **V** can be obtained by the deprotection of trityl and 2-propyl ester groups of the corresponding carbamoylphosphonate **IV** using $(CH_3)_3SiBr$ or Trimethyl-Silyl-Bromide (TMSBr), which in turn can be obtained by the reaction of amine derivative (obtained by the Staudinger reduction of the azide derivative with trimethylphosphine) followed by treatment with 4-nitrophenoxycarbonyl phosphonoformate diisopropyl ester (NPPF-iPr) [12]. This azide derivative III can be obtained by the Mitsunobu reaction of the corresponding alcohol II using triphenylphosphine and hydrazoic acid [13]. The alcohol derivative can be obtained by the reaction of 4-phenoxybenzenesulfonyl chloride with the amino alcohol derivative **I** as shown in Scheme 1.

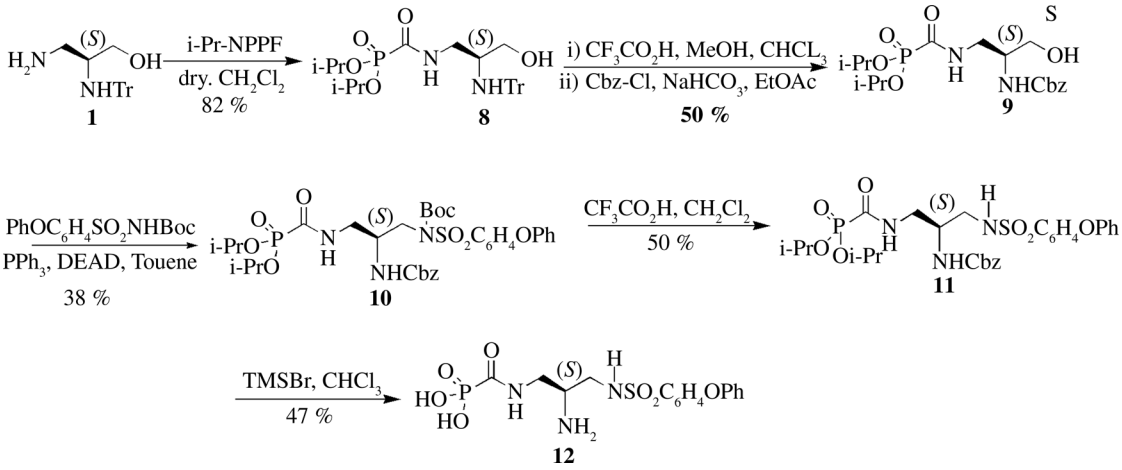
The carbamoylphosphonic acid has been synthesized as shown in Scheme 2. The amino alcohol **1** was prepared according to the published procedure [14] Alcohol **2** was obtained by sulfonylation of the amino alcohol **1** by 4-phenoxybenzenesulfonyl chloride. Since the Mitsunobu reaction of alcohol **2** has gone astray,[i] the trityl group had to be removed

[i] The conversion of alcohol **2** in Scheme 2 to obtain the corresponding azide by the Mitsunobu reaction with HN_3 was tried, but it led to the formation of compound **7** as shown (see Eq. (1)). Therefore, another reaction path was needed.

(1)



SCHEME 2 Synthesis of (*R*)-*N*-[2-amino-3-(4-phenoxybenzenesulfonamido)propyl]carbamoyl phosphonic acid (**6**).

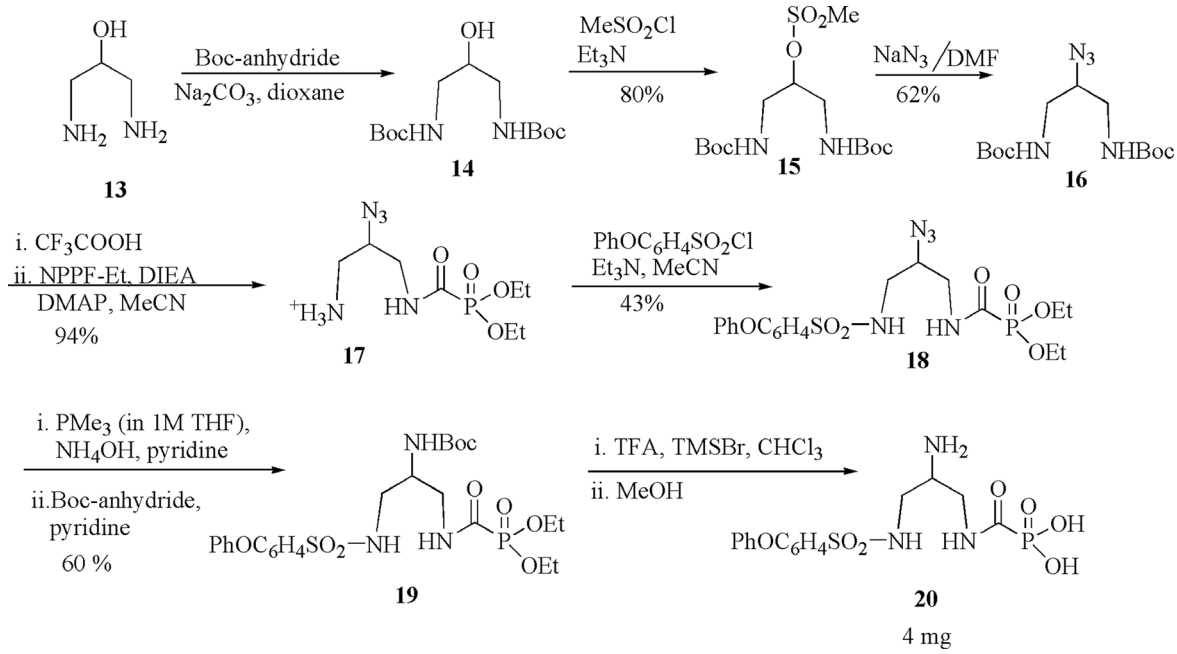


SCHEME 3 Synthesis of (*S*)-*N*-[2-amino-3-(4-phenoxybenzenesulfonamido)propyl]carbamoylphosphonic acid (**12**).

by trifluoroacetic acid (TFA) and the amino group had to be reprotected with the electron-withdrawing benzyloxycarbonyl group as shown in Scheme 2 to compound **3** [15], and then successfully transformed to azide **4** by the Mitsunobu reaction using Ph_3P, followed by the Staudinger reduction and the reaction with NPPF--iPr to provide compound **5**, which was deprotected using TMSBr in $CHCl_3$ to afford the *R* enantiomer, **6**. The structure and the stereochemistry of compound **5** were determined by single crystal X-ray data (Fig. 1).

Similarly, the *S* enantiomer **12** was prepared according to Scheme 3. The treatment of amino alcohol, **1** with NPPF-iPr in dry CH_2Cl_2 provided the compound **8**, which was then treated with TFA to remove the trityl group. The crude amine obtained was again protected with the Cbz group to obtain compound **9**, which was then treated with Boc protected 4-phenoxybenzenesulfonamide under Mitsunobu conditions to compound **10**. Deprotection of the Boc group with TFA provided compound **11**. The final deprotection of Cbz and ester groups with TMSBr in $CHCl_3$ afforded the final compound **12**. The structure of compound **11** was further confirmed by single crystal X-ray data (Fig. 2).

The synthesis of the racemic **20** was based on 1,3-diamino-2-propanol, **13** (Scheme 4). The two amino groups were protected in one step to 1,3-bis-Boc-diaminopropanol **14**. Mesylation of the OH group gave mesylate, **15,** which was converted by S_N2 substitution to azide **16**. This was followed by diethoxyphosphonoformylation of one of the amino groups and the reaction of the second amino group with 4-phenoxybenzenesulfonyl chloride, and finally, the reduction of the azide by Me_3P to the



SCHEME 4 Synthesis of racemic compound **20**.

amino group and by Boc protection to yield the carbamoylphosphonate diethyl ester. Finally, deprotection of the Boc group by TFA and silylation by TMSBr yielded the racemic CPO, **20**.

Stereochemistry

The final enantiomers **6** and **12** had very small angles of rotation; therefore, there was need to find other means to establish the purity or near purity of the compounds. These were found in the next to the last steps in the syntheses, which yielded compounds **5** and **11** and were confirmed by single crystal X-ray data (Figs. 1 and 2 and Table 1), assuming that the final steps kept the steric configurations as confirmed on the basis of the experiment with a chiral shift reagent (see also Fig. 4).

Stereochemistries of compounds **5** and **11** were confirmed by single crystal X-ray data (Figs. 1 and 2). The enantiomeric purities of compounds **5** and **11** were confirmed by chiral shift reagent experiments by ^{1}H NMR (Fig. 3) [16]. The optical purity of compounds **6** and **12** was confirmed by CD data (Fig. 4).

BIOLOGY

Evaluation of Enzyme Inhibition

The IC_{50} values of four CPOs are displayed in Table 2. In addition to the two chiral compounds synthesized in this study (**(*R*)-6** and **(*S*)-12**), two other achiral CPOs (*rac*-**20** and JS-325 [9, 10] (1-(4-phenoxybenzenesulfonamido)-3-[phospho noformamido]propane), which serve as controls, have been added to the table. All compounds in column 3 have relatively poor IC_{50} values of 10–20 μM toward MMP-2. The best MMP inhibitor among all shown is (*R*)-**6** on MMP12. The compound JS-325 [10] lacking an NH_2 group is the most potent MMP2 inhibitor among all CPOs shown [9]. On the other hand, our results concerning CAs fit our experience from our previous study that the molecules' side chains have little influence on their IC_{50} values, which are within about one order of magnitude.

DISCUSSION

It can be concluded from the results that none of the enantiomers or the racemic 1-(4-phenoxybenzenesulfonamido)-2-amino-3-[phosphonoformamido]-propane showed any valuable MMP inhibitory potency. As far as the inhibitory value of the enantiomers or the CPO **JS-325** is concerned, these have the same degree of potency as other CPOs evaluated earlier [10]. The optically active compounds synthesized in this work may become of some value as an analytical tool in a complex biological mixture.

The compounds that have been presented in this article are not the first designed to serve as twofold MMP-CA inhibitors. A number of publications

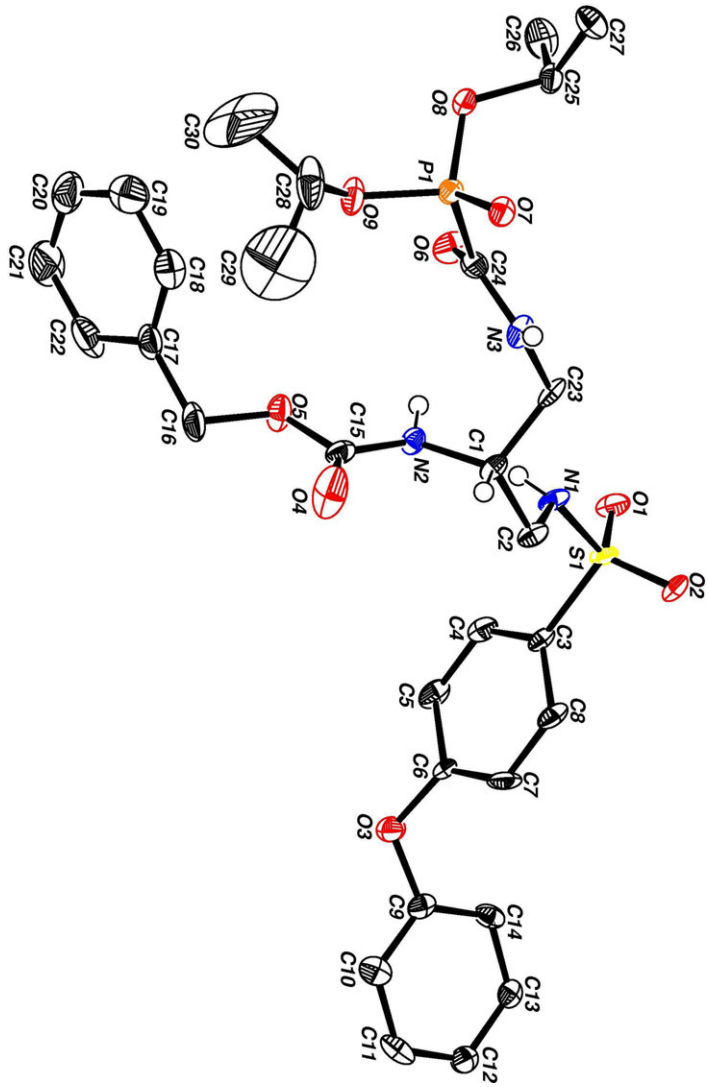


FIGURE 1 ORTEP diagram of compound **5** [(*R*)-Enantiomer]

appeared on hydroxamates, which are powerful zinc-binding groups,but *failed* as in vivo inhibitors because of severe side effects [17–20], which are known as powerful zinc-binding groups but often failed as in vivo inhibitors because of severe side effects. Recently, a promising paper appeared on bisphosphonate based MMP-CA inhibitors as a forerunner of possible drugs for bone cancer [21]. A further development from our laboratory is a threefold inhibitor acting on the enzymes MMP-CA-ATX (autotaxin) based on a CPO [22].

EXPERIMENTAL

Materials

All chemicals were commercially available and used without further purification. Thin layer chromatography (TLC) was performed using Merck silica plates (silica gel 60 F_{254}), and visualization was achieved by ultraviolet (UV) light. Separations by column chromatography were performed using silica gel (0.063–0.200 mm). Melting points (mp) are uncorrected. ^{1}H, ^{13}C, and ^{31}P NMR spectra were obtained with a Varian VXR 300 spectrometer operating at 300, 75, and 121 MHz, respectively. Mass spectra data were recorded by an LTQ-OrbitTrap XL (Thermo Scientific) instrument. Chemical shifts are expressed in δ (ppm) values relative to an internal standard (TMS for proton and carbon; H_3PO_4 for phosphorus), whereas coupling constants (J) are given in hertz. Optical rotations were measured on a Perkin-Elmer model 343 polarimeter. Elemental microanalyses of C, H, and N were performed on a Carlo Erba model 1106 analyzer on the final products **6**, **12**, and **20**.

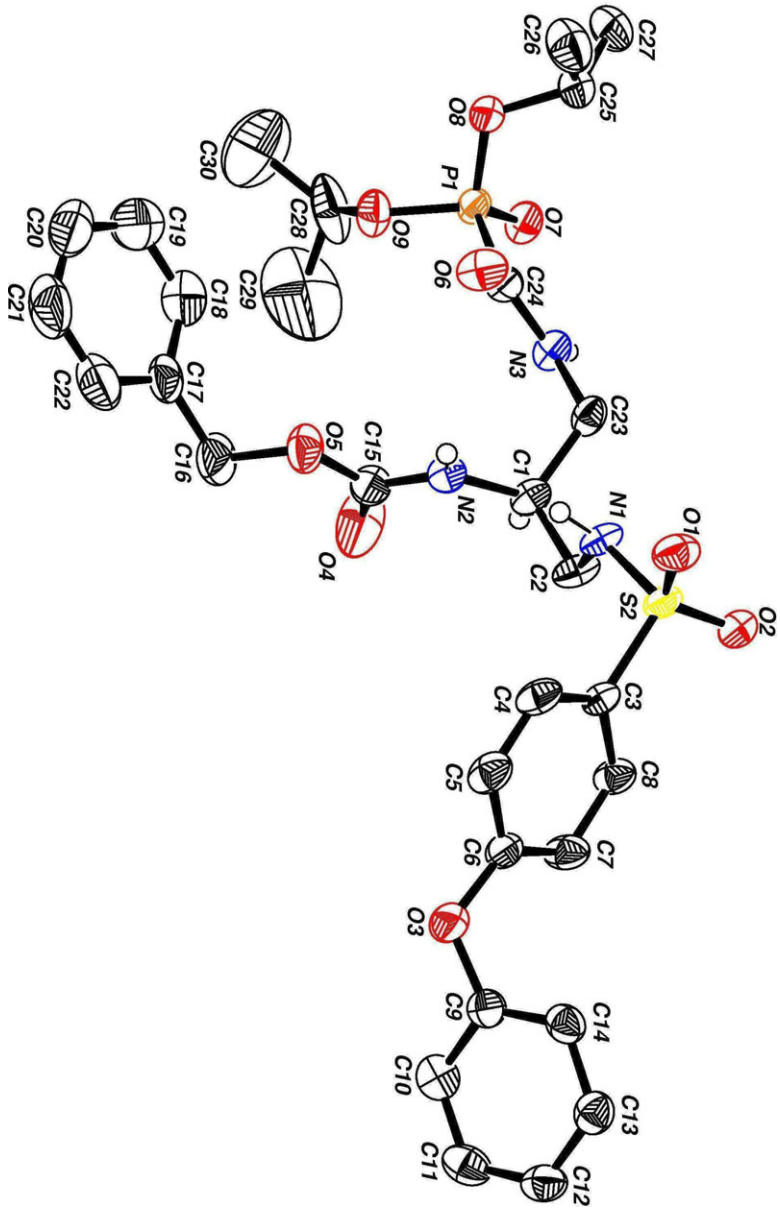


FIGURE 2 ORTEP diagram of compound **11** [(*S*)-Enantiomer]

Syntheses

*(S)-3-(4-Phenoxybenzenesulfonamido)-2-(tritylamino)propanol (**2**).* To a solution of (*S*)-2-triphenylmethylamino-3-aminopropanol (1.99 g, 6 mmol) in CH_2Cl_2 (12 mL) at 0°C, triethylamine (1.7 mL, 12 mmol) was added dropwise followed by the addition of 4-phenoxybenzenesulfonyl chloride (1.61 g in 12 mL CH_2Cl_2). The reaction mixture was stirred at room temperature (RT) for 2 h and then was washed with water (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, concentrated, and purified through column chromatography (silica gel, 35% EtOAc in petroleum ether) to afford (*S*)-3-(4-phenoxybenzenesulfonamido)-2-(tritylamino)propanol **2** (2.52 g, 74% yield) as colorless solid. $[\alpha]_D$ = +64.02 (*c* 1.27, CH_3OH); mp 67–70° C; ^{1}H NMR ($CDCl_3$) δ 2.10 (b s, 1H), 2.37–2.49 (m, 1H), 2.60–2.70 (m, 1H), 2.76–2.87 (m, 2H), 3.13–3.23 (m, 1H), 4.67–4.84 (m, 1H), 6.90–6.96 (m, 2H), 6.97–7.03 (m, 2H), 7.07–7.23 (m, 10H), 7.31–7.44 (m, 9H), 7.61 (d, 2H, J = 9.0 Hz); ^{13}C($CDCl_3$) δ 44.8, 52.1, 62.2, 71.0,117.5, 120.2, 124.9, 126.5, 127.9, 128.4, 129.2, 130.2, 133.1, 146.3, 155.0, 161.4; IR (neat) ν 3302, 3059, 3019 cm^{-1}; MS-ES (*m/z*): 243, 323 (M + 1).

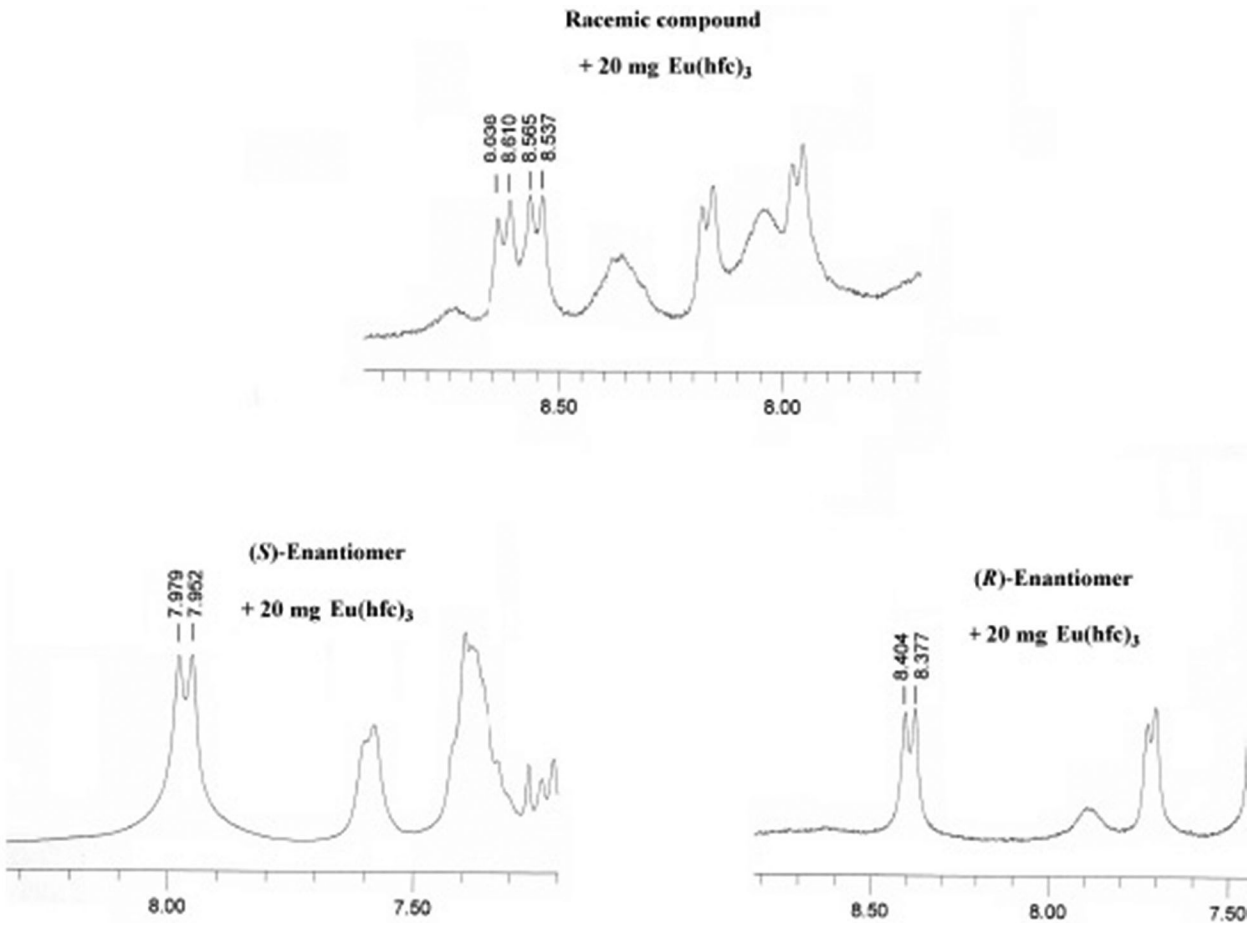


FIGURE 3 300 MHz ^{1}H NMR spectra of 2-Cbz protected carbamoylphosphonate esters in $CDCl_3$ in the presence of $Eu(hfc)_3$ chiral shift reagent ^{1}H NMR spectra of Fig. A (racemate), Fig. B [(*S*)-**11**] and Fig. C [(*R*)-**5**] after addition of chiral shift reagent ($Eu(hfc)_3$).

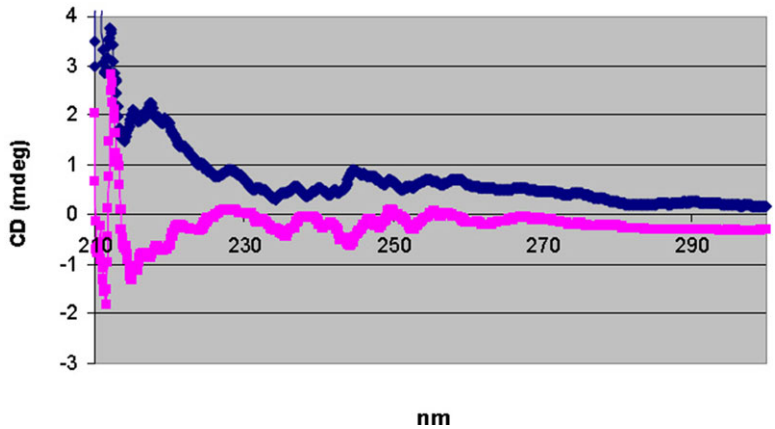


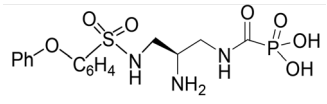
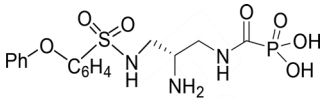
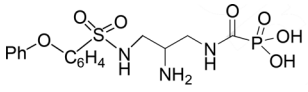
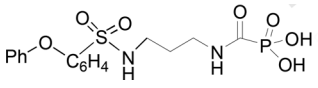
FIGURE 4 Circular dichroism [CD] spectra. Compound (*R*)-**5** purple line, bottom; Compound (*S*)-**11** blue line, top.

(S)-2-(Benzyloxycarbonylamino)-3-(4-phenoxy benzenesulfonamido)propanol (***3***). (*S*)-3-(*4*-phen oxybenzenesulfonamido)-2-(tritylamino)propanol (2.26 g, 4.0 mmol) was dissolved in a mixture of chloroform and methanol (1:1, 8 mL) and cooled to 0°C in an ice bath. The reaction mixture was stirred at 0°C for 2.5 h, after dropwise addition of TFA (5.2 mL, 70 mmol). Solvents were removed, and the residue was azeotroped five times with diethyl ether (5 × 2 mL) and partitioned between

TABLE 1 Crystal Data and Structure Refinement

Compound	*5*	*11*
Empirical formula	$C_{30}H_{38}N_3O_9PS$	
Formula weight	647.66	
Temperature	100(1) K	173(1) K
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	*P*2(1)2(1)2(1)	*P*2(1)2(1)2(1)
Unit cell dimensions		
a, Å	9.886(1)	9.926(1)
b, Å	16.488(2)	16.559(2)
c, Å	20.409(3)	20.395(3)
Volume, Å^3	3326.7(7)	3352.2(8)
Z	4	
Density (calculated), mg/m^3	1.293	1.283
Absorption coefficient, mm^{-1}	0.200	0.198
F(000)	1368	
Crystal size, mm^3	0.32 × 0.10 × 0.06	0.36 × 0.36 × 0.19
Theta range for data collection	2.29–27.00°.	2.28–28.01
Reflections collected	36,379	39,117
Independent reflections	7245 [*R*(int) = 0.1124]	7996 [*R*(int) = 0.0283]
Completeness to theta = 27.00°	99.7%	99.6%
Absorption correction	None	
Refinement method	Full-matrix least-squares on F2	
Data/restraints/parameters	7245/0/411	7996/0/401
Goodness-of-fit on F^2	1.130	1.109
Final *R* indices [$I>2\sigma$ (*I*)]	$R_1 = 0.0791$, $wR_2 = 0.1366$	$R_1 = 0.0484$, $wR_2 = 0.1160$
R indices (all data)	$R_1 = 0.1017$, $wR_2 = 0.1445$	$R_1 = 0.0503$, $wR_2 = 0.1173$
Absolute structure parameter	0.07(11)	0.02(6)
Largest diff. peak and hole, e.Å$^-$	0.458 and -0.414	0.445 and -0.213

TABLE 2 Aminoalkylcarbamoylphosphonates' Inhibition Constants on MMPs and CAs

Symbol	*Structure of CPO*	*MMP2*[a]	*MMP8*[a]	*MMP12*[a]	*hCAI*	*hCAII*	*hCAIX*	*hCAXII*
					IC$_{50}$ (μM)			
(*R*)-6		20	15	12	0.83	0.91	7.00	6.60
(*S*)-12		15	>100	>100	0.79	0.78	6.22	5.01
rac-20		20	>100	>100	ND[b]	ND[b]	ND[b]	ND[b]
JS-325 [10]		10	ND	>100	0.71	0.58	6.80	6.40

Other MMPs showed the following inhibition constants: (*R*)-**6:** MMP-3 >100, MMP-9 >100, MMP-13>100. TACE, >100**. JS-325**, [10] MMP-9, 20, MMP-13, 25, TACE, >100. (*S*)-**12:** MMP-3 ND, MMP-9 >100, MMP13 >100, TACE >100.
[a]Errors for the measurements of MMP IC_{50} values are 5% of the mean values. [b]ND = not determined.

diethyl ether (50 mL) and water (25 mL). The ether layer was washed with water (10 mL), and the combined aqueous fractions are basified with $NaHCO_3$ (1.34 g, 16 mmol). The aqueous fraction was diluted with EtOAc (100 mL), and the mixture was cooled to 0°C in an ice bath. Then benzyl chloroformate (0.57 mL, 4.0 mmol) was added to the reaction mixture and stirred vigorously at RT for 1.5 h. The layers were separated, and the aqueous layer was washed with EtOAc (2 × 20 mL). The combined organic fractions were washed with brine (20 mL), dried over Na_2SO_4, concentrated, and

column purified (silica gel, 5% MeOH in EtOAc) to provide (*S*)-2-(benzyloxycarbonylamino)-3-(*4*-phenoxybenzenesulfonamido)propanol, **3** (1.38 g, 76% yield) as colorless solid. $[\alpha]_D = +4.56$ (*c* 0.98, CH_3OH); mp. 86–89°C; NMR ($CDCl_3$) ^1H, δ 2.66–2.75 (m, 1H), 3.04–3.20 (m, 2H), 3.60–3.92 (m, 3H), 5.05 (s, 2H), 5.44–5.53 (m, 2H), 6.96–7.10 (m, 4H), 7.19–7.27 (m, 1H), 7.29–7.36 (m, 5H), 7.37–7.46 (m, 2H), 7.75 (d, 2H, $J = 9.0$ Hz); $^{13}C(CDCl_3)$: δ 43.4, 51.9, 61.9, 66.9, 117.7, 120.3, 125.0, 127.9, 128.2, 128.5, 129.1, 130.2, 133.0, 136.1, 155.0, 156.6, 161.6; IR (neat) ν 3368, 3295, 3059, 1704 cm^{-1}.

*(R)-2-(Benzyloxycarbonylamino)-3-(4-phenoxy benzenesulfonamido)propyl Azide (**4**)*. To a solution of (*S*)-2-(benzyloxycarbonylamino)-3-(4-phenoxybenzenesulfonamido) -propanol (0.45 g, 1.0 mmol) in toluene (10 mL), PPh_3 (0.28 g, 1.1 mmol) was added and the mixture was stirred at RT for 5 min. After adding hydrazoic acid (1.25 M in toluene, 1.04 mL, 1.3 mmol), **D**i**E**thyl**A**zo**D**icarboxylate (DEAD) (0.17 mL, 1.1 mmol) was added dropwise. The reaction mixture was stirred at RT for 30 min and filtered, and solvent was evaporated. The crude residue was chromatographed (silica gel, 30% EtOAc in *n*-hexane) to afford (*R*)-2-(benzyloxycarbonylamino)-3-(4-phenoxybenzene sulfonamido)-propyl azide **4** (0.29 g, 60% yield) as low melting solid. NMR ($CDCl_3$) ^1H, δ 2.85–3.10 (m, 2H), 3.35–3.55 (m, 2H), 3.70–3.85 (m, 1H), 5.01 (s, 2H), 5.10 (t, 1H, $J = 6.0$ Hz), 5.22 (d, 1H, $J = 9.0$ Hz), 6.90–7.03 (m, 4H), 7.12–7.20 (m, 2H), 7.23–7.30 (m, 4H), 7.31–7.38 (m, 2H), 7.69 (d, 2H, $J = 9.0$ Hz); $[\alpha]_D = +1.00$ (*c* 1.99, CH_3OH); $^{13}C(CDCl_3)$: δ 44.1, 50.1, 51.7, 67.1, 117.6, 120.3, 125.0, 128.0, 128.2, 128.5, 129.2, 130.2, 132.9, 136.0, 155.0, 156.1, 161.7; IR (neat) ν 3296, 2101, 1699 cm^{-1}.

*Diisopropyl (R)-N-[2-(Benzyloxycarbonylamino)-3-(4-phenoxybenzenesulfonami-do)propyl]carbamoy lphosphonate (**5**)*. Trimethylphosphine [0.93 mL (1.0 M solution), 0.933 mmol] was added dropwise to a stirred solution of (*R*)-2-(benzyloxycarbonylamino)-3-(4-phenoxybenzenesul fonam-ido)propyl azide (0.15 g, 0.31 mmol) in dry CH_2Cl_2 (1.0 mL). The reaction mixture was stirred at RT for 1.5 h under nitrogen atmosphere. NPPF-iPr (0.15 g, 0.46 mmol) was added to the reaction mixture and stirred for one more hour at RT. The solvent was evaporated, and the crude residue was column purified (silica gel, 80% EtOAc/*n*-hexane) to afford diisopropyl (*R*)-*N*-[2-(benzyloxycarbonylamino)-3-(4-phenoxybenzenesulfonamido)propyl]carbamoylphosphonate **5** (0.165 g, 82% yield) as colorless solid. $[\alpha]_D = +7.50$ (*c* 1.99, CH_3OH); mp 105–108°C; NMR ($CDCl_3$) ^{31}P, δ -4.00 ppm; ^1H, δ 1.19–1.29 (m, 12H), 2.87–2.97 (m, 2H), 3.34–3.55 (m, 2H), 3.63–3.77 (m, 1H), 4.60–4.77 (m, 2H), 4.99 (s, 2H), 5.75 (d, 1H, $J = 7.2$ Hz), 5.85 (t, 1H, $J = 6.0$ Hz), 6.89–7.03 (m, 4H), 7.12–7.20 (m, 2H), 7.21–7.29 (m, 4H), 7.30–7.38 (m, 2H), 7.63–7.75 (m, 3H); IR (neat) ν 3335, 3177, 2985, 1719, 1649 cm^{-1}; $^{13}C(CDCl_3)$ δ 23.5, 23.6, 23.7, 23.7, 39.9 (d, $J = 7.5$ Hz), 43.5, 50.7, 66.7, 73.9 (d, $J = 6.0$ Hz), 117.5, 120.1, 124.8, 127.8, 128.0, 128.4, 129.0, 130.1, 133.4, 136.1, 155.0, 156.2, 161.3, 167.9 (d, $J =$ 226.3 Hz); MS-ES (*m/z*): 648 (M + 1).

*(R)-N-[2-Amino-3-(4-phenoxybenzenesulfonamido)propyl]carbamoyl Phosphonic Acid (**6**)*. To a stirred solution of (*R*)-benzyl 1-(diisopropylphosphonoformamido)-3-(4-phenoxyphenylsulfonamido)prop-2-ylcarbamate (0.13 g, 0.2 mmol) in $CHCl_3$ (2 mL), TMSBr (0.26 mL, 2.0 mmol) was added dropwise. The reaction mixture was stirred for 12 h at 50°C. Then two portions of 5 equiv of TMSBr were added to the reaction mixture at an interval of 24 h. After completion of the reaction, the solvent was evaporated. Methanol was added to the reaction mixture and stirred at RT for 1 h. Solvents were evaporated, and the required (*R*)-*N*-[2-amino-3-(4-phenoxyphenylsulfonamido)propyl]carbamoylphosphonic acid **6** (0.055 g, 64% yield) solidified to a white solid by adding EtOH, mp 220–235°C; NMR ($D_2O + NaHCO_3$) ^{31}P, δ –1.17 ppm; ^1H, δ 2.56–2.78 (m, 2H), 2.88–3.12 (m, 2H), 3.32–3.46 (m, 1H), 6.80–6.90 (m, 4H), 6.96–7.05 (m, 1H), 7.13–7.24 (m, 2H), 7.52 (dd, 2H, $J = 9.0, 2.4$ Hz); Sodium salt MS-ES (*m/z*): 474 (M + 1). Anal. Calcd. For $2C_{16}H_{20}N_3O_7PS + 3H_2O$: C, 42.11, H, 5.08, N, 9.21. Found: C, 41.85, H, 4.89, N, 9.05.

*(R)-4-Phenoxy-N-((1-tritylaziridin-2-yl)methyl) benzenesulfonamide (**7**)*. To a solution of (*S*)-3-(4-phenoxybenzenesulfonamido)-2-(tritylamino)propanol (1.80 g, 3.2 mmol) in toluene (30 mL), PPh_3 (0.923 g, 3.52 mmol) was added and the mixture was stirred at RT for 5 min. After adding hydrazoic acid (0.62 M in toluene, 5.66 mL, 3.52 mmol), DEAD (0.55 mL, 3.52 mmol) was added dropwise. The reaction mixture was stirred at RT for 30 min and filtered, and the solvent was evaporated. The crude residue was chromatographed (silica gel, 30% EtOAc in *n*-hexane) to afford (*R*)-4-phenoxy-*N*-((1-tritylaziridin-2-yl)methyl)benzenesulfonamide **7** (1.31 g, 75% yield) as white solid. mp. 85–87°C. NMR ($CDCl_3$) ^1H, δ 1.02 (d, 1H, $J = 6.3$ Hz), 1.38–1.46 (m, 1H), 1.74 (d, 1H, $J = 3.0$ Hz), 3.14–3.34 (m, 2H), 4.78 (t, 1H, $J = 5.1$ Hz), 7.02–7.14 (m, 4H), 7.20–7.29 (m,

10H), 7.30–7.36 (m, 6H), 7.40–7.48 (m, 2H), 7.74 (d, 2H, $J = 8.7$ Hz); ^{13}C δ 24.6, 30.5, 44.4, 73.7, 117.5, 120.2, 124.9, 126.8, 127.5, 129.1, 130.1, 133.4, 143.9, 154.9, 161.4; MS-ES (*m/z*): 569 (M + Na).

*(S)-3-Diisopropylphosphonoformamido-2-(tritylamino)propanol (**8**).* A solution of diisopropyl 4-nitrophenoxycarbonylphosphonoformate (NPPF-iPr) (9.27 g, 28 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise to a solution of (*S*)-2-triphenylmethylamino-3-aminopropanol (9.3 g, 28 mmol) in dry CH_2Cl_2 (100 mL) at 0°C. The reaction mixture was stirred at room temperature for 1 h. Workup was done by washing the reaction mixture successively with 0.5 M NaOH and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue obtained was purified by column chromatography (silica gel, 70% EtOAc in petroleum ether) to provide (*S*)-3-diisopropyl phosphonoformamido-2-(tritylamino)propanol, **8** (12.0 g, 82% yield) as gummy solid. $[\alpha]_D = +25.31$ (*c* 1.10, CH_3OH); NMR [$CDCl_3$,D_2O (two drops)] ^{31}P, δ –3.43 ppm; ^{1}H, δ 1.24–1.42 (m, 12H), 2.40–2.55 (m, 1H), 2.64–2.80 (m, 1H), 2.92–3.32 (m, 3H), 4.60–4.90 (m, 2H), 7.08–7.38 (m, 9H), 7.45–7.59 (m, 6H); ^{13}C($CDCl_3$): δ 23.4–23.9 (m, 4C), 41.0 (d, J = 6.7 Hz), 52.8, 61.1, 70.9, 73.5 (t, $J = 6.0$ Hz), 126.3, 127.8, 128.4, 146.4, 167.6 (d, $J = 223.3$ Hz); IR (neat) ν 3396, 3320, 2977, 2939, 1649 cm^{-1}; MS-ES (*m/z*): 243, 283 (M + 1).

*(S)-2-Benzyloxycarbonylamino 3-(diisopropylphosphonoformamido)propanol (**9**).* (*S*)-3-Diisopropylphosphonoformamido-2-(tritylamino)propanol (10.49 g, 20 mmol) was dissolved in a mixture of chloroform–methanol (1:1, 40 mL) and cooled to 0°C in ice bath. The reaction mixture was stirred at RT for 7 h, after dropwise addition of TFA (20.0 mmol, 1.5 mL) at 0°C. The solvents were removed, and the residue was azeotroped five times with diethyl ether (5 × 40 mL) and partitioned between diethyl ether (150 mL) and water (50 mL). The ether layer was washed with water (20 mL), and the combined aqueous fractions were basified with $NaHCO_3$ (80 mmol, 6.72 g). The aqueous fraction was diluted with EtOAc (150 mL), and the mixture was cooled to 0°C in an ice bath. Benzyl chloroformate (2.9 mL, 20 mmol) was then added to the reaction mixture at 0°C and stirred vigorously at RT for 1.5 h. The layers were separated, and the aqueous layer was washed with EtOAc (2 × 30 mL). The combined organic fractions were washed with brine (40 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, 5% MeOH in EtOAc) to afford **(***S*)-2-benzyloxycarbonylamino-3- (diisopropylphosphonoformamido)propanol, **9** (4.16 g, 50% yield) as gummy solid. $[\alpha]_D = -13.44$ (*c* 0.96, CH_3OH); NMR ($CDCl_3$) ^{31}P, δ –3.82 ppm. ^{1}H: δ 1.28–1.37 (m, 12H), 3.44–3.56 (m, 3H), 3.56–3.70 (m, 2H), 3.72–3.86 (m, 1H), 4.66–4.84 (m, 2H), 5.03–5.14 (m, 2H), 5.66–5.74 (m, 1H), 7.30–7.36 (m, 5H), 7.96–8.06 (m, 1H); ^{13}C($CDCl_3$): δ 23.6, 23.6, 23.7, 23.7, 39.8 (d, $J = 6.0$ Hz), 52.1, 61.7, 66.7, 73.9 (d, J = 6.7 Hz), 127.9, 128.0, 128.4, 136.2, 156.3, 167.9 (d, $J = 225.6$ Hz); IR (neat) ν 3313, 2977, 2939, 1713, 1649 cm^{-1}; MS-ES (*m/z*): 417 (M + 1).

*Diisopropyl N-[2-Benzyloxycarbonylamino-3-(-N-tert-butoxycarbonyl)-3-(4-phenoxybenzenesulfonamido)propyl] carbamoylphosphonate (**10**).* To a solution of **(***S*)-2-benzyloxycarbonylamino 3-(diisopropylphosphonoformamido)- propanol (0.208 g, 0.5 mmol) in toluene (5 mL), PPh_3 (0.144 g, 0.55 mmol) was added and the mixture was stirred for 5 min. To this solution, *N*-(*tert*-butoxy)-*N*-(4-phenoxybenzenesulfonyl)carbamate (0.192 g, 0.55 mmol) was added followed by the slow addition of DEAD (0.09 mL, 0.55 mmol). The reaction was stirred at RT for 12 h. The reaction mixture was filtered, the solvent was evaporated, and the obtained residue was purified through column chromatography (silica gel, 30% EtOAc in *n*-hexane) to provide the product **10** (0.140 g, 38% yield) as colorless low melting solid. $[\alpha]_D = +12.36$ (*c* 1.17, CH_3OH); NMR ($CDCl_3$) ^{31}P, δ –3.56 ppm; ^{1}H, δ 1.25–1.32 (m, 21 H), 3.33–3.65 (m, 2H), 3.75–3.95 (m, 2H), 4.05–4.25 (m, 1H), 4.65–4.85 (m, 2H), 4.95–5.15 (m, 2H), 5.49 (d, 1H, J = 7.2 Hz), 6.90 (dd, 2H, J = 9.0, 1.2 Hz), 6.95–6.02 (m, 2H), 7.13–7.20 (m, 2H), 7.20–7.28 (m, 4H), 7.30–7.38 (m, 2H), 7.48–7.58 (m, 1H), 7.73 (d, 2H, J = 7.8 Hz); ^{13}C($CDCl_3$): δ 23.5–23.9 (m, 4C), 27.7, 40.6 (d, J = 7.85 Hz), 47.3, 51.3, 66.6, 73.5 (d, J = 6.33Hz), 84.9, 117.0, 120.1, 125.0, 127.8, 128.3, 130.1, 130.3, 132.8, 136.3, 151.1, 154.7, 156.2, 162.0, 167.6 (d, J = 225.42 Hz); IR (neat) ν 3298, 2973, 1647 cm^{-1}; MS-ES (*m/z*): 748 (M + 1).

*Diisopropyl (S)-2-Benzyloxycarbonylamino-3-(4-phenoxybenzenesulfonamido)-propylcarbamoylphosphonate (**11**).* To a stirred solution of diisopropyl *N*-[2-benzyloxycarbonylamino-3-(-*N*-*tert*-butoxycarbonyl)-3-(-4-phenoxybenzenesulfonamido)propyl]carbamoylphosphonate (0.28 g, 0.37 mmol) in CH_2Cl_2 (1.5 mL), TFA (1.5 mL) was added dropwise. The reaction mixture was stirred at RT for 2 h, quenched with saturated $NaHCO_3$ solution, extracted with EtOAc (3 × 20 mL), and finally washed with brine. Combined organic layers were dried over anhydrous Na_2SO_4, concentrated, and column

purified (silica gel, 70% EtOAc in *n*-hexane) to provide diisopropyl (*S*)-2-benzyloxycarbonylamino-3-(4-phenoxybenzenesulfonamido)propylcarbamoylphosphonate **11** (0.217 g, 90% yield) as colorless solid. $[\alpha]_D = -6.78$ (c 1.99, CH_3OH); mp 106–108°C; NMR ($CDCl_3$) ^{31}P, δ –4.06 ppm; ^1H, δ 1.15–1.35 (m, 12H), 2.85–3.05 (m, 2H), 3.30–3.58 (m, 2H), 3.60–3.80 (m, 1H), 4.60–4.76 (m, 2H), 4.99 (s, 2H), 5.74 (d, 1H, J = 7.4 Hz), 5.83 (t, 1H, J = 6.6 Hz), 6.90–7.02 (m, 4H), 7.12–7.21 (m, 2H), 7.22–7.29 (m, 4H), 7.30–7.38 (m, 2H), 7.59–7.67 (m, 1H), 7.70 (d, 2H, J = 9.0 Hz); IR (neat) ν 3337, 3189, 2978, 1719, 1648 cm^{-1}; $^{13}C(CDCl_3)$: δ 23.7, 23.7, 23.8, 23.8, 39.9 (d, J = 6.7 Hz), 43.5, 50.8, 66.8, 74.0 (d, J = 6.7 Hz), 117.6, 120.2, 124.9, 128.0, 128.1, 128.5, 129.1, 130.2, 133.4, 136.2, 155.1, 156.3, 161.4, 168.2 (d, J = 225.6 Hz); MS-ES (*m/z*): 648.0 (M + 1).

*(S)-N-[2-Amino-3-(4-phenoxybenzenesulfonamido)propyl]carbamoylphosphonic acid (**12**).* To a stirred solution of diisopropyl (*S*)-2-benzyloxycarbonylamino-3-(4-phenoxybenzenesulfonamido)propylcarbamoylphosphonate (0.10 g, 0.15 mmol) in $CHCl_3$ (2 mL), TMSBr (0.20 mL, 1.5 mmol) was added dropwise. The reaction mixture was stirred for 12 h at 50°C. Then two 5 equiv of TMSBr was added to the reaction mixture at an interval of 24 h. After completion of the reaction (^{31}P NMR -20.5 ppm), the solvent was evaporated. Methanol was added to the reaction mixture and stirred at RT for 1 h. Solvents were evaporated, and the product was solidified using MeOH. Mother liquor was evaporated, and the product was solidified from residue using EtOH, to get the second crop of the product (**12**) [0.031 g, 47% yield (including the first crop product)] as white solid. mp. 220–235°C; NMR (D_2O + $NaHCO_3$) ^{31}P, δ –0.89 ppm; ^1H, δ 2.65–2.98 (m, 2H), 3.00–3.28 (m, 2H), 3.42–3.60 (m, 1H), 6.96–7.22 (m, 5H), 7.23–7.42 (m, 2H), 7.69 (d, 2H, J = 9.0 Hz); MS-ES (*m/z*): 430 (M + 1). Anal. Calcd. For $2C_{16}H_{20}N_3O_7PS + 3H_2O$: C 42.11, H 5.08, N 9.21. Found: C 41.83, H, 4.95, N, 9.00.

*1-(4-Phenoxybenzenesulfonamido)-2-amino-3-[phosphonoformamido]propane (**20**)*

*N,N'-di-Boc-2-hydroxy-1,3-diaminopropane (**14**)* To a solution of 2-hydroxy-1,3-diaminopropane (**13**) (5.1 g, 56.6 mmol) in 25 mL water, Boc-anhydride (24.6 g, 113 mmol) was added in 50 mL dioxane followed by Na_2CO_3 (13.76 g, 141 mmol) at 0°C and stirred for 2 h. The reaction mixture was allowed to warm to room temperature and was then stirred for an additional 16 h. Then the solvents were evaporated; the residue was diluted with water and extracted with EtOAc (3 × 50 mL). The organic phase was dried over Na_2SO_4, and the solvent was evaporated. Recrystallization of product from hot cyclohexane gave 13.19 g (80%) white solid. mp = 92°C. NMR ($CDCl_3$) ^1H: 1.46 (s, 18H, Boc), 3.1–3.32 (m, 4H, 2 × CH_2), 3.73 (m, 1H, CH), 5.11 (bs, NH).

*N,N'-di- Boc-2-methanesulfonyloxy-1,3-diaminopropane (**15**)*

To a solution of **14** (13.19 g, 45.4 mmol) and triethylamine (1.5 equiv, 9.5 mL, 68.1 mmol) in dry 50 mL **D**i**C**hloro**M**ethane (DCM), a solution of methanesulfonyl chloride (1.5 equiv, 5.25 mL, 68 mmol) was added dropwise in dry 15 mL DCM over a period of 1 h at 0°C with stirring under nitrogen. After the addition, the reaction was stirred overnight at room temperature. Water was slowly added to quench the reaction; the organic layer was separated and then washed with water (5 × 15 mL), dried over Na_2SO_4, and evaporated to give white solid. Recrystallization from hot acetone and cyclohexane gave 10.36 g (62%) white solid. mp = 127°C. NMR ($CDCl_3$) ^1H: 1.43 (s, 18H, Boc), 3.1 (s, 3H, CH_3), 3.26–3.34 (m, 2H, CH_2), 3.45–3.54 (m, 2H, CH_2), 4.66 (m, 1H, CH), 5.2 (bs, NH).

*2-Azido-N^1,N^3-bis-Boc-1,3-diaminopropane (**16**)*

To a solution of the **15** (10.36 g, 28 mmol) in 60 mL dry DMF, sodium azide (4 equiv, 7.3 g, 112 mmol) was added and the mixture was refluxed for 12 h at 70°C. The reaction mixture was cooled and poured into water. The solid which formed was filtered and washed with water to give 9.45 g (94%) of product. mp = 75–77°C. NMR ($CDCl_3$) ^1H: 1.44 (s, 18H, Boc), 3.10–3.17 (m, 2H, CH_2), 3.3–3.6 (m, 2H, CH_2), 3.64 (m, 1H, CH), 4.72 (bs, NH), 5.08 (bs, NH). IR (NaCl): 2115 cm^{-1}(N_3).

*2-Azido-3-[diethylphosphonoformamido]propylamine (**17**)*

2-Azido-N^1,N^3-di-Boc-1,3-diamino propane (3.34 g, 10.6 mmol) (**16**) was dissolved in 10 mL TFA. The reaction was stirred for 30 min, and the excess of TFA was removed in vacuo with toluene. To the residue in 25 mL of MeCN with **D**i**I**sopropyl**E**thyl**A**mine (DIEA) (2.2 equiv, 3.86 g, 12.7 mmol) and **D**i**M**ethyl**A**mino**P**yridine (DMAP) (0.13 g, 10% mol), very slowly NPPF-E solution (1.2 equiv, 3.86 g, 12.7 mmol) was added in

15 mL MeCN. The reaction was stirred overnight at room temperature. The examination of the reaction mixture by ^{31}P NMR showed that the reaction was finished. The white precipitate that formed was collected by filtration and washed with MeCN to give 1.26 g (43%) solid. mp = 154–156°C. NMR (DMSO-d_6) ^{31}P: –0.75; ^{1}H: 1.28 (dd, J = 7.2 Hz, 6H, 2 × **CH_3**CH_2O), 2.69–2.77 (m, 1H, CH_2), 2.99–3.04 (m, 1H, CH_2), 3.4 (m, J = 7.1Hz, 2H, CH_2), 4.01 (m, 1H, CH), 4.14 (m, 4H, 2 × CH_3**CH_2**O).

*2-Azido-1-(4-phenoxybenzenesulfonamido)-3-[diethylphosphonoformamido] propane (**18**)*

To a solution of **17** (1.26 g, 4.5 mmol) and triethylamine (1.2 equiv, 1.46 g, 5.4 mmol) in 15 mL MeCN, 4-phenoxybenzenesulfonyl chloride solution (1.5 equiv, 68 mmol, 5.25 mL) was added dropwise in 15 mL MeCN at 0°C with stirring. After the addition, the reaction was stirred overnight at room temperature. The solvent was evaporated, and the residue was purified by silica gel chromatography [eluent: DCM /1% MeOH] to yield 1.36 g (60%) a pale yellow oil. NMR ($CDCl_3$) ^{31}P: –2.18; ^{1}H : 1.35 (dd, J = 7.2 Hz, 6H, 2 × **CH_3**CH_2O), 2.91–3.08 (m, 2H, CH_2), 3.41–3.48 (m, 1H, CH_2), 3.57–3.6 (m, 1H, CH_2), 3.8 (m, J = 5.7 Hz, 1H, CH), 4.22 (m, 4H, 2 × CH_3**CH_2**O), 5.75 (t, 1H, NH), 7.05 (dd, J = 8.5 Hz, 4H), 7.22 (t, J = 7.6 Hz, 1H), 7.4 (t, J = 7.9 Hz, 2H), 7.8 (d, J = 8.7 Hz, 2H).

*2-Boc-amino-1-(4-phenoxybenzenesulfonamido)-3-[diethylphosphonoformamido] Propane (**19**). [23]*

To a stirred solution of **18** (0.36 g, 0.7 mmol) in a mixture of pyridine and NH_4OH (7:1, 5 mL), PMe_3 (1 M in THF, 3 equiv, 2.1 mmol, 2.1 mL) was added and the reaction mixture was stirred under nitrogen for 3 h. The solution was diluted with EtOH (5 mL) and water (1 mL) and concentrated. The resulting residue was dissolved in a mixture of toluene and EtOH (1:1, 16 mL) and concentrated again to yield 0.36 g of yellow oil. The absence of the azide group was confirmed by IR analysis. The product was dissolved in 10 mL of pyridine, and to the solution Boc-anhydride was added slowly at 0°C. The reaction was stirred 3 days at room temperature. The examination of the reaction mixture by ^{31}P NMR showed two major peaks at –0.5 and –0.8 ppm. The reaction was stopped, and the solvent was evaporated. The residue was dissolved in 15 mL EtOAc and washed with 0.5 N HCl (2 × 5 mL), $NaHCO_3$ (2 × 5 mL), and water (5 mL). The organic phase was collected and dried over Na_2SO_4. The residue was purified by silica gel column chromatography [eluent: EtOAc/petroleum ether (3:7)] to yield 60 mg (15%) colorless oil. NMR ($CDCl_3$) ^{31}P: –1.88; ^{1}H : 1.35 (dd, J = 6.8 Hz, 6H, 2 × **CH_3**CH_2O), 1.42 (s, 9H, Boc), 2.98 (s, 2H, CH_2), 3.49 (s, 2H, CH_2), 3.76 (s,1H, CH), 4.22 (m, 4H, 2 × CH_3**CH_2**O), 5.45 (s, 1H, NH), 6.12 (s, 1H, NH), 7.05 (dd, J = 8.2 Hz, 4H), 7.23 (t, J = 7.5 Hz, 1H), 7.4 (t, J = 7.7 Hz, 2H), 7.8 (d, J = 8.5 Hz, 2H), 8.02 (s, 1H, NH),

*2-Amino-1-(4-phenoxybenzenesulfonamido)-3-[phosphonoformamido]propane (**20**)*

2-Boc-amino-1-(4-phenoxybenzenesulfonamido-3-[diethylphosphonoformamido] propane (**19**) (50 mg, 0.085 mmol) was dissolved in 2 mL TFA. The reaction was stirred for 30 min, and the excess of TFA was removed in vacuo with toluene. The residue was dissolved in 2 mL $CHCl_3$; and to solution was added TMSBr (10 equiv, 0.12 mL, 0.85 mmol), and reaction was stirred and heated at 50°C for 3 h. The examination of the reaction mixture by ^{31}P NMR showed that the reaction was completely finished. The solvent was evaporated, and to the residue was added MeOH (2 mL) for 30 min. The solvent was evaporated to give brown foam, which was solidified from EtOH. The white solid was filtered and washed with MeCN to give 4 mg of product. NMR (D_2O+$NaHCO_3$) ^{31}P: –1.09; ^{1}H : 2.81–2.96 (m, 2H, CH_2), 3.13–3.28 (m, 2H, CH_2), 3.58 (m, 1H, CH), 7.08 (dd, J = 9.0 Hz, 4H), 7.2 (t, J = 7.5 Hz, 1H), 7.4 (t, J = 8.0 Hz, 2H), 7.58 (d, J = 9.0 Hz, 2H). Anal. Calcd. For $C_{16}H_{20}N_3O_7PS$, C, 44.76, H, 4.69, N, 9.79. Found. C, 44.49, H, 4.90, N, 10.05.

Determination of MMP Inhibitory Potency

Recombinant enzymes, human MMP-2, -3, -8, -9, -12, -13, and TACE (R&D Systems, Minneapolis, MN, USA) were incubated at four different concentrations with the relevant colorimetric or fluorescent peptide substrates (R&D Systems) and monitored every 5 min for 3 h. The examined compounds were added at four to six different concentrations, and the inhibitory potencies, expressed in a colorimetric change, were measured by an Enzyme-Linked ImmunoSorbent Assay (ELISA) or fluorescent reader. Each experiment was repeated twice.

Carbonic Anhydrase Inhibition

Assaying the CA-catalyzed CO_2 hydration activity was done by using an Applied Photophysics stopped flow instrument as described previously [24].

Crystallography

A single crystal of compound **5** or **11** was attached on a MiTeGen micromesh mount with Gargille immersion oil, and transferred to a Bruker SMART APEX CCD X-ray diffractometer equipped with a graphite-monochromator. Maintaining the crystal at low temperature, was done with a Bruker KRYOFLEX nitrogen cryostat. The system was controlled by a pentium-based PC running the SMART software package [25]. Data were collected using MoKα radiation ($\lambda = 0.71073$ Å). Immediately after collection, the raw data frames of each enantiomer were transferred to a second PC computer for integration and reduction by the SAINT program package [26]. The structures were solved and refined by the SHELXTL software package [27]. Results are summarized in Table 1.

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