ACS OMEGA

Article

Cite This: ACS Omega 2018, 3, 4441−4452

Preparation of Phosphonic Acid Analogues of Proline and Proline Analogues and Their Biological Evaluation as δ^1-Pyrroline-5-carboxylate Reductase Inhibitors

Renzhe Qian,[†] Thomas Kalina,[†] Jeannie Horak,[‡] Samuele Giberti,[§] Giuseppe Forlani,[§] and Friedrich Hammerschmidt*[,†]

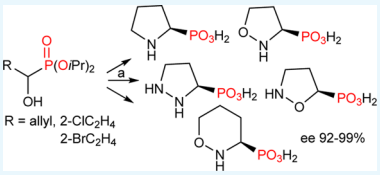
[†]Institute of Organic Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna, Austria

[‡]Institute of Pharmaceutical Sciences, Pharmaceutical (Bio-)Analysis, Eberhard-Karls-University Tübingen, Auf der Morgensstelle 8, 72076 Tübingen, Germany

[§]Department of Life Science and Biotechnology, University of Ferrara, via L. Borsari 46, 44121 Ferrara, Italy

Supporting Information

ABSTRACT: Racemic 1-hydroxy-3-butenyl-, 3-chloro-1-hydroxypropyl-, and 3-bromo-1-hydroxypropylphosphonate and the corresponding (*S*)-enantiomers obtained by lipase-catalyzed resolution of the respective racemic chloroacetates were subjected to functional group manipulations. These comprised ozonolysis, Mitsunobu reactions with hydrazoic acid and *N*-hydroxyphthalimide, alkylation of hydrazine derivative, removal of phthaloyl group followed by intramolecular substitution, and global deprotection to deliver the racemates and (*R*)-enantiomers (ee 92−99% by chiral high-performance liquid chromatography) of pyrrolidin-2-yl-, oxazolidin-3-yl-, oxazolidin-5-yl-, pyrazolidin-3-yl-, and 1,2-oxazinan-3-ylphosphonic acids. These phosphonic acids were evaluated as analogues of proline and proline analogues for the ability to inhibit γ-glutamyl kinase, δ^1-pyrroline-5-carboxylate synthetase, and δ^1-pyrroline-5-carboxylate reductase. Only the latter enzyme was inhibited by two of them at concentrations exceeding 1 mM.



INTRODUCTION

Herbicides with favorable properties such as high activity, crop tolerance, and low toxicity to insects and mammals are essential for weed control in modern agriculture to secure food supply for the growing world population.[1] Furthermore, persistence of these agrochemicals should be minimal and biodegradation by the soil microflora should be complete in a short time span. Rapid development of resistant weeds in combination with diffusion of the acquired resistance in the biosphere is a public concern and has forced companies to search for new herbicide targets and active ingredients. Amino acid metabolism is an attractive target for herbicide development.[2] However, little attention has so far been paid to interference with proline biosynthesis. It is accessed by two routes, the ornithine and glutamate pathways, the latter being the main one in plants. Both the pathways share the last reaction step catalyzed by the NAD(P)H-dependent δ^1-pyrroline-5-carboxylate (P5C) reductase.[3] Luckily, this fact allows to block both ways with only one inhibitor.

Forlani and Kafarski et al. found that *N*-phenyl-substituted aminomethylenebisphosphonic acids are inhibitors of P5C reductase with activity in the micromolar to millimolar range.[4−6] These compounds also demonstrated phytotoxicity in vivo, which could be reversed by exogenously supplied amino acids.[7] We reasoned that phosphonic acid analogues of L-proline and proline analogues could be inhibitors of the reductase and other enzymes in the proline metabolism. To test this idea, a series of racemic, chiral nonracemic, and achiral phosphonic acids were synthesized for evaluation first with P5C reductase of plants (Figure 1). The selection of compounds comprised five- (**1**−**4**) and six-membered (**5** and **6**) ring systems with one or two heteroatoms, all with an attached phosphonic acid group. The (*R*)-configured enantiomers of **1**−**6** correspond to the (*S*)- or L-configured ones in the carboxyl acid series because of the higher priority of PO_3H_2 compared to CO_2H according to the Cahn−Ingold−Prelog rules. Many azaheterocyclic phosphonates have been synthesized and biologically evaluated in the past.[8]

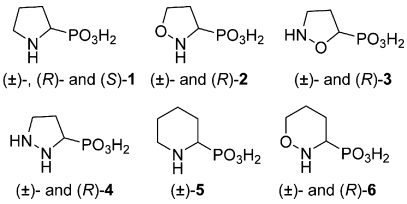


Figure 1. Phosphonic acid analogues of proline and proline analogues.

RESULTS AND DISCUSSION

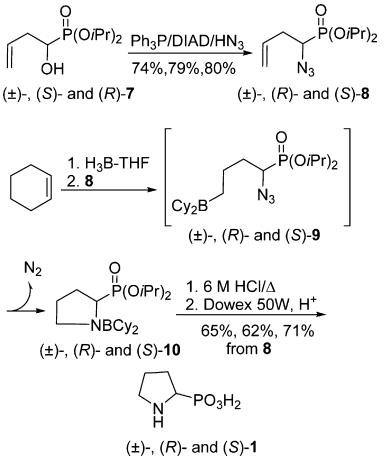
Synthesis of (±)-, (*R*)-, and (*S*)-Phosphaproline. Although racemic, (*R*)-, and (*S*)-phosphaproline [(±)-, (*R*)-, and (*S*)-**1**] have been described in the literature,[9−17] we present

Received: February 27, 2018
Accepted: April 11, 2018
Published: April 24, 2018

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

here a new access from recently prepared racemic and enantiomeric 1-hydroxy-3-butenylphosphonate **7** (Scheme 1).[18] It also served as a starting material for phosphonic acids

Scheme 1. Preparation of (±)-, (*R*)-, and (*S*)-Proline [(±)-, (*R*)-, and (*S*)-1]

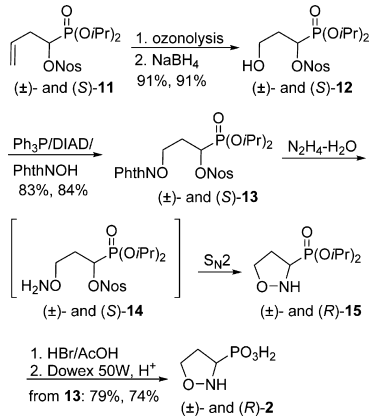


racemic **2** and **6**. Hydroxyphosphonate (±)-**7** was resolved by lipase-catalyzed kinetic hydrolysis of the chloroacetic ester and delivered the enantiomers with ee >97%.[18] The enantiomer (*S*)-**7** was transformed into (*R*)-**1**, (*R*)-**2**, and (*R*)-**6** and the enantiomer (*R*)-**7** into phosphaproline (*S*)-**1** by functional group manipulation. Furthermore, (±)- and (*S*)-**7** were already converted to phosphaaspartic acids via azides (±)- and (*S*)-**8** prepared by Mitsunobu reaction.[18] Evans and Weber[19] and later others[20,21] described the formation of pyrrolidines from homoallyl azides upon hydroboration with dicyclohexylborane. As we hoped that azides **8** prepared by the Mitsunobu reaction[18,22] would undergo the same cylization, we reacted them with dicyclohexylborane.[23] Trialkylboranes **9** were unstable and underwent migration of an alkyl group from the boron to nitrogen atom, with loss of nitrogen after attack of the azido group on the boron atom.[19] The phosphaproline derivatives **10** were globally deprotected by refluxing 6 M HCl. Phosphaprolines **1** were isolated by cation-exchange chromatography (Dowex 50W × 8, H^+) and crystallized. The enantiomers of **8** delivered the enantiomers of **1** of known configuration, correctly assigned[17] recently.

The ee of (*R*)- and (*S*)-**1** was the same (>99%) as that of the starting α-hydroxyphosphonates **7** (>99%), which was proven by chiral high-performance liquid chromatography (HPLC) (Figure S1).

Synthesis of (±)- and (*R*)-Isoxazolidin-3-ylphosphonic Acid. These phosphonic acid analogues of structural analogues of proline were obtained by functional group manipulation of (±)- and (*S*)-**11**[18] (Scheme 2). Ozonolysis gave both hydroxyphosphonates **12** in 91% yield. The following Mitsunobu reaction[22] with *N*-hydroxyphthalimide delivered protected *O*-alkylhydroxylamines (±)- and (*S*)-**13** in yields of 83 and 84%, respectively. Hydrazinolysis in *i*PrOH effected removal of the phthaloyl group. The open-chain *O*-alkylhydroxylamines (±)- and (*S*)-**14** with a leaving group at C-1 immediately cyclized to isoxazolidin-3-ylphosphonates **15**. As the 4-nitrobenzenesulfonyloxy (nosyloxy) group was replaced with inversion of configuration, the (*S*)-enantiomer of **13** was transformed into (*R*)-**15**. The crude isoxazolidin-3-ylphosphonates **15** were deprotected with HBr in AcOH at room temperature in 16

Scheme 2. Preparation of (±)- and (*R*)-Isoxazolidin-3-ylphosphonic Acid [(±)- and (*R*)-2][a]

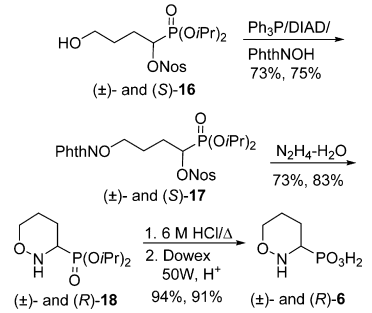


[a]Nos = 4-nitrobenzenesulfonyl; DIAD = diisopropyl azodicarboxylate, PhthNOH = *N*-hydroxyphthalimide.

h.[24] The phosphonic acids were purified by cation-exchange chromatography (Dowex 50W × 8, H^+) and crystallization. The overall yields of (±)- and (*R*)-**2**[25] starting from **13** were 79 and 74% (ee 99%, Figure S2), respectively. Surprisingly, deprotection of **15** with bromotrimethylsilane (TMSBr)/allyltrimethylsilane (allylTMS)[26] had a detrimental effect on the yield of (*R*)-**2** (15%).

Synthesis of Phosphapipecolic Acid (±)-5 and (±)- and (*R*)-1,2-Oxazinan-3-ylphosphonic Acid [(±)- and (*R*)-6]. Phosphonic acids **5** and **6** differ from phosphaproline by replacing the five-membered ring by a six-membered ring containing an additional CH_2 group or an oxygen atom. The preparation of racemic phosphapipecolic acid was recently published.[14] The synthesis of analogues **6** was accomplished starting from 4-hydroxybutylphosphonates **16**, derived from nosylates (±)- and (*S*)-**11** (ee 85%) by hydroboration with H_3B × tetrahydrofuran (THF), followed by oxidative cleavage of the B−C bond with $H_2O_2/NaHCO_3$ (Scheme 3).[18] The Mitsunobu

Scheme 3. Preparation of (±)- and (*R*)-(−)-1,2-Oxazinan-3-ylphosphonic Acid [(±)- and (*R*)-6][a]



[a]Nos = 4-nitrobenzenesulfonyl; DIAD = diisopropyl azodicarboxylate, PhthNOH = *N*-hydroxyphthalimide.

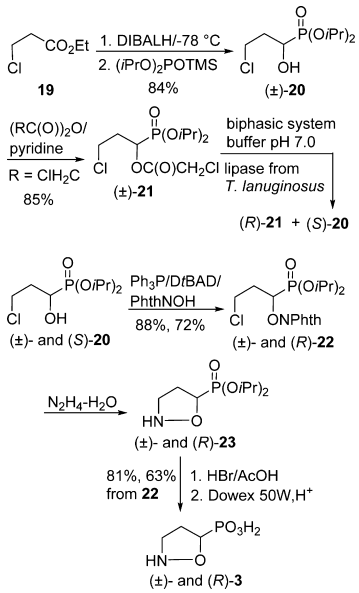
reaction and the ensuing cyclization induced by hydrazine hydrate were similarly performed for the transformation of **12** into **15** in Scheme 2 and delivered 1,2-oxazinanes (±)- and (*R*)-**18**, cyclic oxime ethers.[27] However, this time the hydroxylamine derivatives, the two 1,2-oxazinan-3-ylphosphonates (±)- and (*R*)-**18**, were first isolated as homogeneous compounds by flash column chromatography, fully characterized and finally depro-

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

tected. Although the five-membered analogue **15** suffered partial decomposition on attempted deprotection with refluxing 6 M HCl, 1,2-oxazinan-3-ylphosphonates (±)- and (*R*)-**18** were smoothly deprotected, as evidenced by their high yields of 94 and 91%, respectively. The ee of 83% for phosphonic acid (*R*)-**6** was increased to 92% upon crystallization from H_2O/EtOH (Figure S5).

Synthesis of (±)- and (*R*)-(+)-Isoxazolidin-5-ylphosphonic Acid [(±)- and (*R*)-3]. For the preparation of these heterocyclic phosphonic acids, isomeric to (±)- and (*R*)-**2**, a separate entry had to be developed (Scheme 4). We reasoned

Scheme 4. Preparation of (±)- and (*R*)-(+)-Isoxazolidin-5-ylphosphonic Acid [(±)- and (*R*)-3][a]

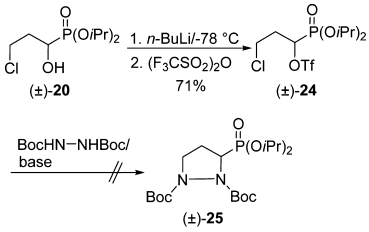


[a]DIAD = diisopropyl azodicarboxylate, PhthNOH = *N*-hydroxyphthalimide.

that racemic 3-chloro-1-hydroxypropylphosphonate (±)-**20** could be the key intermediate for both isoxazolidin-5-ylphosphonic acids, as it could be easily prepared as racemate and resolved enzymatically. Ethyl *β*-chloropropionate (**19**) was reduced to the aldehyde with diisobutylaluminium hydride (DIBALH) in dry toluene at −78 °C, to which diisopropyl trimethylsilyl phosphite was added to give *α*-hydroxyphosphonate (±)-**20** in 84% yield in a one-pot reaction.[28] Chloroacetylation with $(ClH_2CC(O))_2O$/pyridine furnished *α*-chloroacetoxyphosphonate (±)-**21**. This ester was subjected[14] to lipase-catalyzed kinetic hydrolysis in a biphasic system on a preparative scale with 16.3 mmol of substrate. When the enzymatic hydrolysis was stopped at 40% conversion, 5.14 mmol of *α*-hydroxyphosphonate (+)-**20** with an ee of 97% and (*S*)-configuration were obtained, determined[28,29] by using (*R*)-(+)-*t*-Bu(Ph)P(O)SH as the chiral solvating agent in combination with ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Satisfyingly, *α*-hydroxyphosphonates (±)- and (*S*)-**20** were converted to *N*-protected *O*-alkylhydroxylamines (±)- and (*R*)-**22** in yields of 88 and 72% (ee 95% by HPLC, Figure S6), respectively. Luckily, the chloride on the primary C-3 position was a much weaker leaving group than Ph_3PO and did not compete with the displacement reaction at C-1. The cleavage of the phthaloyl group was again induced with hydrazine hydrate. Precursors **22** were cyclized to isoxazolidin-5-ylphosphonic acids (±)- and (*R*)-**23**, which were directly used for the next step. Global deprotection was accomplished as outlined above. The overall yields of (±)- and (*R*)-**3** starting from **23** were 81 and 63% (ee 97%, Figure S3), respectively. The crystalline isoxazolidinylphosphonic acids **2** and **3** had to be stored at −18 °C to prevent gradual decomposition at room temperature.

Synthesis of (±)- and (*R*)-(−)-Pyrazolidin-3-ylphosphonic Acids [(±)- and (*R*)-4]. Initially, we reasoned that these phosphonic acids could easily be prepared by the reaction of a modified propylphosphonate, with leaving groups at C-1 and C-3, with a protected hydrazine derivative, but were convinced of the contrary. At first, 3-chloro-1-hydroxypropylphosphonate (±)-**20** was converted to triflate (±)-**24** in 71% yield (Scheme 5). It was added to a mixture of *N*,*N*′-bis(Boc)-hydrazine[31] and

Scheme 5. Attempted Preparation of Protected (±)-Pyrazolidin-3-ylphosphonic Acid [(±)-27]

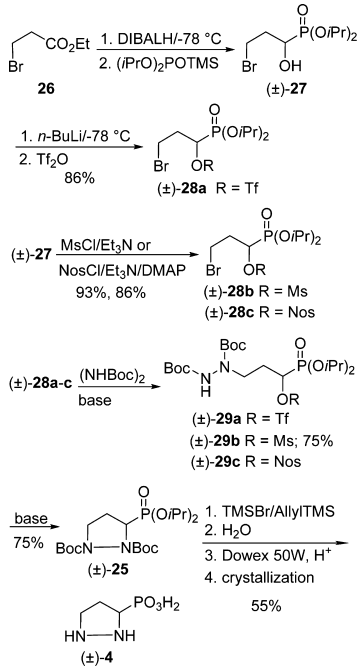


NaH in dimethylformamide (DMF), which had been stirred for 30 min at room temperature and was allowed to react for 18 h at 20 °C and 2 h at 50 °C. The crude product, which did not contain the starting material, was a complex mixture and was therefore discarded. The failure of this experiment was attributed to a combination of the low reactivity from Cl^- as the leaving group at C-3 and the high reactivity[32] of TfO^- at C-1. The first step, that is, the intermolecular reaction of the deprotonated hydrazine derivative will prefer attack at C-3. The cyclization of the N-substituted hydrazine intermediate should easily proceed to pyrazolidin-3-ylphosphonate (±)-**25**, as it is an intramolecular process, and TfO^- is an excellent leaving group. Substitution at C-1 of phosphonates was first considered unlikely for steric reasons and low reactivity in general. The size of the attacking nucleophile and the shielding of C-1 by the isopropyl-protecting groups at the phosphorus atom disfavor a S_N2 reaction. Base-induced elimination of TfOH was more likely than substitution. Consequently, a better leaving group had to be placed at C-3 or/and a less reactive one at C-1.

To replace chloride by bromide, *β*-bromopropionate **26** was transformed into 3-bromo-1-hydroxyphosphonate (±)-**29** and then into bromo triflate (±)-**30a** by the same procedures as used for the chloro derivative (Scheme 6). It was reacted with *N*,*N*′-bis(Boc)-hydrazine in a biphasic system[33] (20% NaOH/toluene) under phase-transfer conditions at room temperature. The extractively obtained crude product was again a complex mixture without a starting material, but contained elimination products as judged by NMR spectroscopy. As found later, when we had compound (±)-**25** in hand, this mixture already contained some of it. Importantly, this experiment demonstrated that the trifluoromethanesulfonyloxy group had to be replaced by a less reactive leaving group such as a mesyloxy or 4-nitrobenzenesulfonyloxy (nosyloxy) group to interfere with elimination. The corresponding mesylate (±)-**28b** and nosylate (±)-**28c** were obtained in 93 and 86% yield, respectively, by esterification of 3-bromo-1-hydroxyphosphonate (±)-**27** with mesyl chloride/

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

Scheme 6. Preparation of (±)-Pyrazolidin-3-ylphosphonic Acid [(±)-4][a]

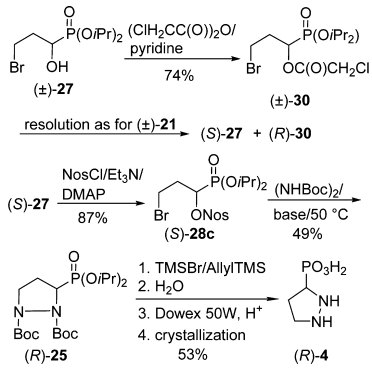


[a]Nos = 4-nitrobenzenesulfonyl, Tf = trifluoromethanesulfonyl, Ms = methanesulfonyl.

Et_3N or $NosCl/Et_3N$/dimethylaminopyridine (DMAP) (Scheme 6). When the experiment with bis(Boc)-hydrazine/NaOH was repeated with mesylate (±)-**28b** instead of the triflate at 0 °C and followed by thin-layer chromatography (TLC) monitoring, no new product could be detected besides the starting material after a reaction time of 1 h. Then, the temperature was increased to 19−20 °C. After 1 h, a spot of a new compound less polar than the substrate appeared on the TLC plate, and its intensity increased with the reaction time. After 5 h at 19−20 °C, the reaction mixture was worked up. The ^{31}P NMR spectrum of the crude product displayed resonances for the cyclic phosphonate (±)-**25**, the open chain product (±)-**29b**, a compound of unknown structure, and the starting material (±)-**28b** in molar ratios of 4:100:16:1. Flash column chromatography gave the open chain hydrazine derivative (±)-**29b** in 75% yield as a colorless gum. As expected, the first substitution occurred at C-3, and the second one at C-1 virtually did not take place at 20 °C. When hydrazine derivative (±)-**29b** was heated at 80 °C under the same reaction conditions as before except for the higher reaction temperature, cyclization smoothly proceeded to pyrazolidine (±)-**25** and was finished after 2.5 h. Workup and purification delivered pyrazolidin-3-ylphosphonate (±)-**25** in 75% yield. The reaction with nosylate (±)-**29c** was performed analogously (56% yield) except that the reaction temperature for both steps was 50 °C instead of 20 °C at the beginning and 80 °C later, owing to the nosyloxy group being a better leaving group than the mesyloxy one. When cyclization was carried out in DMF with KO*t*Bu as the base for 1.5 h at 0 °C and 1.5 h at 20 °C, the yield was somewhat higher (65%). The yield could not be improved any further despite many experiments. It seemed likely that the partial removal of an isopropyl group would give a water-soluble salt and thus decrease the yield. Global deprotection of (±)-**25** was more sensitive to forcing conditions such as refluxing 6 M HCl than to the milder conditions with TMSBr/allylTMS at 50 °C. Purification of the crude product by cation-exchange chromatography (Dowex 50W × 8, H^+) and crystallization furnished racemic pyrazolidin-3-ylphosphonic acids [(±)-**4**] in 55% yield.

To prepare its enantiomer (*R*)-**4**, bromohydroxyphosphonate (±)-**27** was chloroacetylated and kinetically resolved in the same way as the chloro analogue (±)-**21** (Scheme 7). Stopping

Scheme 7. Preparation of (*R*)-Pyrazolidin-3-ylphosphonic Acid [(*R*)-4][a]



[a]Nos = 4-nitrobenzenesulfonyl.

hydrolysis at a conversion of 40% delivered (+)-α-hydroxyphosphonate (*S*)-**27** of 95% ee, as determined via (*R*)-Mosher ester in combination with ^{31}P NMR spectroscopy. This enantiomer was nosylated (ee 95% by HPLC, Figure S7) and then cyclized with inversion of configuration at C-1 in analogy to the racemate in a basic biphasic system mediated by a phase-transfer catalyst at 50 °C. Global deprotection of (*R*)-**25** delivered pyrazolidin-3-ylphosphonic acid [(*R*)-**4**, ee 92%, Figure S4] by isolation from the crude product as for the racemate.

Inhibition of Plant P5C Reductase by Phosphonic Acid Analogues of Proline and Proline Analogues. The ability of all obtained compounds to interfere with the catalytic activity of P5C reductase, purified from *Arabidopsis thaliana* cultured cells,[34] was then evaluated. In the range from 10^{-4} to 10^{-3} M, their addition to the assay mixture was found ineffective. On the contrary, when millimolar concentrations of compounds (*R*)-, (*S*)-**1**, and(*R*)-**6** were used, a significant inhibition was evident that was proportional to the dose (Figure 2). Interestingly, their effectiveness was higher than that of proline, which exerts product inhibition in the range from 10 to 200 mM.[34,35] The calculated IC_{50} values were 84 ± 3, 24 ± 4, 21 ± 2, and 45 ± 19 mM for proline, (*R*)-, (*S*)-**1**, and (*R*)-**6**, respectively. However, these concentrations were 3−4 orders of magnitude higher than those found for bisphosphonate inhibitors of P5C reductases from higher plants.[4−7] Therefore, although potentially useful for molecular-docking studies aimed at a better comprehension of the product inhibition mechanism, the use of these analogues for weed control seems unfeasible. The ability of phosphonic acid analogues of proline and proline analogues to interfere with the activity of the enzymes that catalyze the first step in proline biosynthesis in bacteria and plants, namely γ-glutamyl kinase and P5C synthetase from *Escherichia coli* and rice, respectively, was also assessed. However, in no case was their catalytic rate significantly inhibited (data not shown).

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

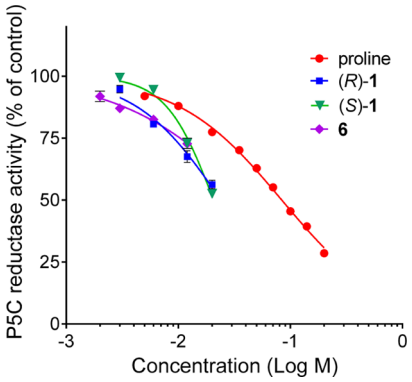


Figure 2. Effect of millimolar concentrations of compounds (*R*)-, (*S*)-**1**, and (*R*)-**6** on the activity of *A. thaliana* P5C reductase. The inhibition brought about by the physiological product of the enzyme, proline, is also shown as a term of comparison.

CONCLUSIONS

In summary, we have prepared the racemic 1-hydroxy-3-butenyl-, 3-chloro-1-hydroxypropyl-, and 3-bromo-1-hydroxypropylphosphonate and converted them to chloroacetates for lipase-catalyzed enantioselective hydrolysis. The racemates and (*S*)-enantiomers of these α-hydroxyphosphonates were transformed into racemic and (*R*)-configured pyrrolidin-2-yl-, oxazolidin-3-yl-, oxazolidin-5-yl-, pyrazolidin-3-yl-, and 1,2-oxazinan-3-ylphosphonic phosphonic acids by a variety of functional group manipulations. The ee (92−99%) of the (*R*)-enantiomers were determined after derivatization using chiral HPLC on quinine and quinidine-derived anion-exchange columns. These phosphonic acids, structural analogues of proline and proline analogues, were tested as P5C reductase inhibitors, but displayed negligible activity. They also did not interfere with the enzymes of bacteria and plants catalyzing the first step in proline biosynthesis. These phosphonic acids may also be evaluated as inhibitors of proline-metabolizing enzymes.

EXPERIMENTAL SECTION

General Information. ^{1}H, ^{13}C (*J*-modulated) and ^{31}P NMR spectra were recorded in $CDCl_3$ or D_2O on a Bruker AV 400 (^{1}H: 400.13 MHz, ^{13}C: 100.61 MHz, ^{31}P: 161.98 MHz), AV III 400 (^{1}H: 400.27 MHz, ^{13}C: 100.65 MHz, ^{31}P: 162.03 MHz), AV II^{+} 500 (^{1}H: 500.32 MHz, ^{31}P: 202.53 MHz), and AV III 600 (^{1}H: 600.25 MHz, ^{13}C: 150.93 MHz, ^{31}P: 242.99 MHz) at 25 °C unless otherwise indicated. Chemical shifts (δ) are reported in parts per million (ppm) relative to $CHCl_3/CDCl_3$ (δ_H 7.24; δ_C 77.00), toluene-d_8 (δ_H 2.09 for HD_2C of toluene-d_7), HOD (δ_H 4.80) and external H_3PO_4 (85%; δ_P 0.00) and coupling constants (*J*) in Hz. Infrared (IR) spectra were recorded on a Bruker VERTEX 70 IR spectrometer in the attenuated total reflection (ATR) mode or of films on a silicon disc.[36] High-resolution mass spectra (HRMS) were obtained using a Brucker Maxis Q-TOF mass spectrometer [electrospray ionization (ESI)]. Optical rotations were measured on a PerkinElmer 341 polarimeter in a 1 dm quartz cell. Analytical HPLC: Shimadzu system comprising components LC-20AT, SIL-20A HT, CTO-20AC, SPD-20A, CMB-20A, column: CHIRALPAK IA (250 mm × 4.6 mm, particle size 5 μm, 1 mL/min, 25 °C). Melting points were measured on a Leica Galen III Thermovar instrument and are uncorrected.

Anhydrous THF was refluxed over potassium and distilled prior to use. Pyridine was dried by refluxing over powdered CaH_2, then distilled, and stored over molecular sieves (4 Å). All other solvents, also dry ones, and chemicals were used as purchased. Flash column chromatography was performed using silica gel (particle size 0.040−0.063 mm). Reactions were monitored by analytical TLC using precoated silica gel plates (60 F_{254}, 250 μm thickness). Spots were visualized by ultraviolet (UV) and/or dipping into a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25.0 g) and $Ce(SO_4)_2\cdot 4H_2O$ (1.0 g) in 10% aqueous H_2SO_4 (500 mL), followed by heating with a heat gun. The solvent for TLC of phosphonic acids was *i*PrOH/H_2O/NH_3 (25%) in the ratio of 6:3:1. Spots of phosphonic acids were visualized by dipping the silica gel plate into a solution of 2% ninhydrin in EtOH and heating with a heat gun.

Determination of Enantiomeric Excesses of Aminophosphonic Acids. The analytical determination of enantiomeric excesses was performed with a commercial CHIRALPAK *tert*-butyl-QD-AX (150 mm × 4 mm, 5 μm) column and an in-house prepared underivatized OH-QN-AX column[37] (150 mm × 4 mm, 5 μm; CSP1). The mobile phase comprised an aqueous H_3PO_4 solution in MeOH in the ratio of 1:9 (v/v). Note that the molarity of the aqueous phase as well as the apparent pH (adjusted with triethylamine) of the polar organic mobile phase and the flow rates were optimized depending on the amino phosphonic acid, the column type, and the derivatization type. These chromatographic conditions are provided in the respective figure legends of the corresponding chromatograms summarized in the Supporting Information.

The instrumentation used were a thermoshaker PHMT with PSC24N from Grant-bio (Cambridgeshire, UK) and an Agilent 1100 HPLC−UV−fluorescence detector (FLD) system from Agilent (Waldbronn, Germany), which comprised a binary pump, a temperature controlled column oven, an autosampler, a multiwavelength detector (MWD) and a FLD. For the FLD, a gain of 10, an excitation wavelength of 254 nm, and an emission wavelength of 395 nm were chosen, whereas the MWD was set to 254 nm.

For compounds **1** and **2**, a derivatization with the Sanger's reagent (1-fluoro-2,4-dinitrobenzene, DNFB from Sigma Aldrich) and chiral separation on the CHIRALPAK *tert*-butyl-QD-AX column were chosen. For compounds **3**, **4**, and **6**, a derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC from Synchem) and chiral separation on an underivatized OH-QN-AX column provided the best results.

AQC Derivatization. Phospha amino acid (10 μL, 20 mM in water) was added to 0.2 M borate buffer (pH 8.8, 70 μL), followed by the addition of AQC (3 mg/mL in dry acetonitrile, 20 μL). The reaction mixture was immediately heated at 55 °C for 10 min.

DNFB Derivatization. To a solution (150 μL, 10 mM) of phospha amino acid in sodium carbonate buffer (0.1 M, pH 9.5), Sanger reagent [2.5% (v/v) in acetonitrile, 50 μL] was added and heated for 15 min at 50 °C. After reaching room temperature and centrifugation, the respective mobile phase (350 μL) was added.

Chromatographic results are summarized in the Supporting Information in Figure S1 [compounds (*R*)-, (*S*)-, and (*R*,*S*)-**1**], Figure S2 [compounds (*R*)- and (*R*,*S*)-**2**], Figure S3 [compounds (*R*)- and (*R*,*S*)-**3**], Figure S4 [compound (*R*)-**4**], and Figure S5 [compounds (*R*)- and (*R*,*S*)-**5**].

Note that for DNFB-derivatized compounds **1** and **2**, an elution order of the (*S*)-enantiomer before the (*R*)-enantiomer was observed on the CHIRALPAK *tert*-butyl-QD-AX column with separation factors of 1.32 and 1.23, respectively. Concerning enantiomeric excess, an ee of 99.3% was determined for

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

compound (*R*)-**1**, 99.99% for (*S*)-**1**, and 99.2% for (*R*)-**2**. The same elution order, (*S*)- before (*R*)-enantiomer, was also observed for AQC-derivatized compounds **3** and **4** using the unmodified QN-AX column, providing separation factors of 1.14 and 1.38, respectively. The determined ee values were 97.2% for compound (*R*)-**3** and 98.8% for (*R*)-**4** after crystallization (values for mother liquor and before crystallization: 76.9 and 92.2%). Although compound **6** was an analogue of compound **2**, an elution order of (*R*) before (*S*) was observed with a separation factor of 1.23 and an ee value of 91.6% after crystallization (values for mother liquor and before crystallization: 56.3 and 83.1%) using AQC derivatization and separation on an unmodified QN-AX column. A control experiment for compound **2** derivatized with AQC and separation on the same unmodified QN-AX column showed the same elution order, (*R*) before (*S*), as observed for compound **6**; however, no baseline separation could be achieved under standard test conditions (data not shown). The deviation in the elution order was the result of using different derivatization reagents combined with different chiral selector types (QN-AX vs QD-AX) and selector modifications (*tert*-butyl type vs unmodified CSP) for enantiomer separation.

(±)-, (*R*)-(+)-, and (*S*)-(−)-Pyrrolidin-2-ylphosphonic Acid (Phosphaproline) [(±)-, (*R*)-, and (*S*)-1]. Cyclohexene (598 mg, 0.74 mL, 7.28 mmol, 4 equiv) was added to a solution of $H_3B \times Me_2S$ (277 mg, 0.35 mL, 3.64 mmol, 2 equiv) in freshly distilled dry 1,2-dimethoxyethane (DME, 5.5 mL) under an argon atmosphere at 0 °C. The reaction mixture was stirred for 15 min at 0 °C and 1 h at room temperature. The resulting suspension of dicyclohexylborane[23] was again cooled at 0 °C. Racemic 1-azido-3-butenylphosphonate (±)-**8**[18] (475 mg, 1.82 mmol) dissolved in dry DME (1 mL) was added. After stirring for 1 h at 0 °C and 2 h at room temperature, the reaction was quenched with concd HCl (2 mL) and water (5 mL). The organic phase was removed, and the aq one was concentrated under reduced pressure. The residue was refluxed with a mixture of concd HCl (8 mL) and water (5 mL) for 18 h. The solution was cooled and concentrated under reduced pressure. The residue was dried over KOH in a vacuum desiccator and purified by cation-exchange chromatography (Dowex 50W × 8, H^+, elution with water). Fractions containing the product (TLC: *i*PrOH/H_2O/NH_3 (25%), 6:3:1, R_f = 0.25) were pooled and concentrated under reduced pressure to furnish racemic phosphaproline [(±)-**1**] (179 mg, 65%) as colorless crystals; mp 270−273 °C (H_2O/EtOH). Similarly, (*R*)-(−)-1-azido-3-butenylphosphonate (*R*)-**8**[18] {533 mg, 2.04 mmol; $[\alpha]_D^{20}$ −31.3 (*c* 1.2, acetone)} was converted to (*R*)-phosphaproline [(*R*)-**1**] (191 mg, 62%); $[\alpha]_D^{22}$ −46.6 (*c* 0.5, 1 M NaOH) {lit.:[14] $[\alpha]_D^{21}$ −49.1 (*c* 1.1, 1 M NaOH); lit.:[17] $[\alpha]_D^{21}$ −49.8 (*c* 1.1, 1 M NaOH); lit.:[9] $[\alpha]_{578}^{20}$ +64 (*c* 1.0, 1 M NaOH), +64 (*c* 1.0, 1 M NaOH)}. Similarly, (*S*)-(+)-azide [(*S*)-**8**] [1.09 g, 4.17 mmol; $[\alpha]_D^{20}$ +32.0 (*c* 1.08, acetone)] was converted to (*S*)-(+)-phosphaproline [(*S*)-**1**] (451 mg, 71%): $[\alpha]_{578}^{23}$ +49.5 (*c* 0.55, 1 M NaOH); $[\alpha]_D^{20}$ +47.3 (*c* 0.55, 1 M NaOH); mp 280 °C (decomp). Azide (*S*)-**8** (1.670 g, 80%) was prepared from 1-hydroxy-3-butenylphosphonate (*R*)-**7** (1.89 g, 8.0 mmol, 99% ee) by a literature procedure;[18] $[\alpha]_D^{20}$ +32.0 (*c* 1.08, acetone). The NMR spectra of (±)-, (*R*)-, and (*S*)-phosphaproline were identical to those reported in the literature.[14]

(±)- and (*S*)-(+)-3-Hydroxy-1-(4-nitrobenzenesulfonyloxy)propylphosphonate [(±)- and (*S*)-12]. Racemic nosylate (±)-**11**[18] (993 mg, 2.36 mmol) was dissolved in a mixture of methanol (5 mL) and CH_2Cl_2 (5 mL). Ozone was bubbled through the stirred solution at −78 °C until the blue color persisted. Excess ozone was removed by passing air through the solution. $NaBH_4$ (107 mg, 2.83 mmol, 1.2 equiv, dissolved in 1 mL of ethanol) was added and stirring was continued for 2.5 h at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in water (10 mL) and EtOAc (10 mL). The organic layer was separated, and the aq one was extracted with EtOAc (2 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1, R_f 0.17) to yield 3-hydroxypropylphosphonate (±)-**12** (912 mg, 91%) as colorless crystals; mp 103 °C (CH_2Cl_2/hexanes). Similarly, nosylate (*S*)-**11**[18] (1.334 g, 3.166 mmol) was converted to hydroxypropylphosphonate (*S*)-**12** (1.227 g, 91%) as colorless crystals; mp 93 °C (CH_2Cl_2/hexanes); $[\alpha]_D^{20}$ +25.5 (*c* 1.15, acetone). The NMR spectra of (±)- and (*S*)-**12** were identical.

IR (ATR) of (*S*)-enantiomer ν: 3358, 2985, 1608, 1534, 1375, 1350, 1242, 1186, 988 cm^{-1}. ^1H NMR (400.13 MHz, $CDCl_3$): δ 8.40−8.34 (m, 2H), 8.20−8.13 (m, 2H), 5.08 (td, *J* = 9.4, 4.5 Hz, 1H), 4.68 (2 oct overlapping to a dec, *J* = 6.4 Hz, 2H), 3.79 (td, *J* = 11.8, 4.4 Hz, 1H), 3.70 (td, *J* = 11.8, 3.5 Hz, 1H), 2.55 (br s, 1H, OH), 2.22−2.07 (m, 1H), 2.01−1.93 (m, 1H), 1.298 (d, *J* = 6.4 Hz, 3H), 1.294 (d, *J* = 6.4 Hz, 3H), 1.264 (d, *J* = 6.4 Hz, 3H), 1.248 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 150.8, 142.3, 129.5 (2C), 124.2 (2C), 75.1 (d, *J* = 172.6 Hz), 72.6 (d, *J* = 6.3 Hz), 72.6 (d, *J* = 6.5 Hz), 57.3 (d, *J* = 10.3 Hz), 33.5, 24.0 (d, *J* = 5.2 Hz), 24.0 (d, *J* = 5.2 Hz), 23.9 (d, *J* = 5.1 Hz), 23.7 (d, *J* = 4.9 Hz); ^{31}P NMR (162.03 MHz, $CDCl_3$): δ 16.7. Anal. Calcd for $C_{15}H_{24}NO_9PS$: C, 42.35; H, 5.69; N, 3.29. Found: C, 42.40; H, 5.72; N, 3.29.

(±)- and (*S*)-(+)-Diisopropyl 1-(4-nitrobenzenesulfonyloxy)-3-(phthalimidooxy)propylphosphonate [(±)- and (*S*)-13]. Racemic 3-hydroxypropylphosphonate (±)-**12** (1.260 g, 2.96 mmol), *N*-hydroxyphthalimide (507 mg, 3.11 mmol, 1.05 equiv) and Ph_3P (1.010 g, 3.85 mmol, 1.3 equiv) were dissolved with stirring in dry THF (12 mL) and dry CH_2Cl_2 (1.5 mL) under argon. DIAD (779 mg, 0.76 mL, 3.85 mmol, 1.3 equiv) was added dropwise at 0 °C, and the solution was slowly warmed to room temperature in a cooling bath and stirred overnight. Water (a few drops) was added, and after 15 min, the reaction mixture was concentrated under reduced pressure. The residue was flash-chromatographed (hexanes/EtOAc, 3:2, R_f 0.33 for hexanes/EtOAc, 1:1) to yield racemic phthalimidooxyphosphonate (±)-**13** (1.399 g, 83%) as a colorless foam. Similarly, (*S*)-hydroxypropylphosphonate (*S*)-**12** (1.572 g, 2.96 mmol; 97% ee) was converted to (*S*)-(+)-phthalimidooxyphosphonate (*S*)-**13** (1.399 g, 84%) as a colorless foam; $[\alpha]_D^{20}$ +15.1 (*c* 0.35, acetone). The NMR spectra of (±)- and (*S*)-**13** were identical.

IR (ATR) of (±)-**15** ν: 2981, 1732, 1531, 1373, 1349, 1255, 1185, 984 cm^{-1}. ^1H NMR (600.25 MHz, $CDCl_3$): δ 8.45−8.39 (m, 2H), 8.30−8.24 (m, 2H), 7.89−7.84 (m, 2H), 7.81−7.76 (m, 2H), 5.35 (td, *J* = 9.1, 4.2 Hz, 1H), 4.74 (oct, *J* = 6.2 Hz, 1H), 4.70 (oct, *J* = 6.2 Hz, 1H), 4.45−4.38 (m, 1H), 4.32 (ddd, *J* = 10.2, 8.2, 5.5 Hz, 1H), 2.48−2.39 (m, 1H), 2.27−2.18 (m, 1H), 1.34 (d, *J* = 6.2 Hz, 3H), 1.32 (d, *J* = 6.2 Hz, 3H), 1.31 (d, *J* = 6.2 Hz, 3H), 1.30 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (150.93 MHz, $CDCl_3$): δ 163.4 (2C), 150.7, 142.1, 134.6 (2C), 129.7 (2C), 128.7 (2C), 124.2 (2C), 123.6 (2C), 74.1 (d, *J* = 172.4 Hz), 73.5 (d, *J* = 10.9 Hz), 72.7 (d, *J* = 6.1 Hz), 72.6 (d, *J* = 6.4 Hz), 29.7, 24.0 (d, *J* = 3.6 Hz), 24.0 (d, *J* = 3.7 Hz), 23.8 (d, *J* = 5.0 Hz), 23.7 (d, *J* = 5.0 Hz); ^{31}P NMR (242.99 MHz, $CDCl_3$): δ 14.2. HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ calcd $C_{23}H_{27}N_2O_{11}PSNa$, 593.0966; found, 593.0961.

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

(±)- and (*R*)-(+)-Isoxazolidin-3-ylphosphonic Acid [(±)- and (*R*)-2]. Racemic phthalimidooxyphosphonate (±)-**13** (762 mg, 1.34 mmol) was dissolved in *i*PrOH (4 mL) and heated and stirred at 60 °C after addition of $N_2H_4 \times H_2O$ (334 mg, 6.68 mmol, 0.33 mL). A crystalline solid was formed rapidly. After 1 h, the solvent was removed at reduced pressure (1 mbar). Diisopropyl ether was added, and the residue was converted to a paste by stirring and breaking up the lumps with a spatula. The mixture was filtered through a Celite pad with suction and carefully washed with iPr_2O. The filtrate was concentrated under reduced pressure. The oily, faint yellow residue (355 mg) was mixed with AcOH/HBr[24] (7 mL, 30%) under argon at room temperature and stirred for 16 h. Volatile compounds were removed under vacuum (1 mbar). The residue was purified by cation-exchange chromatography (Dowex 50W × 8, H^+; column: o.d. 2 cm × 38 cm, elution with water, fractions of 10 mL; R_f 0.33). Ninhydrin-positive fractions were pooled and concentrated under reduced pressure to give racemic isoxazolidin-3-ylphosphonic acid (±)-**2** (162 mg, 79% combined yield for two steps) as colorless crystals; mp 148 °C (decomp) (H_2O/*i*PrOH). Similarly, (*S*)-(+)-phthalimidooxyphosphonate (*S*)-**13** (928 mg, 1.63 mmol) was converted to (*R*)-(+)-isoxazolidin-3-ylphosphonic acid (*R*)-**2** (148 mg, 74%) as colorless crystals; mp 180 °C (decomp) (water/ethanol); ee 99% (Figure S2); $[\alpha]_D^{20}$ +6.4 (*c* 0.63, water) {lit.:[25] $[\alpha]_D^{20}$ +30.8 (*c* 1.0, CF_3CO_2H)}. The NMR spectra of (±)- and (*R*)-**2** were identical.

IR (ATR) of (±)-**2** ν: 3100−1500 (very br), 1447, 1232, 1169, 1076, 963, 927, 858 cm^{-1}. ^1H NMR (600.25 MHz, D_2O): δ 4.34 (qd, J = 8.0, 4.0 Hz, 1H), 4.18 (qd, J = 8.0, 0.5 Hz, 1H), 3.84 (td, J = 9.9, 8.4 Hz, 1H), 2.76−2.68 (m, 1H), 2.53−2.42 (m, 1H); ^{13}C NMR (150.93 MHz, D_2O): δ 71.1 (d, J = 8.2 Hz), 56.8 (d, J = 139.9 Hz, 30.7); ^{31}P NMR (242.99 MHz, D_2O): δ 7.8. Anal. Calcd for $C_3H_8NO_4P$: C, 23.54; H, 5.27; N, 9.15. Found: C, 23.56; H, 5.05; N, 25.

(±)- and (*S*)-(+)-Diisopropyl 1-(4-Nitrobenzenesulfonyloxy)-4-(phthalimidooxy)butylphosphonate [(±)- and (*S*)-17]. The mixture of racemic ω-hydroxynosylate (±)-**16**[18] (2.334 g, 5.31 mmol), *N*-hydroxyphthalimide (1.039 g, 6.37 mmol, 1.2 equiv) and dry toluene (30 mL) was concentrated under reduced pressure and dried in vacuo. Triphenylphosphane (1.95 g, 7.43 mmol, 1.4 equiv) was added to the residue, followed by dry THF (20 mL) under argon atmosphere. After cooling at 0 °C, DIAD (1.502 g, 7.43 mmol, 1.4 equiv, 1.46 mL) was added dropwise. Stirring was continued for 3 min at 0 °C and 4 h at room temperature before H_2O (0.5 mL) was added. After 5 min, the reaction mixture was concentrated under reduced pressure. The residue was flash-chromatographed (heptanes/EtOAc, 1:1, R_f 0.38) to yield racemic phthalimidooxyphosphonate (±)-**17** (2.275 g, 73%) as colorless crystals; mp 130−132 °C [1,2-dichloroethane (DCE)/heptanes]. Similarly, (*S*)-(+)-ω-hydroxynosylate (*S*)-**18** {2.580 g, 5.87 mmol; $[\alpha]_D^{27}$ +7.7 (*c* 1.25, acetone), derived from 1-hydroxy-3-butenylphosphonate[18] of 85% ee} was converted to (*S*)-phthalimidooxyphosphonate (*S*)-**17** (2.574 g, 75%) as colorless crystals; mp 128 °C (CH_2Cl_2/hexanes); $[\alpha]_D^{20}$ +5.0 (*c* 1.29, acetone). The NMR spectra of (±)- and (*S*)-**19** were identical.

IR (ATR) of (±)-**19** ν: 2978, 2960, 1732, 1367, 1345, 1268, 1182, 1007, 981 cm^{-1}. ^1H NMR (600.25 MHz, $CDCl_3$): δ 8.38−8.34 (m, 2H), 8.21−8.16 (m, 2H), 7.83−7.79 (m, 2H), 7.76−7.71 (m, 2H), 4.99 (td, J = 9.1, 4.4 Hz, 1H), 4.72 (oct, J = 6.2 Hz, 1H), 4.63 (oct, J = 6.2 Hz, 1H), 4.22−4.14 (m, 2H), 2.28−2.19 (m, 1H), 2.10−2.02 (m, 1H), 2.02−1.94 (m, 1H), 1.91−1.82 (m, 1H), 1.30 (d, J = 6.2 Hz, 3H), 1.298 (d, J = 6.2 Hz, 3H), 1.27 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H); ^{13}C NMR (150.93 MHz, $CDCl_3$): δ 163.5 (2C), 150.7, 142.5, 134.5 (2C), 129.4 (2C), 128.8 (2C), 124.2 (2C), 123.3 (2C), 77.7 (d, J = 171.5 Hz), 77.0, 72.4 (d, J = 7.1 Hz), 72.4 (d, J = 7.1 Hz), 26.7, 24.2 (d, J = 10.0 Hz), 24.1 (d, J = 3.6 Hz), 24.0 (d, J = 3.7 Hz), 23.9 (d, J = 5.0 Hz), 23.7 (d, J = 5.0 Hz); ^{31}P NMR (242.99 MHz, $CDCl_3$): δ 14.4. Anal. Calcd for $C_{24}H_{29}N_2O_{11}PS$: C, 49.31; H, 5.00; N, 4.79. Found: C, 49.40; H, 5.13; N, 4.98.

(±)- and (*R*)-Diisopropyl 1,2-Oxazinan-3-ylphosphonate [(±)- and (*R*)-18]. A mixture of racemic phthalimidooxyphosphonate (±)-**17** (873 mg, 1.49 mmol), $N_2H_4 \times H_2O$ (374 mg, 7.47 mmol, 5 equiv, 0.36 mL) and *i*PrOH (5 mL) were heated at 80 °C for 1 h. The mixture was cooled and diluted with EtOAc (5 mL). The crystals were collected by suction and washed with EtOAc. The combined filtrates were concentrated under reduced pressure. The residue was flash-chromatographed (EtOAc, R_f 0.36) to give racemic 1,2-oxazinan-3-ylphosphonate [(±)-**18**] (273 mg, 73%) as a colorless liquid. Similarly, (*S*)-(+)-phthalimidooxyphosphonate (*S*)-**17** (1.40 g, 2.4 mmol) was converted to (*R*)-(−)-1,2-oxazinan-3-ylphosphonate (*R*)-**18** (500 mg, 83%); $[\alpha]_D^{23}$ −38.9 (*c* 0.9, MeOH). The NMR spectra of (±)- and (*R*)-(−)-**18** were identical.

IR (ATR) ν: 3242, 2978, 2939, 1375, 1232, 1058, 974 cm^{-1}. ^1H NMR (600.25 MHz, $CDCl_3$): δ 5.42 (br s, 1H), 4.76−4.65 (m, 2H), 3.99−3.93 (m, 1H), 3.73 (td, J = 11.3, 3.1 Hz, 1H), 3.43 (ddd, J = 15.4, 11.2, 3.0 Hz, 1H), 2.00−1.93 (m, 1H), 1.82−1.65 (m, 3H), 1.32 (d, J = 5.8 Hz, 3H), 1.31 (d, J = 6.6 Hz, 3H), 1.30 (d, J = 6.4 Hz, 6H); ^{13}C NMR (150.93 MHz, $CDCl_3$): δ 71.1 (d, J = 6.7 Hz), 71.0 (d, J = 7.0 Hz), 70.5, 56.5 (d, J = 150.7 Hz), 24.5 (d, J = 11.7 Hz), 24.1 (d, J = 3.6 Hz), 24.1 (d, J = 3.7 Hz), 24.0 (d, J = 4.5 Hz), 23.95 (d, J = 4.5 Hz), 23.64 (d, J = 3.7 Hz); ^{31}P NMR (242.99 MHz, $CDCl_3$): δ 20.1. Anal. Calcd for $C_{10}H_{22}NO_4P$: C, 47.80; H, 8.83; N, 5.57; O, 25.47. Found: C, 47.78; H, 9.19; N, 5.74; O, 25.53.

(±)- and (*R*)-(−)-1,2-Oxazinan-3-ylphosphonic Acid [(±)- and (*R*)-6]. Racemic 1,2-oxazinan-3-ylphosphonate (±)-**18** (479 mg, 1.906 mmol) was dissolved in 6 M HCl (10 mL) and refluxed for 4 h. The solution was concentrated under reduced pressure. The residue was purified by cation-exchange chromatography (Dowex 50W × 8, H^+, water as eluent, R_f 0.61) to furnish racemic 1,2-oxazinan-3-ylphosphonic acid (±)-**6** (301 mg, 94%) as crystals; mp 183−86 °C (decomp.) (H_2O/EtOH). Similarly, (*R*)-(−)-1,2-oxazinan-3-ylphosphonate (*R*)-**18** (480 mg, 1.91 mmol) was converted to (*R*)-(−)-1,2-oxazinan-3-ylphosphonic acid [(*R*)-**6**] (289 mg, 91%) as crystals; mp 183−85 °C (decomp) (H_2O/EtOH); $[\alpha]_D^{25}$ −22.3 (*c* 0.95, H_2O) before crystallization (ee 83.1%; Figure S5); $[\alpha]_D^{27}$ −23.2 (*c* 0.95, H_2O) after crystallization (ee 91.6%). The NMR spectra of (±)- and (*R*)-**6** were identical.

IR (ATR) of (±)-**6** ν: 3500−1700 (very br), 1234, 1186, 1168, 1069, 1040, 978, 960, 913 cm^{-1}. ^1H NMR (600.25 MHz, D_2O): δ 4.31−4.23 (m, 1H), 4.15 (td, J = 12.1, 2.5 Hz, 1H), 3.59 (ddd, J = 13.7, 12.3, 2.5 Hz, 1H), 2.22−2.12 (m, 1H), 1.99−1.78 (m, 3H); ^{13}C NMR (150.93 MHz, D_2O): δ 71.4, 56.1 (d, J = 134.5 Hz), 21.8 (d, J = 9.8 Hz), 20.8 (d, J = 2.5 Hz); ^{31}P NMR (242.99 MHz, D_2O): δ 9.1. Anal. Calcd for $C_4H_{10}NO_4P$: C, 28.75; H, 6.03; N, 8.38; O, 38.30. Found: C, 28.79; H, 5.94; N, 8.21; O, 38.63.

(±)-Diisopropyl 3-Chloro-1-hydroxypropylphosphonate [(±)-20]. Ethyl 3-chloropropionate (**19**) (4.097 g, 30 mmol) was dissolved in dry toluene (40 mL) and cooled to −78 °C under argon atmosphere. A solution of DIBALH (33 mL, 1 M, toluene) was added dropwise over 10 min.[28] After 2 h of additional stirring at −78 °C, diisopropyl trimethylsilyl

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

phosphite (7.150 g, 30 mmol) was added. The cooling bath was removed and the reaction mixture was stirred at room temperature for 18 h. HCl (2 M, 10 mL, exothermic!) was added dropwise, and after 10 min, more HCl (90 mL) was added and stirring was continued for 30 min (TLC: the silylated hydroxyphosphonate should be absent). The organic phase was separated and the aq one was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:3; R_f 0.24) to give racemic α-hydroxyphosphonate (±)-**20** (6.558 g, 84%) as a colorless oil.

IR (ATR) ν: 3272, 2979, 1386, 1376, 1178, 1077, 978 cm^{-1}. ^1H NMR (400.27 MHz, $CDCl_3$): δ 4.90−4.50 (m and br s, 3H), 3.99 (td, J = 9.7, 4.4 Hz, 1H), 3.78−3.65 (m, 2H), 2.18−1.99 (m, 2H), 1.30 (d, J = 6.0 Hz, 3H), 1.29 (d, J = 6.2 Hz, 9H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 71.5 (d, J = 7.4 Hz), 71.3 (d, J = 7.4 Hz), 64.6 (d, J = 165.7 Hz), 41.2 (d, J = 16.7 Hz), 34.4 (d, J = 2.5 Hz), 24.1 (d, J = 3.7 Hz), 24.0 (d, J = 3.8 Hz), 23.9 (d, J = 4.7 Hz, 2C); ^{31}P NMR (162.04 MHz, $CDCl_3$): δ 22.7. Anal. Calcd for $C_9H_{20}ClO_4P$: C, 41.79; H, 7.79; O, 24.74. Found: C, 41.84; H, 7.76; O, 24.84.

(±)-Diisopropyl 3-Chloro-1-(chloroacetoxy)-propylphosphonate [(±)-21]. Racemic 3-chloro-1-hydroxy-propylphosphonate (±)-**20** (4.967 g, 19.2 mmol) was dissolved in dry CH_2Cl_2 (30 mL) under Ar atmosphere. After cooling to 0 °C, dry pyridine (4.556 g, 57.6 mmol, 4.649 mL, 3 equiv) was added, and the solution was stirred for 10 min. Chloroacetic anhydride (4.924 g, 28.8 mmol, 1.5 equiv) dissolved in dry CH_2Cl_2 (16 mL) was added and stirring was continued at 0 °C.[38] After 2.5 h (TLC monitoring), when the starting material was consumed, the reaction was quenched with water (4 mL), and stirring was continued for 5 min before more water (20 mL) was added. The organic phase was separated, and the aqueous one was extracted with EtOAc (30 mL). The combined organic phases were washed with HCl (30 mL, 2 M), water (10 mL), saturated aqueous solution of $NaHCO_3$ (20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1, R_f 0.33) to give chloroacetate (±)-**21** (5.463 g, 85%) as a colorless oil.

IR (ATR) ν: 2981, 2937, 1768, 1387, 1386, 1376, 1246, 1157, 1103, 979 cm^{-1}. ^1H NMR (600.25 MHz, $CDCl_3$): δ 5.42 (td, J = 9.2, 4.5 Hz, 1H), 4.74 (sept, J = 6.2 Hz, 1H), 4.73 (sept, J = 6.2 Hz, 1H), 4.10 (AB system, J = 14.8 Hz, 2H), 3.64−3.58 (m, 1H), 3.50 (ddd, J = 11.2, 8.1, 6.6 Hz, 1H), 2.36−2.23 (m, 2H), 1.32, 1.316, 1.309, 1.299 (4 × d, each J = 6.2 Hz, 3H); ^{13}C NMR (150.93 MHz, $CDCl_3$): δ 166.2 (d, J = 4.9 Hz), 72.2 (d, J = 6.7 Hz), 72.0 (d, J = 7.2 Hz), 67.5 (d, J = 171.4 Hz), 40.5, 40.1 (d, J = 14.4 Hz), 32.6, 24.1 (d, J = 3.5 Hz), 24.0 (d, J = 3.9 Hz), 23.95 (d, J = 5.0 Hz), 23.8 (d, J = 5.1 Hz); ^{31}P NMR (242.99 MHz, $CDCl_3$): δ 16.3. Anal. Calcd for $C_{11}H_{21}Cl_2O_5P$: C, 39.42; H, 6.32; O, 23.87. Found: C, 39.39; H, 6.23; O, 24.06.

Resolution of Racemic Diisopropyl 3-Chloro-1-(chloroacetoxy)propylphosphonate [(±)-21]. (±)-3-Chloro-1-(chloroacetoxy)propylphosphonate (±)-**21** (5.463 g, 16.3 mmol) was dissolved in a mixture of *t*-BuOMe and hexanes (36 mL, 1:1) and phosphate buffer (25 mM, 120 mL).[14] After the pH had been adjusted to 7.0 using the autotitrator, lipase from *Thermomyces lanuginosus* (0.4 mL, ≥100 000 U/g, [3.1.1.3], Sigma) was added. The mixture was vigorously stirred at room temperature, and pH of 7.0 was maintained by the addition of NaOH (0.5 M). At a conversion of 40% by consumption of the base (13.04 mL), the pH was brought to 4 by the addition of HCl (2 M). The reaction mixture was extracted with EtOAc (3 × 100 mL). The combined organic phases were washed with a saturated aq solution of $NaHCO_3$ (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:2, chloroacetate: R_f 0.44; hydroxyphosphonate: R_f 0.15) to give chloroacetate (*R*)-**21** {2.918 g, 53%; $[\alpha]_D^{21}$ −21.4 (*c* 1.01, acetone)} and hydroxyphosphonate (*S*)-**20** {1.332 g, 5.14 mmol, 63%; $[\alpha]_D^{21}$ +31.7 (*c* 1.67, acetone)} as colorless liquids. The ee of 97% for (+)-**22** was determined by using(*R*)-(+)-(*t*-Bu)(Ph)P(O)(SH) as CSA and ^{31}P NMR spectroscopy:[29,30] major singlet (1.00) at 22.9 ppm and minor one (0.015) at 22.7 ppm.

(±)- and (*R*)-(−)-Diisopropyl 3-Chloro-1-(phthalimidooxy)propylphosphonate [(±)- and (*R*)-22]. Dry toluene (20 mL) was added to a mixture of (±)-3-chloro-1-hydroxypropylphosphonate (±)-**20** (1.484 g, 5.74 mmol), Ph_3P (2.107 g, 8.03 mmol, 1.4 equiv), and *N*-hydroxyphthalimide (1.310 g, 8.03 mmol, 1.4 equiv) under argon atmosphere. A solution of di-*tert*-butyl azodicarboxylate (1.850 g, 8.03 mmol, 1.4 equiv) in dry THF (6 mL) was dropwise added under cooling with water at room temperature. After stirring for 2 h, while the color of the reaction mixture had changed from an intense red brown to a faint orange brown, water was added (10 drops). Ten minutes later, the reaction mixture was concentrated under reduced pressure. The residue was flash-chromatographed (heptanes/EtOAc, 1:1; R_f 0.33) to yield phthalimidooxyphosphonate (±)-**22** (2.05 g, 88%) as a colorless heavy oil, which eventually crystallized; mp 94−95 °C (CH_2Cl_2/heptanes). Similarly, (*S*)-3-chloro-1-hydroxypropylphosphonate (*S*)-**20** (575 mg, 2.5 mmol; ee 96%) was converted to (*R*)-(−)-3-chloro-1-(phthalimidooxy)propylphosphonate (*R*)-**22** (727 mg, 72%); $[\alpha]_D^{15}$ −28.8 (*c* 2.4, acetone); mp 92−93 °C (hexanes/EtOAc); ee 95%, determined by chiral HPLC (Figure S6). The NMR spectra of (±)- and (*R*)-**22** were identical.

IR (ATR) of crystalline (±)-**24** ν: 2977, 1788, 1729, 1374, 1358, 1273, 1251, 1188, 1173, 1135, 1123, 1107, 982 cm^{-1}. ^1H NMR (400.27 MHz, $CDCl_3$): δ 7.83−7.77 (m, 2H), 7.74−7.68 (m, 2H), 4.98 (septd, J = 7.3, 6.2 Hz, 1H), 4.80−4.67 (m, 2H), 3.95 (td, J = 11.0, 7.3 Hz, 1H), 3.84 (dddd, J = 11.0, 6.7, 5.8, 0.9 Hz, 1H), 2.46−2.29 (m, 2H), 1.38 (d, J = 6.2 Hz, 3H), 1.33 (d, J = 6.2 Hz, 3H), 1.32 (d, J = 6.2 Hz, 3H), 1.31 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100.65 MHz, $CDCl_3$): δ 163.0 (2C), 134.5 (2C), 128.9 (2C), 123.6 (2C), 79.3 (d, J = 163.8 Hz), 72.5 (d, J = 6.7 Hz), 71.9 (d, J = 7.1 Hz), 40.7 (d, J = 12.4 Hz), 33.0, 24.249 (d, J = 2.9 Hz), 24.01 (d, J = 3.8 Hz, CH_3), 23.98 (d, J = 4.7 Hz), 23.8 (d, J = 5.7 Hz); ^{31}P NMR (162.04 MHz, $CDCl_3$): δ 15.0. Anal. Calcd for $C_{17}H_{23}ClNO_6P$: C, 50.57; H, 5.74; N, 3.47; O, 23.77. Found: C, 50.69; H, 5.52; N, 3.45; O, 23.79.

(±)- and (*R*)-(+)-Isoxazolidin-5-ylphosphonic Acid [(±)- and (*R*)-(+)-3]. (±)-3-Chloro-1-(phthalimidooxy)-propylphosphonate (±)-**22** (848 mg, 2.1 mmol) was converted to (±)-isoxazolidin-5-ylphosphonic acid (±)-**3** (259 mg, 81%) by the same procedure as used for the preparation of (±)-isoxazolidin-3-ylphosphonic acid (±)-**2** from the respective nosylate (±)-**13**; mp 173 °C (decomp) (H_2O/*i*PrOH). Similarly, protected (*R*)-(−)-α-aminooxyphosphonate (*R*)-**22** (662 mg, 1.64 mmol) was converted to (*R*)-(+)-isoxazolidin-5-ylphosphonic acid [(*R*)-**3**] (159 mg, 63%); $[\alpha]_D^{15}$ +20.3 (*c* 1.67, H_2O); mp 192 °C (decomp) (H_2O/EtOH); ee 97.2% before crystallization (Figure S3). The NMR spectra of (±)- and (*R*)-(+)-**3** were identical.

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

IR (ATR) of (±)-**3** ν: 3250−1500 (very br), 1453, 1283, 1240, 1136, 1077, 1024, 949, 928, 907, 890 cm^{-1}. ^1H NMR (400.27 MHz, D_2O): δ 4.47 (dd, J = 9.5, 7.0 Hz, 1H), 3.84 (ddd, J = 10.9, 8.5, 4.2 Hz, 1H), 3.71 (td, J = 10.9, 8.1 Hz, 1H), 2.89−2.77 (m, 1H), 2.66−2.51 (m, 1H); ^{13}C NMR (150.93 MHz, D_2O): δ 78.4 (d, J = 155.9 Hz), 47.0 (d, J = 7.8 Hz), 30.0; ^{31}P NMR (162.04 MHz, D_2O): δ 10.8. Anal. Calcd for $C_3H_8NO_6P$: C, 23.54; H, 5.27; N, 9.15; O, 41.81. Found: C, 23.57; H, 5.23; N, 9.01; O, 41.38.

(±)-Diisopropyl 3-Chloro-1-(trifluoromethanesulfonyloxy)propylphosphonate [(±)-24]. (±)-3-Chloro-1-hydroxypropylphosphonate (±)-**20** (573 mg, 2.22 mmol, dried by azeotropic distillation with toluene) was dissolved in dry Et_2O (9 mL) and cooled at −78 °C under an Ar atmosphere. *n*-BuLi (0.98 mL, 2.5 M in hexanes, 2.44 mmol, 1.1 equiv) was added, and the solution was stirred for 5 min. Then, $(CF_3SO_2)_2O$ (691 mg, 0.41 mL, 2.44 mmol, 1.1 equiv) was added, and the reaction mixture was stirred for 1 h at −78 °C. The reaction was quenched by adding water (5 mL) and a saturated aq solution of $NaHCO_3$ (5 mL), and stirring was continued for 5 min. EtOAc (20 mL) was added, and the phases were separated. The aq layer was extracted with EtOAc (2 × 15 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (heptanes/EtOAc, 2:1, R_f 0.40) to yield racemic 3-chloro-1-(trifluoromethanesulfonyloxy)-propylphosphonate (±)-**24** (618 mg, 71%) as a colorless oil.

IR (ATR) ν: 2984, 1414, 1242, 1205, 1140, 1103, 986, 923 cm^{-1}. ^1H NMR (400.27 MHz, $CDCl_3$): δ 5.15 (td, J = 8.2, 5.0 Hz, 1H), 4.88−4.73 (m, 2H), 3.78−3.70 (m, 1H, 1H), 3.60 (ddd, J = 11.5, 7.5, 6.4 Hz, 1H), 2.53−2.33 (m, 2H), 1.369 (d, J = 6.0 Hz, 3H), 1.362 (d, J = 6.2 Hz, 6H), 1.356 (d, J = 5.9 Hz, 3H); ^{13}C NMR (100.65 MHz, $CDCl_3$): δ 118.3 (q, J = 319.6 Hz), 78.7 (d, J = 170.8 Hz), 73.5 (d, J = 6.9 Hz), 73.3 (d, J = 7.3 Hz), 39.1 (d, J = 10.7 Hz), 33.5, 24.0 (d, J = 3.7 Hz), 23.9 (d, J = 3.7 Hz), 23.8 (d, J = 4.6 Hz), 23.6 (d, J = 5.0 Hz); ^{31}P NMR (162.03 MHz, $CDCl_3$): δ 11.9. Anal. Calcd for $C_{10}H_{19}ClF_3O_6PS$: C, 30.74; H, 4.90; O, 24.57; S, 8.21. Found: C, 30.60; H, 4.98; O, 23.45; S, 8.24.

(±)-Diisopropyl 3-Bromo-1-hydroxypropylphosphonate [(±)-27]. Ethyl 3-bromopropionate (5.431 g, 30 mmol) was converted to (±)-3-bromo-1-hydroxypropylphosphonate (±)-**27** (6.729 g, 74%) by the procedure used for the preparation of the chloro analogue (±)-**20**; flash chromatography (hexanes/EtOAc, 1:3; R_f 0.24).

IR (ATR) ν: 3267, 2979, 1386, 1376, 1253, 1222, 1106, 987 cm^{-1}. ^1H NMR (400.27 MHz, $CDCl_3$): δ 4.80−4.65 (m, 2H), 4.04−3.92 (m, 1H), 3.59 (dd, J = 7.9, 5.7 Hz, 2H), 3.29 (br s, 1H), 2.28−2.10 (m, 2H), 1.32 (d, J = 6.2 Hz, 6H), 1.31 (d, J = 6.2 Hz, 6H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 71.6 (d, J = 7.4 Hz), 71.4 (d, J = 7.3 Hz), 65.7 (d, J = 165.0 Hz), 34.5 (d, J = 2.5 Hz), 29.9 (d, J = 17.1 Hz), 24.1 (d, J = 3.2 Hz), 24.1 (d J = 3.3 Hz), 24.0 (d, J = 4.4 Hz), 23.9 (d, J = 4.3 Hz); ^{31}P NMR (162.03 MHz, $CDCl_3$): δ 22.4. Anal. Calcd for $C_9H_{20}BrO_4P$: C, 35.66; H, 6.65. Found: C, 35.91; H, 6.50.

(±)-Diisopropyl 3-Bromo-1-(chloroacetoxy)-propylphosphonate [(±)-30]. Racemic 3-bromo-1-hydroxypropylphosphonate (±)-**27** (7.578 g, 25.0 mmol) was converted to (±)-3-bromo-1-(chloroacetoxy)propylphosphonate (±)-**30** (7.022 g, 74%; hexanes/EtOAc, 1:1; R_f 0.33) by the procedure used for the preparation of (±)-**21**.

IR (ATR) ν: 2981, 1725, 1387, 1377, 1181, 995 cm^{-1}. ^1H NMR (400.13 MHz, $CDCl_3$): δ 5.39 (ddd, J = 9.1, 8.1, 5.2 Hz, 1H), 4.79 (sepd, J = 7.1, 6.2 Hz, 2H), 4.09 (AB-system, J = 14.8 Hz, 2H), 3.45 (dddd, J = 10.3, 6.5, 5.8, 1.1 Hz, 1H), 3.34 (td, J = 10.3, 7.5 Hz, 1H), 2.43−2.33 (m, 2H), 1.32 (d, J = 6.2 Hz, 3H), 1.312 (d, J = 6.2 Hz, 3H), 1.31 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 166.2 (d, J = 4.8 Hz), 72.3 (d, J = 6.8 Hz), 72.0 (d, J = 7.3 Hz), 68.6 (d, J = 171.0 Hz), 40.5, 32.8, 27.8 (d, J = 14.6 Hz), 24.0 (d, J = 3.6 Hz), 24. (d, J = 3.0 Hz), 23.95 (d, J = 4.6 Hz), 23.8 (d, J = 5.0 Hz); ^{31}P NMR (162.03 MHz, $CDCl_3$): δ 16.0. Anal. Calcd for $C_{11}H_{21}BrClO_5P$: C, 34.80; H, 5.58. Found: C, 34.52; H, 5.36.

Lipase-Catalyzed Resolution of Racemic Diisopropyl 3-Bromo-1-(chloroacetoxy)propylphosphonate [(±)-30]. (±)-3-Bromo-1-(chloroacetoxy)propylphosphonate (±)-**30** (4.144 g, 10.92 mmol) was enzymatically resolved (15 mL of *t*-BuOMe, 15 mL of hexanes, 50 mL of 25 mM phosphate buffer pH 7.0, room temperature; stopped after addition of 8.37 mL of 0.5 M NaOH, corresponding to 40% conversion in 7 h 40 min) using lipase from *T. lanuginosus* (0.60 mL) by the method used for racemic 1-chloroacetoxy-3-butenylphosphonate (±)-**21**. The crude mixture of hydroxyphosphonate and chloroacetate (molar ratio by ^{31}P NMR: 37:63) was flash-chromatographed (hexanes/EtOAc, 1:2, chloroacetate: R_f 0.44, hydroxyphosphonate: R_f 0.15) to give (*R*)-(−)-chloroacetate (*R*)-**30** {2.40 g, 58%; $[\alpha]_D^{26}$ −24.9 (*c* 2.04, acetone)} and (*S*)-(+)-hydroxyphosphonate (*S*)-**27** {1.171 g, 3.86 mmol, 35%; $[\alpha]_D^{26}$ +29.5 (*c* 2.37, acetone), 95% ee and (*S*)-configuration by ^{31}P NMR spectroscopy of (*R*)-(+)-Mosher ester} as colorless oils.

(±)-Diisopropyl 3-Bromo-1-(trifluoromethanesulfonyloxy)propylphosphonate [(±)-28a]. (±)-3-Bromo-1-hydroxypropylphosphonate (±)-**27** (1.515 g, 5.0 mmol) was converted to racemic 3-bromo-1-(trifluoromethanesulfonyloxy)propylphosphonate (±)-**28a** (1.8.71 g, 86%) as a colorless oil by the procedure used for the preparation of the chloro analogue (±)-**24**.

IR (ATR) ν: 2984, 1414, 1242, 1204, 1140, 1102, 988, 921 cm^{-1}. ^1H NMR (400.27 MHz, $CDCl_3$): δ 5.11 (td, J = 8.2, 5.2 Hz, 1H), 4.86−4.71 (m, 2H), 3.61−3.53 (m, 1H), 3.41 (td, J = 10.6, 7.3 Hz, 1H), 2.60−2.39 (m, 2H), 1.36 (d, J = 6.2 Hz, 3H), 1.36 (d, J = 6.2 Hz, 6H), 1.34 (d, J = 5.2 Hz, 3H); ^{13}C NMR (150.93 MHz, $CDCl_3$): δ 118.2 (q, J = 319.5 Hz), 79.7 (d, J = 170.4 Hz), 73.5 (d, J = 6.8 Hz), 73.3 (d, J = 7.3 Hz), 33.6, 26.7 (d, J = 11.1 Hz), 24.0 (d, J = 3.8 Hz), 23.9 (d, J = 3.7 Hz), 23.85 (d, J = 4.5 Hz), 23.6 (d, J = 5.0 Hz); ^{31}P NMR ($CDCl_3$, 162.03 MHz): δ 11.7. Anal. Calcd for $C_{10}H_{19}BrF_3O_6PS$: C, 27.60; H, 4.40; O, 22.06; S, 7.37. Found: C, 27.69; H, 4.44; O, 22.22; S, 7.42.

(±)-Diisopropyl 3-Bromo-1-(methanesulfonyloxy)-propylphosphonate [(±)-28b]. Et_3N (1.518 g, 2.08 mL, 15.0 mmol, 1.5 equiv) and CH_3SO_2Cl (1.489 g, 1.01 mL, 13.0 mmol, 1.3 equiv, dissolved in 5 mL of dry CH_2Cl_2) were added to a stirred solution of racemic 3-bromo-1-hydroxypropylphosphonate (±)-**27** (3.031 g, 10 mmol) in dry CH_2Cl_2 (35 mL) at 0 °C under Ar. After 1 h, the cooling bath was removed, and water (10 mL) was added. Stirring was continued for 10 min, before the organic phase was separated, and the aq one was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were washed with HCl (2 M, 10 mL), washed with water (5 mL), then dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (heptanes/EtOAc, 1:1, R_f 0.37) to yield mesyloxyphosphonate (±)-**28b** (3.535 g, 93%) as a colorless oil.

IR (ATR) ν: 2981, 2936, 1359, 1260, 1240, 1174, 979 cm^{-1}. ^1H NMR (400.27 MHz, $CDCl_3$): δ 4.90 (td, J = 8.5, 4.7 Hz, 1H), 4.83−4.68 (m, 2H), 3.59−3.51 (m, 1H), 3.49−3.40 (m, 1H), 3.20 (s, 3H), 2.44−2.28 (m, 2H), 1.34 (d, J = 6.2 Hz, 9H), 1.35

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

(d, J = 6.2 Hz, 3H); ^{13}C NMR (150.93 MHz, $CDCl_3$): δ 75.1 (d, J = 171.8 Hz), 72.7 (d, J = 6.8 Hz), 72.5 (d, J = 6.9 Hz), 39.1, 34.0, 27.8 (d, J = 14.8 Hz), 24.1 (d, J = 3.5 Hz), 24.0 (d, J = 4.0 Hz), 23.95 (d, J = 5.0 Hz), 23.8 (d, J = 5.0 Hz); ^{31}P NMR (242.99 MHz, $CDCl_3$): δ 14.9. Anal. Calcd for $C_{10}H_{22}BrO_6PS$: C, 31.51; H, 5.82; O, 25.18; S, 8.41. Found: C, 31.53; H, 5.76; O, 25.43; S, 8.61.

(±)- and (*S*)-(+)-Diisopropyl 3-Bromo-1-(4-nitrobenzenesulfonyloxy)propylphosphonate [(±)- and (*S*)-(+)-28c]. A solution of 4-nitrobenzenesulfonyl chloride (1.441 g, 6.5 mmol, 1.3 equiv, = NosCl) and DMAP (40 mg, 0.33 mmol) in dry CH_2Cl_2 (5 mL) and Et_3N (1.214 g, 1.66 mL, 12 mmol, 2.4 equiv) was added to a stirred solution of racemic 3-bromo-1-hydroxypropylphosphonate (±)-**27** (1.516 g, 5 mmol, dried by evaporation of a solution in dry toluene) in dry CH_2Cl_2 (20 mL) at 0 °C under Ar. The reaction mixture was stirred for 4.5 h at 0 °C and then quenched with H_2O (5 mL) and concd HCl. (1 mL) 10 min later. The phases were separated, and the aqueous one was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1, R_f 0.22) to give nosylate (±)-**28c** (2.148 g, 86%) as a yellowish oil. Similarly, (*S*)-(+)-3-bromo-1-hydroxypropylphosphonate (*S*)-**27** {1.649 g, 5.44 mmol; ee 95% by Mosher; $[\alpha]_D^{18}$ +30.5 (*c* 0.95, acetone)} was converted to (*S*)-(+)-nosyloxyphosphonate (*S*)-**28c** {2.313 g, 87, 95.4% ee by chiral HPLC (Figure S7); $[\alpha]_D^{23}$ +19.7 (*c* 1.86, acetone)}. The spectroscopic data of (±)- and (*S*)-**28c** were identical.

IR (ATR) ν: 2982, 1536, 1404, 1377, 1261, 1188, 992 cm^{-1}. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 8.40−8.35 (m, 2H), 8.22−8.17 (m, 2H), 5.06 (ddd, J = 9.4, 8.3, 4.7 Hz, 1H), 4.70 (sepd, J = 7.0, 6.2 Hz, 1H), 4.64 (sepd, J = 7.2, 6.2 Hz, 1H), 3.54 (ddd, J = 10.4, 6.4, 5.8 Hz, 1H), 3.35 (ddd, J = 10.4, 8.2, 7.0 Hz, 1H), 2.46−2.29 (m, 2H), 1.30 (d, J = 6.2 Hz, 3H), 1.19 (d, J = 6.2 Hz, 3H), 1.27 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 150.8, 142.2, 129.6 (2C), 124.2 (2C), 75.6 (d, J = 172.2 Hz), 72.7 (d, J = 6.8 Hz, 2C), 33.8, 27.9 (d, J = 13.0 Hz), 24.0 (d, J = 4.0 Hz), 23.97 (d, J = 4.9 Hz), 23.9 (d, J = 4.9 Hz), 23.7 (d, J = 4.8 Hz); ^{31}P NMR (161.98 MHz, $CDCl_3$): δ 15.1. Anal. Calcd for $C_{15}H_{23}BrNO_8PS$: C, 36.90; H, 4.75; S, 6.57. Found: C, 37.09; H, 4.75; S, 6.59.

(±)-Diisopropyl 3-[(*N*,*N*′-Di-*tert*-butoxycarbonyl)-hydrazino]-1-(methanesulfonyloxy)propylphosphonate [(±)-29b]. Bis(Boc)-hydrazine[31] (799 mg, 3.42 mmol, 1.5 equiv), Bu_4NHSO_4 (116 mg, 0.34 mmol, 0.15 equiv), toluene (10 mL), and NaOH (4 mL, 20 w/v %) were added to mesyloxyphosphonate (±)-**28b** (870 mg, 2.28 mmol) at 0 °C. The mixture was stirred vigorously. After 1 h, a sample for the ^{31}P NMR spectrum was withdrawn and diluted with $CDCl_3$. As only the starting product was detected, the cooling bath was replaced by a water bath of 19 °C (the temperature was not allowed to surpass 20 °C). After 2 h at 19−20 °C, another sample was withdrawn for ^{31}P NMR spectroscopy. This time, beside the resonance for the starting material (δ_P 15.3), a new signal (δ_P 16.1) appeared which was assigned to the hydrazine derivative (±)-**29b** (their ratio was 1.0:0.54). Within 5 h at 19−20 °C, the starting material decreased to 1%. Now, the reaction mixture was worked up. EtOAc (20 mL) and a saturated aq solution of $NaHCO_3$ (20 mL) were added. The organic phase was removed, and the aq one was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with HCl (10 mL, 1 M), dried (Na_2SO_4), and concentrated under reduced pressure. The residue [(±)-**25**/(±)-**29b**/unknown compound/(±)-**28b** = 4:100:16:1, by ^{31}P NMR] was purified by flash chromatography (heptanes/EtOAc, 1:1, R_f 0.15, starting material 0.22) to give *N*,*N*′-bis(Boc)-hydrazinophosphonate (±)-**29b** (911 mg, 75%) as a colorless gum.

IR (ATR) ν: 3254, 2979, 2935, 1707, 1363, 1248, 1173, 1143, 985, 933 cm^{-1}. ^{1}H NMR (500.32 MHz, toluene-d_8, 80 °C): δ 6.26 (br s, 1H), 4.94 (br s, 1H), 4.73−4.51 (m, 2H), 3.83−3.71 (m, 1H), 3.67−3.57 (m, 1H), 2.77−2.70 (m, 3H), 2.43−2.30 (m, 1H), 2.20−2.08 (m, 1H), 1.45−1.40 (m, 9H), 1.40−1.36 (m, 9H), 1.21−1.07 (m, 12H); ^{31}P NMR (202.53 MHz, toluene-d_8, 80 °C): δ 16.1, 15.2; ratio: 91:9. Anal. Calcd for $C_{20}H_{41}N_2O_{10}PS$: C, 45.10; H, 7.76; N, 5.26; O, 30.04; S, 6.02. Found: C, 44.71; H, 8.10; N, 4.94; O, 29.59; S, 5.76.

(±)-Diisopropyl 1,2-(Bis-*tert*-butoxycarbonyl)-pyrazolidin-3-ylphosphonate [(±)-25] from (±)-29b. Bu_4NHSO_4 (64 mg, 0.19 mmol, 0.15 equiv), toluene (5 mL), and NaOH (2 mL, 20 w/v %) were added to 3-hydrazinophosphonate (±)-**29b** (666 mg, 1.25 mmol). The mixture was heated at 80 °C and stirred vigorously. After 3 h, the mixture was cooled and diluted with EtOAc (20 mL) and water (8 mL). The organic phase was separated, and the aq one was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with HCl (10 mL, 1 M), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was flash-chromatographed (heptanes/EtOAc, 1:2, R_f 0.29) to furnish protected pyrazolidinylphosphonate (±)-**25** (411 mg, 75%) as a colorless, very viscous oil.

IR (ATR) of (±)-**25** ν: 2977, 1702, 1366, 1252, 1166, 1141, 986 cm^{-1}. ^{1}H NMR (400.13 MHz, $CDCl_3$, 50 °C): δ 4.84 (oct, J = 6.2 Hz, 1H), 4.71 (sepd, J = 7.3, 6.2 Hz, 1H), 4.42 (td, J = 9.6, 3.3 Hz, 1H), 3.97 (td, J = 9.5, 7.1 Hz, 1H), 3.21 (td, J = 9.8, 5.3 Hz, 1H), 2.44−2.30 (m, 1H), 2.30−2.11 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H), 1.31 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 1.29 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100.61 MHz, $CDCl_3$, 50 °C): δ 156.6, 156.5, 81.8, 80.7, 72.0 (d, J = 7.0 Hz), 70.8 (d, J = 7.2 Hz), 54.1 (d, J = 173.3 Hz), 45.3 (br s), 28.3 (3C), 28.1 (3C), 27.2 (d, J = 1.1 Hz), 24.4 (d, J = 2.4 Hz), 24.1 (d, J = 4.8 Hz), 24.0 (d, J = 3.6 Hz), 23.7 (d, J = 6.1 Hz); ^{31}P NMR (162.04 MHz, $CDCl_3$, 50 °C): δ 21.8. Anal. Calcd for $C_{19}H_{37}N_2O_7P$: C, 52.28; H, 8.54; N, 6.42. Found: C, 51.99; H, 8.40; N, 6.34.

(±)- and (*R*)-(−)-Diisopropyl 1,2-(Bis-*tert*-butoxycarbonyl)pyrazolidin-3-ylphosphonate [(±)- and (*R*)-25] from 28c. *Method A.* (±)-3-Bromo-1-(4-nitrobenzenesulfonyloxy)propylphosphonate (±)-**28c** (1.465 g, 3 mmol) was dissolved in toluene (10 mL) under Ar. An aq solution of NaOH (6 mL, 20 w/v %), BocNH−NHBoc (1.394 g, 6 mmol) and Bu_4NHSO_4 (290 mg, 0.9 mmol, dissolved in 5 mL of toluene) was added at room temperature. The reaction mixture was vigorously stirred and heated at 50 °C for 7 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc (10 mL) and neutralized with HCl (2 M). The organic phase was separated, and the aq one was extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1, R_f 0.15) to yield pyrazolidin-3-ylphosphonate (±)-**25** (728 mg, 56%) as a yellowish gum.

Method B. BocNH−NHBoc (1.237 g, 5 mmol, 2.5 equiv) and KO*t*Bu (561 mg, 5 mmol, 2.5 equiv) were dissolved in dry DMF (5 mL) under Ar atmosphere. A solution of (±)-3-bromo-1-(4-nitrobenzenesulfonyloxy)propylphosphonate (±)-**28c** (977 mg,

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

2 mmol) in dry DMF (5 mL) was added at 0 °C. The reaction mixture was stirred for 1.5 h at 0 °C and at room temperature until the starting material was virtually consumed (1.5 h). Acetic acid (6 drops) was added, and the volatile components were removed under reduced pressure (0.5 mbar). The residue was taken up in H_2O/EtOAc (30 mL, 1:1). The organic phase was separated, and the aq one was extracted with EtOAc (2 × 10 mL). The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Flash chromatography (hexanes/EtOAc, 3:2, R_f 0.23) of the residue gave the desired racemic pyrazolidin-3-ylphosphonate (±)-**25** (570 mg, 65%) as a yellowish oil.

Cyclization by Method B with Alternative Work Up. (±)-Bromonosylate (±)-**28c** (2.300 g, 4.71 mmol) was converted to protected pyrazolidin-3-ylphosphonate (±)-**25**. When cyclization was finished, the reaction mixture was cooled to room temperature, and HCl (2 M, 25 mL) and water (25 mL) were added (color changed from dark red/brown to yellow). The mixture was extracted with EtOAc (4 × 25 mL). The combined organic phases were washed with HCl (1 M, 2 × 20 mL), $NaHCO_3$ (saturated aq solution, 10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Flash chromatography of the residue gave the desired (±)-pyrazolidin-3-ylphosphonate (±)-**25** (1.250 g, 61%) as a yellowish oil.

(*S*)-(+)-Bromonosylate (*S*)-**28c** {1.464 g, 3 mmol; $[\alpha]_D^{23}$ +19.7 (*c* 1.86, acetone); ee 96% by chiral HPLC} was converted to protected (*R*)-(−)-pyrazolidin-3-ylphosphonate (*R*)-**25** {635 mg, 49%; $[\alpha]_D^{26}$ −25.5 (*c* 1.40, acetone)} by method A. The spectroscopic data were identical to those of the racemate.

(±)- and (*R*)-(+)-Pyrazolidin-3-ylphosphonic Acid [(±)- and (*R*)-4]. AllylTMS (1.333 g, 1.85 mL, 11.67 mmol, 3 equiv) and TMSBr (5.955 g, 5.13 mL, 38.9 mmol, 10 equiv) were added to a solution of protected (±)-pyrazolidin-3-ylphosphonate (±)-**25** (1.700 g, 3.89 mmol) in dry DCE (20 mL) under Ar atmosphere.[26] After stirring at 50 °C for 14 h and cooling to room temperature, volatile components were removed under reduced pressure (0.5 mbar). The residue was dissolved in DCE (10 mL), and the solvent was again removed under reduced pressure (0.5 mbar). Water (20 mL) was added to the residue, and the mixture was stirred for 10 min before it was extracted with EtOAc (2 × 15 mL). The aq phase was concentrated (5 mL) and applied to a column filled with Dowex 50W × 8, H^+ (o. d. 1.5 × 14 cm, water as eluent, fractions of 25 mL). Fractions containing the product (TLC: R_f 0.42) were pooled and concentrated under reduced pressure to give (±)-pyrazolidinylphosphonic acid [(±)-**4**] (323 mg, 55%); mp 129−131 °C (H_2O/EtOH). Similarly, protected (*R*)-(−)-pyrazolidin-3-ylphosphonate (*R*)-**25** {739 mg, 1.69 mmol; $[\alpha]_D^{26}$ −25.5 (*c* 1.40, acetone)} was converted to (*R*)-(+)-pyrazolidin-3-ylphosphonic acid [(*R*)-**4**] {136 mg, 53%; $[\alpha]_D^{18}$ +5.09 (*c* 0.51, H_2O)} by the procedure used for the racemic compound; mp 118−119 °C (H_2O/EtOH); ee 92.2% before crystallization, after crystallization 98.8% ee (Figure S4). The NMR data of (±)-and (*R*)-**4** were identical.

IR (ATR) of (±)-**4** ν: 3277, 1626, 1446, 1234, 1190, 1145, 1028, 957, 924, 894 cm^{-1}. ^1H NMR (400.13 MHz, D_2O): δ 3.56 (ddd, J = 11.3, 9.2, 4.6 Hz, 1H), 3.53 (td, J = 8.8, 8.0 Hz, 1H), 3.45 (td, J = 11.3, 8.2 Hz, 1H), 2.56−2.44 (m, 1H), 2.34−2.19 (m, 1H); ^{13}C NMR (100.61 MHz, D_2O): δ 56.6 (d, J = 151.2 Hz), 47.3 (d, J = 8.1 Hz), 28.327; ^{31}P NMR (161.98 MHz, D_2O): δ 16.13. Anal. Calcd for $C_3H_9N_2O_3P$: C, 23.69; H, 5.96; N, 18.42. Found for (±)-**4**: C, 23.60; H, 5.70; N, 18.34.

P5C Reductase Purification and Assay. *A. thaliana* P5C reductase was purified from a suspension of cultured cells, as previously described.[34] Enzyme activity was measured at 35 °C as the P5C-dependent oxidation of NADH. The assay mixture contained 50 mM Tris-HCl buffer, pH 7.5, 0.4 mM NADH, 2 mM DL−P5C and 1 mM $MgCl_2$ in a final volume of 1 mL. A limiting amount of enzyme (about 0.2 nkat) was added to the prewarmed mixture, and the decrease in absorbance at 340 nm was determined for up to 10 min by continuous monitoring of the sample. Activity was calculated on the assumption of an extinction coefficient for NADH of 6220 M^{-1} cm^{-1}. The concentrations causing 50% inhibition (IC_{50}) of P5C reductase activity and their confidence intervals were estimated by nonlinear regression analysis using Prism 6 for Windows (version 6.07; GraphPad Software Inc., San Diego, CA, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00354.

Chiral HPLC chromatograms of racemic and chiral phosphonic acids **1**−**4** and **6**, phosphonates **22** and **28c**, and ^1H, ^{13}C, and ^{31}P NMR spectra of all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: hammerschmidt@univie.ac.at (F.H.).

ORCID

Friedrich Hammerschmidt: 0000-0003-2193-1405

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank S. Felsinger for recording NMR spectra, E. Macoratti for the determination of the ee of phosphonates by chiral HPLC, and J. Theiner for combustion analyses.

■ REFERENCES

(1) Rüegg, W. T.; Quadranti, M.; Zoschke, A. Herbicide research and development: challenges and opportunities. *Weed Res.* **2007**, *47*, 271−275.

(2) Tan, S.; Evans, R.; Singh, B. Herbicidal inhibitors of amino acid biosynthesis and herbicide-tolerant crops. *Amino Acids* **2006**, *30*, 195−204.

(3) Kavi Kishor, P. B.; Sangam, S.; Amrutha, R. N.; Laxmi, P. S.; Naidu, K. R.; Rao, K. R. S. S.; Rao, S.; Reddy, K. J.; Theriappan, P.; Sreenivasulu, N. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr. Sci.* **2005**, *88*, 424−438.

(4) Kafarski, P.; Lejczak, B.; Forlani, G. Herbicidally active amino-methylenebisphosphonic acids. *Heteroat. Chem.* **2000**, *11*, 449−453.

(5) Forlani, G.; Occhipinti, A.; Berlicki, Ł.; Dziędzioła, G.; Wieczorek, A.; Kafarski, P. Tailoring the Structure of Aminobisphosphonate to Target Plant P5C Reductase. *J. Agric. Food Chem.* **2008**, *56*, 3193−3199.

(6) Forlani, G.; Berlicki, Ł.; Duò, M.; Dziędzioła, G.; Giberti, S.; Bertazzini, M.; Kafarski, P. Synthesis and Evaluation of Effective Inhibitors of Plant δ^1-Pyrroline-5-carboxylate Reductase. *J. Agric. Food Chem.* **2013**, *61*, 6792−6798.

(7) Giberti, S.; Bertazzini, M.; Liboni, M.; Berlicki, Ł.; Kafarski, P.; Forlani, G. Phytotoxicity of aminobisphosphonates targeting both δ^1-pyrroline-5-carboxylate reductase and glutamine synthetase. *Pest Manage. Sci.* **2017**, *73*, 435−443.

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452

(8) Moonen, K.; Laureyn, I.; Stevens, C. V. Synthetic Methods for Azaheterocyclic Phosphonates and Their Biological Activity. *Chem. Rev.* **2004**, *104*, 6177−6215.
(9) Lejczak, B.; Kafarski, P.; Mastalerz, M. Chromatographic assignment of the configuration of 1-aminoalkanephosphonic acids. *J. Chromatogr. A* **1985**, *324*, 455−461.
(10) Zecchini, G. P.; Paradisi, M. P.; Torrini, I.; Lucente, G. A convenient route to pyrrolidine-2-phosphonic acid dipeptides. *Int. J. Pept. Protein Res.* **2009**, *34*, 33−36.
(11) Katritzky, A. R.; Cui, X.-L.; Yang, B.; Steel, P. J. Asymmetric Syntheses of 2-Substituted and 2,5-Disubstituted Pyrrolidines from (3*S*,5*R*,7a*R*)-5-(Benzotriazol-1-yl)-3-phenyl[2,1-*b*]oxazolopyrrolidine. *J. Org. Chem.* **1999**, *64*, 1979−1985.
(12) Kaname, M.; Arakawa, Y.; Yoshifuji, S. Synthesis of novel (*R*)- and (*S*)-piperidazine-3-phosphonic acids and transformation into (*R*)- and (*S*)-pyrrolidine-2-phosphonic acids. *Tetrahedron Lett.* **2001**, *42*, 2713−2716.
(13) Dinér, P.; Amedjkouh, M. Aminophosphonates as organocatalysts in the direct asymmetric aldol reaction: towards syn selectivity in the presence of Lewis bases. *Org. Biomol. Chem.* **2006**, *4*, 2091−2096.
(14) Wuggenig, F.; Schweifer, A.; Mereiter, K.; Hammerschmidt, F. Chemoenzymatic Synthesis of Phosphonic Acid Analogues of L-Lysine, L-Proline, L-Ornithine, and L-Pipecolic Acid of 99% ee—Assignment of Absolute Configuration to (−)-Proline. *Eur. J. Org. Chem.* **2011**, 1870−1879.
(15) Hirata, S.; Kuriyama, M.; Onomura, O. Facile synthesis of both enantiomers of (pyrrolidin-2-yl)phosphonate from l-proline. *Tetrahedron* **2011**, *67*, 9411−9416.
(16) Kaboudin, B.; Kato, J.; Aoyama, H.; Yokomatsu, T. A novel and simple method for the preparation of (*R*)- and (*S*)-pyrrolidine-2-phosphonic acids: phosphonic acid analogues of proline. *Tetrahedron: Asymmetry* **2013**, *24*, 1562−1566.
(17) Arizpe, A.; Rodríguez-Mata, M.; Sayago, F. J.; Pueyo, M. J.; Gotor, V.; Jiménez, A. I.; Gotor-Fernández, V.; Cativiela, C. Enzymatic and chromatographic resolution procedures applied to the synthesis of the phosphoproline enantiomers. *Tetrahedron: Asymmetry* **2015**, *26*, 1469−1477.
(18) Qian, R.; Kuliszewska, E.; Macoratti, E.; Hammerschmidt, F. Chemoenzymatic Synthesis of Racemic and Enantiomerically Pure Phosphaaspartic Acid and Phosphaarginine. *Eur. J. Org. Chem.* **2017**, 4836−4845.
(19) Evans, D. A.; Weber, A. E. Synthesis of the Cyclic Hexapeptide Echinocandin D. New Approaches to the Asymmetric Synthesis of *β*-Hydroxy *α*-Amino Acids. *J. Am. Chem. Soc.* **1987**, *109*, 7151−7157.
(20) Waid, P. P.; Flynn, G. A.; Huber, E. W.; Sabol, J. S. Constrained amino acids. An approach to the synthesis of 3-substituted prolines. *Tetrahedron Lett.* **1996**, *37*, 4091−4094.
(21) Salmon, A.; Carboni, B. Hydroboration−azide alkylation as efficient tandem reactions for the synthesis of chiral non racemic substituted pyrrolidines. *J. Organomet. Chem.* **1998**, *567*, 31−37.
(22) Kumara Swamy, K. C.; Bhuvan Kumar, N. N.; Balaraman, E.; Pavan Kumar, K. V. P. Mitsunobu and Related Reactions: Advances and Applications. *Chem. Rev.* **2009**, *109*, 2551−2651.
(23) Hoffmann, R. W.; Dresely, S. Preparation of 3-Substituted (*E*)-1-Alkenylboronic Esters. *Synthesis* **1988**, 103−106.
(24) (a) Drag, M.; Dlugosz, K.; Oleksyszyn, J. Facile Synthesis of bis-*α*-Aminoalkylphosphinates. *Synth. Commun.* **2006**, *36*, 2787−2795. (b) Atherton, F. R.; Hassall, C. H.; Lambert, R. W. Synthesis and Structure-Activity Relationships of Antibacterial Phosphonopeptides Incorporating (1-Aminoethyl)phosphonic Acid and (Aminomethyl)-phosphonic Acid. *J. Med. Chem.* **1986**, *29*, 29−40. (c) Lejczak, B.; Kafarski, P.; Soroka, M.; Mastalerz, P. Synthesis of the Phosphonic Acid Analog of Serine. *Synthesis* **1984**, 577−580.
(25) Vaseila, A.; Voeffray, R. Asymmetric Synthesis of *α*-Aminophosphonic Acids by Cycloaddition of *N*-Glycosyl-*C*-dialkoxyphosphonoylnitrones. *Helv. Chim. Acta* **1982**, *65*, 1953−1964.
(26) Hammerschmidt, F. Addition von Dialkylphosphiten und Dialkyl(trimethylsilyl)phosphiten an 2-(Benzyloxy)propanal - Darstellung aller vier stereoisomeren (1,2-Dihydroxy-[1-$^{2}H_{1}$]propyl)-phosphonsäuren aus chiralen Lactaten. *Liebigs Ann. Chem.* **1991**, *1991*, 469−475.
(27) Cheng, S.; Yu, S. Enantioselective synthesis of 3-substituted 1,2-oxazinanes via organocatalytic intramolecular aza-Michael addition. *Org. Biomol. Chem.* **2014**, *12*, 8607−8610.
(28) Hammerschmidt, F.; Lindner, W.; Wuggenig, F.; Zarbl, E. Enzymes in organic chemistry. Part 10: Chemo-enzymatic synthesis of L-phosphaserine and L-phosphaisoserine and enantioseparation of amino-hydroxyethylphosphonic acids by non-aqueous capillary electrophoresis with quinine carbamate as chiral ion pair agen. *Tetrahedron: Asymmetry* **2000**, *11*, 2955−2964.
(29) Drescher, M.; Felsinger, S.; Hammerschmidt, F.; Kählig, H.; Schmidt, S.; Wuggenig, F. Enzymes in Organic Chemistry 7. Evaluation of Homochiral *t*-Butyl(phenyl)phosphinothioic Acid for the Determination of Enantiomeric Excesses and Absolute Configurations of *α*-Substituted Phosphonates. *Phosphorus, Sulfur Silicon Relat. Elem.* **1998**, *140*, 79−93.
(30) Żymańczyk-Duda, E.; Skwarczyński, M.; Lejczak, B.; Kafarski, P. Accurate assay of enantiopurity of 1-hydroxy- and 2-hydroxyalkylphosphonate esters. *Tetrahedron: Asymmetry* **1996**, *7*, 1277−1280.
(31) Ling, K. B.; Smith, A. D. *α*-Aroyloxyaldehydes: scope and limitations as alternatives to *α*-haloaldehydes for NHC-catalysed redox transformations. *Chem. Commun.* **2011**, *47*, 373−375.
(32) Stang, P. J.; White, M. R. Triflic Acid and Its Derivatives. *Aldrichimica Acta* **1983**, *16*, 15−22.
(33) Brazier, J. B.; Cavill, J. L.; Elliott, R. L.; Evans, G.; Gibbs, T. J. K.; Jones, I. L.; Platts, J. A.; Tomkinson, N. C. O. The *α*-effect in cyclic secondary amines: new scaffolds for iminium ion accelerated transformations. *Tetrahedron* **2009**, *65*, 9961−9966.
(34) Giberti, S.; Funck, D.; Forlani, G. Δ^{1}-pyrroline-5-carboxylate reductase from *Arabidopsis thaliana*: stimulation or inhibition by chloride ions and feedback regulation by proline depend on whether NADPH or NADH acts as co-substrate. *New Phytol.* **2014**, *202*, 911−919.
(35) Forlani, G.; Bertazzini, M.; Zarattini, M.; Funck, D.; Ruszkowski, M.; Nocek, B. Functional properties and structural characterization of rice δ^{1}-pyrroline-5-carboxylate reductase. *Front. Plant Sci.* **2015**, *6*, 565.
(36) Mikenda, W. Solid films and layers deposited on silicon—a versatile infrared sampling technique. *Vib. Spectrosc.* **1992**, *3*, 327−330.
(37) Mandl, A.; Nicoletti, L.; Lämmerhofer, M.; Lindner, W. Quinine- versus carbamoylated quinine-based chiral anion exchangers: A comparison regarding enantioselectivity for *N*-protected amino acids and other chiral acids. *J. Chromatogr. A* **1999**, *858*, 1−11.
(38) Drescher, M.; Li, Y.-F.; Hammerschmidt, F. Enzymes in organic chemistry, part 2: Lipase-catalysed hydrolysis of 1-acyloxy-2-arylethylphosphonates and synthesis of phosphonic acid analogues of L-phenylalanine and L-tyrosine. *Tetrahedron* **1995**, *51*, 4933−4946.

DOI: 10.1021/acsomega.8b00354
ACS Omega 2018, 3, 4441−4452