Preparation of Phosphonic Acid Analogues of Proline and Proline Analogues and Their Biological Evaluation as δ¹-Pyrroline-5-carboxylate Reductase Inhibitors

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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, δ¹-pyrroline-5-carboxylate synthetase, and δ¹-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 diuron 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 δ¹-pyrroline-5-carboxylate (PSC) 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 PSC 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 PSC 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 i-configured ones in the carboxyl acid series because of the higher priority of PO₃H₂ compared to CO₂H according to the Cahn–Ingold–Prelog rules. Many azaheterocyclic phosphonates have been synthesized and biologically evaluated in the past.8

RESULTS AND DISCUSSION

Synthesis of (+)-, (R)-, and (S)-Phosphaproline. Although racemic, (R)-, and (S)-phosphoproline [(±)-, (R)-, and (S)-1] have been described in the literature,9–17 we present...
here a new access from recently prepared racemic and enantiomeric 1-hydroxy-3-butenylphosphonate 7 (Scheme 1). It also served as a starting material for phosphonic acids reaction22 with assigned17 recently.

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

1. H₂B-THF
2. N₂

N₂

PO₂H₂

PO₂H₂

N₂

Phosphonic acids 5 and 6 differ from phosphoproline by replacing the five-membered ring by a six-membered ring containing an additional CH₂ group or an oxygen atom. The preparation of racemic phosphapiepecolic acid was recently published.14 The synthesis of analogues 6 was accomplished starting from hydroxybutylphosphonates 16, derived from nosylates (±)- and (S)-11 (ee 85%) by hydroboration with H₂B × tetrahydrofuran (THF), followed by oxidative cleavage of the (S)-11 dibenzyl ether from phosphaproline by functional group manipulation of the boron to nitrogen atom, with loss of nitrogen after attack of the azido group on the boron atom.19 The phosphoproline 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 assigned20 recently.

The ee of (R)- and (S)-1 was the same (>99%) as that of the starting 60-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)-1118 (Scheme 2). Ozonolysis gave both hydroxyphosphonates 12 in 19% yield. The following Mitsunobu reaction21 with N-hydroxyphthalimide delivered protected O-alkylhydroxylamines (±)- and (S)-13 in yields of 83 and 84%, respectively. Hydrazinolysis in iPrOH 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 (noslyoxy) 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 h.24 The phosphonic acids were purified by cation-exchange chromatography (Dowex 50W × 8, H⁺) and crystallization. The overall yields of (±)- and (R)-215 starting from 13 were 79 and 74% (ee 99%, Figure S2), respectively. Surprisingly, deprotection of 15 with bromotrimethylsilane (TMSBr)/allyltrimethylsilane (allyTMS)26 had a detrimental effect on the yield of (R)-2 (15%).

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

Nos = 4-nitrobenzenesulfonyl; DIAD = disopropyl 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 hydroxyamine 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-
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 H2O/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 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 and resolved enzymatically. Ethyl (±)-bromo-1-hydroxyphosphonate (±)-20 was converted to triflate (±)-24 in 71% yield (Scheme 5). It was added to a mixture of N,N′-bis(Boc)-hydrazine31 and 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 reactivity32 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 S2,2 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 system33 (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 trifluoromethanesulfonylexoy group had to be replaced by a less reactive leaving group such as a mesyloxy or 4-nitrobenzenesulfonylexoy (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/...
Scheme 6. Preparation of (±)-Pyrazolidin-3-ylphosphonic Acid [(±)-4]  

Et$_2$N or NosCl/ Et$_3$N/dimethylamino pyridine (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 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 at $±$25% yield as a colorless gum. As expected, the calculated IC$_{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. 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 catalytic activity of P5C reductase, purified from Arabidopsis thaliana cultured cells, was then evaluated. In the range from 10$^{-4}$ to 10$^{-2}$ 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 potent inhibition in the range from 10 to 200 mM. 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 reductase from higher plants. 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 $\gamma$-glutamyl kinase and PSC synthetase from Escherichia coli and rice, respectively, was also assessed. However, in no case was their catalytic rate significantly inhibited (data not shown).
CONCLUSIONS

In summary, we have prepared the racemic 1-hydroxy-3-butenyl-, 3-chloro-1-hydroxymethyl-, and 3-bromo-1-hydroxymethylphosphonate and converted them to chloroacetates for lipase-catalyzed enantioselective hydrolysis. The racemates and (S)-enantiomers of these α-hydroxymethylphosphonates were transformed into racemic and (R)-configured pyrrolidin-2-yl-, oxazolidin-3-yl-, oxazolidin-3-yl-, pyrazolidin-3-yl-, and 1,2-oxazinan-3-ylphosphonic acid analogues 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\(_2\)O 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 (\(\delta\)) are reported in parts per million (ppm) relative to CHCl\(_3\) (CDCl\(_3\), \(\delta\)_H 7.26, \(\delta\)_C 77.10), toluene-d\(_8\) (\(\delta\)_H 6.98, \(\delta\)_C 49.00) and external H\(_2\)PO\(_4\) (85%; \(\delta\)_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. \(^{13}\)C High-resolution mass spectra (HRMS) were obtained using a Bruker 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 Fluka 250 μm thickness). Spots were visualized by ultraviolet (UV) and/or dipping into a solution of (NH\(_4\))\(_2\)MoO\(_4\)·4H\(_2\)O (25.0 g) and Ce(SO\(_4\))\(_2·4\)H\(_2\)O (1.0 g) in 10% aqueous H\(_2\)SO\(_4\) (500 mL), followed by heating with a heat gun. The solvent for TLC of phosphonic acids was iPrOH/H\(_2\)O/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 Amino-phosphonic 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\(_2\)PO\(_4\) 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 thermostaker PHMT with PSC24N from Grant-bio (Cambridgeshire, UK) and an Agilent 1100 HPLC–UV–fluorescence detector (FLD) system from Agilent (Waldbrohn, Germany), which comprised a binary pump, a temperature controlled column oven, an autosampler, a multilength 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-aminooquinolyl-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 phospho 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...
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 AQCl-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 AQCl derivatization and separation on an unmodified QN-AX column. A control experiment for compound 2 derivatized with AQCl 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)(−)-Pyrrrolidin-2-yl phosphonic Acid (Phosphopline) [(+)-, (R)-, and (S)-1]. Cyclohexene (598 mg, 0.74 mL, 7.28 mmol, 4 equiv) was added to a solution of H2B × Me2S (277 mg, 0.35 mL, 3.64 mmol, 2 equiv) in freshly distilled dry 1,2-dimethoxyethane (DME, 5.5 mL) under 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 diclohydroxylborane23 was again cooled at 0 °C. Racemic 1-azido-3-butenylphosphonate (±)-818 (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 rechromatographed (hexanes/EtOAc, 3:2, acetone). Similarly, 1.15, acetone). The NMR data of (±)- and (S)-13 were identical. IR (ATR) of (±)-enantiomer ν: 3358, 2985, 1608, 1534, 1375, 1350, 1242, 1186, 988 cm−1. 1 H NMR (400.13 MHz, CDCl3): 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 (bs 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), 1.3C NMR (100.61 MHz, CDCl3): 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);31P NMR (162.03 MHz, CDCl3): δ 16.7. Anal. Calcd for C11H16NO3PS: 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-(phthalimidoxy)propyl phosphonate ([±]- and (S)-13). Racemic 3-hydroxypropylphosphonate (±)-12 (1.26 g, 2.96 mmol), N-hydroxyphthalimide (507 mg, 3.11 mmol, 1.05 equiv) and Ph3P (1.010 g, 3.85 mmol, 1.3 equiv) were dissolved in dry THF (12 mL) and dry CH2Cl2 (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, J = 1.15, acetone) 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; [α]20D +15.1 (c 0.35, acetone). The NMR spectra of (±)- and (S)-13 were identical.

IR (ATR) of (±)-enantiomer ν: 2981, 1732, 1531, 1373, 1349, 1255, 1185, 984 cm−1. 1 H NMR (600.25 MHz, CDCl3): 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), 5.53 (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 (dd, 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), 1.3C NMR (150.93 MHz, CDCl3): 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, 74.3 (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);31P NMR (242.99 MHz, CDCl3): δ 14.2. HRMS (ESI-TOF) m/z: [M + Na]+ calc C13H12N2O3PSNa, 593.0966; found, 593.0961.
(±)- and (R)-(+) -Isoxazolidin-3-ylphosphonic Acid ([±] and (R)-2). Racemic phthalimidoxyphosphonate ([±]-13 (762 mg, 1.34 mmol) was dissolved in iPrOH (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 Celite pad with suction and carefully washed with iPr$_2$O. The filtrate was concentrated under reduced pressure. The oily, faint yellow residue (355 mg) was reduced to (R)-[18] (273 mg, 73%) as a colorless liquid. Similarly, (S)-(+) -phthalimidoxyphosphonate (S)-17 (928 mg, 1.63 mmol) was converted to (R)-[18] (1.906 mmol) and 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$^+$; column: 0.2 cm × 38 cm, elution with water, fractions of 10 mL; Rt 0.33). Ninhydrin-positive fractions were pooled and concentrated under reduced pressure to give racemic isoxazolidin-3-ylphosphonic acid ([±]-2 (1.40 g, 2.4 mmol) was added dropwise over 10 min. After 2 h of stirring at 60°C and 4 h at room temperature before crystallization (ee 83.1%; Figure S5); [α]$_D^{20}$ -3.08 (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), 7.46–6.45 (m, 2H), 3.93–3.99 (m, 1H), 3.73 (td, J = 11.3, 3.1 Hz, 1H), 3.43 (dd, 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), 13C NMR (150.93 MHz, CDCl$_3$): δ 77.1, 71.0 (d, J = 7.0 Hz), 70.5, 65.6 (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), 31P NMR (242.99 MHz, CDCl$_3$): δ 20.1. Anal. Calcd for C$_6$H$_6$NO$_5$P·C: 47.80; H, 8.83; N, 5.57; O, 38.47. Found: C, 47.87; H, 9.19; N, 5.74; O, 25.53.

(±)- and (S)-(+) -Diisopropyl 1-(4-Nitrobenzenesulfonyloxy)-4-(phthalimidoxy)butylphosphonate ([±] and (S)-[17]. Racemic 1,2-oxazinan-3-ylphosphonic Acid ([±] and (R)-[6]. Racemic 1,2-oxazinan-3-ylphosphonic acid ([±]-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, Rt 0.61) to furnish racemic 1,2-oxazinan-3-ylphosphonic acid ([±]-6 (301 mg, 94%) as crystals; mp 183–86 °C (decomp.) (H$_2$O/EOH). Similarly, (R)-(−)-1,2-oxazinan-3-ylphosphonic acid (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$_2$O/EOH); [α]$_D^{20}$ -23.2 (c 0.95, H$_2$O) before crystallization (ee 83.1%; Figure S5); [α]$_D^{20}$ -23.2 (c 0.95, H$_2$O) after crystallization (ee 91.6%). The NMR spectra of (±) and (R)-[6] were identical.

IR (ATR) ν: 3500–1700 (very br), 1234, 1186, 1168, 1069, 1040, 978, 960, 913 cm$^{-1}$. 1H NMR (600.25 MHz, D$_2$O): δ 4.31–4.23 (m, 1H), 4.15 (td, J = 12.1, 2.5 Hz, 1H), 3.59 (d, J = 13.7, 2.5 Hz, 1H), 2.22–2.12 (m, 1H), 1.99–1.78 (m, 3H); 13C NMR (150.93 MHz, D$_2$O): δ 71.4, 56.1 (d, J = 134.5 Hz), 21.8 (d, J = 9.8 Hz), 20.8 (d, J = 2.5 Hz), 31P NMR (242.99 MHz, D$_2$O): δ 9.1. Anal. Calcd for C$_6$H$_6$NO$_5$P·C: 47.85; 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-hydroxyphosphonylphosphonic Acid ([±]-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. After 2 h of additional stirring at −78 °C, diisopropyl trimethylsilyl
phosphate (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 phases were washed with a saturated aq solution of NaHCO3 (50 mL), dried (MgSO4), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:2; chloroacete: Rf: 0.44; hydroxyphosphonate: Rf: 0.15) to give chloroace (R)-21 (2.183 g, 53%: [α]20D +21.4 (c 1.01, acetone) and hydroxyphosphonate (S)-20 (1.332 g, 5.14 mmol, 63%: [α]20D +31.7 (c 1.67, acetone) as colorless liquids. The ee of 97% for (S)-20 was determined by using (S)-(−)-3-(Bu)2P(O)(O)(SH) as CSA and 31P NMR spectroscopy.29,30 major singlet (1.00) at 22.9 ppm and minor one (0.015) at 22.7 ppm.

**Dipropyl 3-Chloro-1-(phthalimidoxy)propylphosphonate (±) and (R)-(−)-Diisopropyl 3-Chloro-1-(phthalimidoxy)propylphosphonate (±) and (R)-(−)-22.**

Dry toluene (20 mL) was added to a mixture of (±)-3-chloro-1-hydroxyphosphonate (±)-20 (1.484 g, 5.74 mmol) Ph3P (2.107 g, 8.03 mmol, 1.4 equiv) and N-hydroxylamine (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 has 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; Rf: 0.33) to yield phthalimidooxyphosphonate (±)-22 (2.05 g, 88%) as a colorless heavy oil, which eventually crystallized; mp 94–95 °C (CHCl3/heptanes). Similarly, (S)-3-chloro-1-hydroxyphosphonate (S)-20 (0.35 mg, 2.5 mmol, ee 96%) was converted to (R)-(−)-3-chloro-1-(phthalimidoxy)propylphosphonate (R)-22 (727 mg, 72%) and 31P NMR spectroscopy:29,30 major singlet (1.00) at 22.9 ppm and minor one (0.015) at 22.7 ppm.

**Resolution of Racemic Diisopropyl 3-Chloro-1-(phthalimidoxy)propylphosphonate (±) and (R)-(−)-3-Chloro-1-(phthalimidoxy)propylphosphonate (±)-22.**

**Resolution of Racemic Diisopropyl 3-Chloro-1-(phthalimidoxy)propylphosphonate (±)-22.** The racemic diisopropyl 3-chloro-1-(phthalimidoxy)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 resolution of (±)-isoxazolidin-3-ylphosphonic acid (±)-2 from the respective nosylate (±)-13; mp 173 °C (decomp) (H2O/PrOH). Similarly, protected (R)-(−)-α-aminoxyphosphonate (R)-22 (662 mg, 1.64 mmol) was converted to (R)-(−)-isoxazolidin-5-ylphosphonic acid [(R)-3] (159 mg, 63%; [α]20D +20.3 (c 1.67, H2O); mp 192 °C (H2O/EtOH); ee 97.2% before crystallization (Figure S3). The NMR spectra of (±)- and (R)-(−)-3 were identical.
IR (ATR) of (±)-3: v = 3250–1500 (very br), 1453, 1283, 1240, 1136, 1077, 1024, 949, 928, 907, 890 cm⁻¹. ¹H NMR (400.27 MHz, D₂O): δ 4.47 (dd, J = 9.5, 7.0 Hz, 1H), 3.88 (dd, 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); ¹³C NMR (150.93 MHz, D₂O): δ 67.84 (d, J = 155.9 Hz), 47.0 (d, J = 7.8 Hz), 30.0; ³¹P NMR (162.04 MHz, D₂O): δ 10.8. Anal. Calc for C₅H₃NO₃P: C, 23.54; H, 5.27; N, 9.15; O, 41.38.

(±)-Diisopropyl 3-Chloro-1-(trifluoromethanesulfonyloxy)propylphosphonate ([±]-24). (±)-3-Chloro-1-hydroxypropylphosphonate (±-20) (753 mg, 2.22 mmol, dried by azotropic distillation with toluene) was dissolved in dry Et₂O (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, (CF₃SO₂)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 NaHCO₃ (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 (MgSO₄) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 2:1, Rf 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, CDCl₃): 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); ¹³C NMR (100.65 MHz, CDCl₃): δ 118.3 (q, J = 31.96 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); ³¹P NMR (162.03 MHz, CDCl₃): δ 11.9. Anal. Calc for C₅H₃ClF₃O₆PS: 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-ButOMe, 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-butylphosphonate (±-21). The crude mixture of hydroxyphosphonate and chloroacetate (molar ratio by ³¹P NMR: 37:63) was flash-chromatographed (hexanes/EtOAc, 1:2, chloroacetate: Rf 0.44, hydroxyphosphonate: Rf 0.15) to give (R)-(−)-chloroacetate (R)-30 (2.40 g, 58%; [α]D⁻²⁸ = −24.9° (c 2.04, acetone) and (S)-(+)hydroxyphosphonate (S)-31 (1.71 g, 38% mmol, 35%; [α]D⁺²⁸ = +29.5° (2.37, acetone), 95% ee and (S)-configuration by ³¹P 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) (1.871 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, CDCl₃): δ 5.11 (td, J = 8.2, 5.0 Hz, 1H), 4.86–4.71 (m, 2H), 3.61–3.53 (m, 1H, 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); ¹³C NMR (100.65 MHz, CDCl₃): δ 118.2 (q, J = 31.95 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); ³¹P NMR (162.03 MHz, CDCl₃): δ 11.7. Anal. Calc for C₅H₃BrO₂Cl₃P: 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). Et₂N (1.518 g, 20.8 mL, 15.0 mmol, 1.5 equiv) and CH₂SO₂Cl (1.489 g, 1.01 mL, 13.0 mmol, 1.3 equiv, dissolved in 5 mL of dry CH₂Cl₂) were added to a stirred solution of racemic 3-bromo-1-(methanesulfonyloxy)-propylphosphonate (±-27) (3.031 g, 10 mmol) in dry CH₂Cl₂ (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 CH₂Cl₂ (2 × 15 mL). The combined organic layers were washed with HCl (2 M, 10 mL), washed with water (5 mL), then dried (MgSO₄), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1, Rf 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, CDCl₃): δ 4.90 (td, J = 8.5, 4.7 Hz, 1H), 4.83–4.68 (m, 2H), 3.59–3.51 (m, 1H, 1H), 3.49–3.40 (m, 1H, 1H), 3.20 (s, 3H), 2.44–2.28 (m, 2H), 1.34 (d, J = 6.2 Hz, 9H), 1.35
(±)- and (S)-(±)-Diisopropyl 3-Bromo-1-(4-nitrobenzenesulfonyloxy)proplyphosphonate [(±)-28b]. A solution of 4-nitrobenzenesulfonyl chloride (1.444 g, 6.55 mmol, 1.3 equiv, = NosCl) and DMA (40 mg, 0.33 mmol) in dry CH₂Cl₂ (5 mL) and Et₂N (1.214 g, 1.66 mmol, 12 mmol, 2.4 eq) was added to a stirred solution of racemic 3-bromo-1-hydroxyproplyphosphonate (±)-27 (1.516 g, 5 mmol, dried by evaporation of a solution in dry toluene) in dry CH₂Cl₂ (20 mL) at 0 °C under Ar. The reaction mixture was stirred for 4.5 h at 0 °C and then quenched with H₂O (5 mL) and concd HCl (1 mL) 10 min later. The phases were separated, and the aq one was extracted with CH₂Cl₂ (2 × 20 mL). The combined organic layers were washed with a saturated aqueous solution of NaHCO₃ (30 mL), dried (MgSO₄), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1, Rf 0.15) to furnish (±)-nosyloxyphosphonate (±)-28b (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 (Na₂SO₄), and concentrated under reduced pressure. The residue [(±)-25]/(±)-29b/unknown compound [(±)-28b = 4:100:16:1, by 31P NMR] was purified by flash chromatography (heptanes/EtOAc, 1:1, Rf 0.15, starting material 0.22) to give N,N’-bis(Boc)-hydrazinophosphonate (±)-29b (911 mg, 75%) as a colorless gum.

IR (ATR) ν 2977, 2935, 1703, 1606, 1513, 1460, 1419, 1365, 1363, 1248, 1173, 1143, 985, 933 cm⁻¹. 1H NMR (500.12 MHz, toluene-d₈, 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, 2H), 2.20–2.08 (m, 1H), 1.45–1.40 (m, 9H), 1.40–1.36 (m, 9H), 1.21–1.07 (m, 12H). 31P NMR (202.53 MHz, toluene-d₈, 80 °C): δ 16.1, 15.2, ratio: 9:1. Analytical data for C₂₀H₄₁N₂O₁₀PS: C, 51.99; H, 8.40; N, 6.34.

(±)-Diisopropyl 1,2-(Bis-tert-butoxycarbonyl)-pyrazolidin-3-ylphosphonate [(±)-25] from (±)-29b. Bu₄NHSO₄ (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. At 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 (Na₂SO₄), and concentrated under reduced pressure. The residue was flash-chromatographed (heptanes/EtOAc, 1:2, Rf 0.29) to furnish protected pyrazolidinophosphonate (±)-25 (411 mg, 75%) as a colorless, very viscous oil.

IR (ATR) of (±)-25 ν: 2977, 1702, 1366, 1252, 1166, 1141, 986 cm⁻¹. 1H NMR (400.13 MHz, CDCl₃, 50 °C): δ 4.84 (oct, J = 6.2 Hz, 1H), 4.71 (sept, 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), 1.27 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H). 13C NMR (100.61 MHz, CDCl₃): δ 150.8, 140.2, 129.6, 124.2 (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). 31P NMR (161.98 MHz, CDCl₃): δ 15.1. Analytical data for C₂₁H₄₃N₂O₃P: 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 [(±)-25] from 28c. **Method A.** (±)-3-Bromo-1-(4-nitrobenzenesulfonyloxy)proplyphosphonate (±)-28c (1.465 g, 3 mmol) was dissolved in toluene (10 mL) under Ar. An aq solution of NaOH (6 mL, 20 w/v %), Boc₂NH—NH—Boc (1.394 g, 6 mmol) and Bu₄NHSO₄ (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 (MgSO₄), and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1, Rf 0.15) to yield pyrazolidin-3-ylphosphonate (±)-25 (728 mg, 56%) as a yellowish gum.

**Method B.** Boc₂NH—NH—Boc (1.237 g, 5 mmol, 2.5 equiv) and KO(t-Bu)₂ (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)proplyphosphonate (±)-28c (977 mg,
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 H₂O/EtOH (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 (MgSO₄), and concentrated under reduced pressure. Flash chromatography (hexanes/EtOAc, 3:2, Rf 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), NaHCO₃ (saturated aq solution, 10 mL), dried (MgSO₄), 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.

(+)-Bromonosylate (S)-28c (1.464 g, 3 mmol; [α]D₂⁰ +19.7 (c 1.86, acetone); ee 96% by chiral HPLC) was converted to protected (R)-(−)-pyrazolidin-3-ylphosphonate (R)-25 [635 mg, 49%; [α]D₂⁰ −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, 38.99 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. 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⁺ (ø. d. 1.5 × 14 cm, water as eluent, fractions of 25 mL). Fractions containing the product (TLC: Rf 0.42) were pooled and concentrated under reduced pressure to give (+)-pyrazolidinylphosphonic acid [(+)-4] (323 mg, 55%); mp 129–131 °C (H₂O/EtOH). Similarly, protected (R)-(−)-pyrazolidin-3-ylphosphonate (R)-25 [739 mg, 1.69 mmol; [α]D₂⁰ +31.2 (c 1.40, acetone)] was converted to (R)-(+)–pyrazolidin-3-ylphosphonic acid [((R)-4] (136 mg, 53%; [α]D₂⁰ +0.9 (0.51, H₂O₂)) by the procedure used for the racemic compound; mp 118–119 °C (H₂O/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, D₂O): δ 3.56 (ddd, J = 11.3, 9.2, 4.6 Hz, 1H), 3.33 (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); ¹³C NMR (100.61 MHz, D₂O): δ 56.6 (d, J = 15.1 Hz), 47.3 (d, J = 8.1 Hz), 38.327; ³¹P NMR (161.98 MHz, D₂O): δ 16.13. Anal. Calcd for C₇H₁₃NO₃P: C, 23.69; H, 5.96; N, 18.42. Found for (±)-4: C, 23.60; H, 5.70; N, 18.34.

PSC Reductase Purification and Assay. A. thaliana PSC reductase was purified from a suspension of cultured cells, as previously described.³⁴ Enzyme activity was measured at 35 °C as the PSC-dependent oxidation of NADH. The assay mixture contained 50 mM Tris-HCl buffer, pH 7.5, 0.4 mM NADH, 2 mM L-PSC and 1 mM MgCl₂ 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 (IC₅₀) of PSC 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, ¹³C, and ³¹P NMR spectra of all new compounds (PDF)

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Notes
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