

Short Note

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Posted Date: 9 September 2024

doi: 10.20944/preprints202409.0592.v1

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Short Note

(R)-2-Amino-1-Hydroxyethylphosphonic Acid

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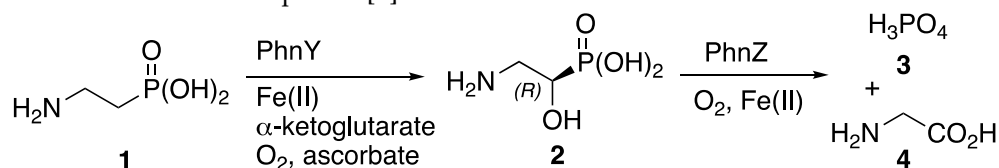
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Abstract: (R)-2-Amino-1-hydroxyethylphosphonic acid **2** was prepared by hydrolytic kinetic resolution of *rac*-diethyl oxiran-2-ylphosphonate followed by reaction with benzylamine, acid hydrolysis, catalytic hydrogenolysis and anion exchange chromatography. Recrystallization from water-ethanol gave pure **2**, which was characterized by IR, ¹H NMR, ¹³C NMR, ³¹P NMR, polarimetry, elemental microanalysis, high-resolution mass spectrometry and single-crystal X-ray diffraction. The acid **2** crystallized in the orthorhombic chiral space group *P*2₁2₁2₁ with cell constants *a* = 6.303(2) Å, *b* = 7.104(2) Å, *c* = 11.627(3) Å. The X-ray crystal structure confirmed the (*R*)-configuration of **2** and revealed that **2** is zwitterionic in the solid state, with extensive intermolecular hydrogen bonding between the hydroxyl, ammonium cation and phosphonate anion groups.

Keywords: X-ray structure; amino phosphonic acid; hydrogen bonding

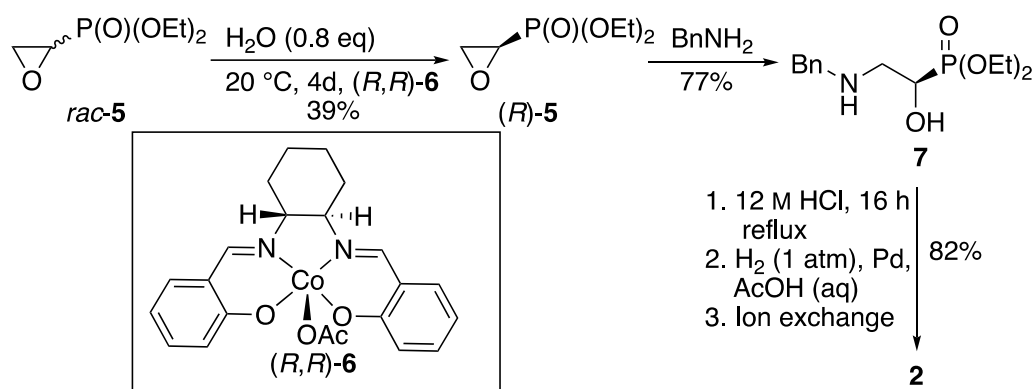
1. Introduction

Phosphorus is essential for all life on Earth, but the low bioavailability of this vital element in many marine environments places stress on biological systems [1]. Microorganisms can use phosphonic acid derivatives to manage scarce phosphorus resources. Thus, in phytoplankton, oxidation of 2-aminoethylphosphonic acid **1** (Scheme 1), mediated by the iron-dependent enzyme PhnY, leads to stereoselective formation of (*R*)-2-amino-1-hydroxyethylphosphonic acid **2**. This catabolic intermediate **2** is then used as a reservoir for supplying inorganic phosphate **3**, which is released alongside glycine **4** following further stereoselective oxidation, mediated by a second iron-dependent enzyme, PhnZ [2]. A 2.1 Å resolution X-ray crystal structure of the PhnZ enzyme bound to its substrate **2** has been reported [3].



Scheme 1. Catabolic pathway in which (*R*)-2-amino-1-hydroxyethylphosphonic acid **2** acts as a reservoir supplying inorganic phosphate **3**.

We have shown [4] that enantioselective chemical synthesis of the natural product **2** can be achieved using hydrolytic kinetic resolution of racemic epoxyphosphonate **5** in the presence of the Jacobsen [5] cobalt catalyst **6** (Scheme 2). The unchanged (*R*)-enantiomer of the epoxide **5** was ring-opened by benzylamine to give the intermediate **7**, then all protecting groups were removed to afford (*R*)-**2**, with physical data consistent with those of material obtained from natural sources [6], by preparation of diastereoisomeric salts [6] and by kinetic resolution of an ester precursor using a lipase [7]. Recently the formation of **2** by borane reduction of a nitrile precursor was used to determine the absolute configuration of the natural product (–)-hydroxynitralaphos [8].



Scheme 2. Chemical synthesis of (*R*)-2-amino-1-hydroxyethylphosphonic acid **2** from racemic epoxyphosphonate **5** using an enantioselective hydrolytic kinetic resolution [4].

There have been only a few crystal structures reported for aminophosphonic acids. These include compounds **1** [9] and **8** - **11** (Figure 1) [10–13]. In this article we provide experimental details and characterization for the chemical synthesis of the acid **2** and we report a high resolution (0.7 Å) single crystal X-ray structure determination of **2**, thus defining the hydrogen bond network that exists in the solid state for pure (*R*)-**2**, a small but highly functionalized chiral molecule.

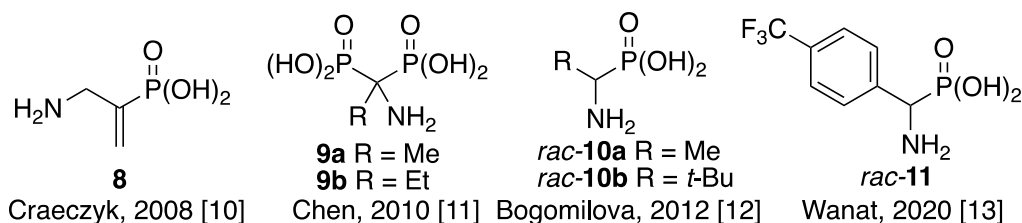


Figure 1. Aminophosphonic acids **8** - **11** that have been characterized by X-ray crystallography. .

2. Results

The synthesis of **2** proceeded as previously outlined [4]; product **2** was recrystallized from aqueous ethanol, then dried under vacuum. Physical data for **2**, including NMR spectra, melting point and optical rotation matched existing literature. High resolution mass spectrometry confirmed the molecular formula and elemental analysis demonstrated that the crystals did not contain residual solvent.

Single-crystal X-ray diffraction (Figure 2) revealed the structure of **2**, which exists in the chiral space group $P2_12_12_1$. The presence of the relatively heavy phosphorus atom within the compound allowed the absolute configuration to be confirmed with confidence: a Flack [14] absolute structure parameter of 0.023(13) was calculated for the (*R*)-enantiomer. Molecules of **2** exist as zwitterions with transfer of one proton from each phosphonic acid group to the amino group generating phosphonate anions and ammonium cations. The conformation of each molecule is such that the phosphorus and nitrogen atoms are antiperiplanar to one another, with P–C(1)–C(2)–N torsion angles of 173.8°, similar to the value of 174.2° found in the crystal structure for **2** that is bound to the PhnZ enzyme [3] and to the conformations of compounds **1** and **8** observed in previous reports [9, 10]. The polar functional groups of **2** engage in complex networks of hydrogen bonds between neighboring molecules of **2**; no intramolecular hydrogen bonds are observed and, in accord with the microanalysis results, no solvent molecules are retained within the crystals. This absence of solvation contrasts with compounds *rac*-**10b**, which crystallized as a hydrate, and *rac*-**11**, which contained ethanol of crystallization.

For each molecule of acid **2** in the crystal, there were four N–H...O and two O–H...O type intermolecular hydrogen bonds to adjacent molecules of **2** (Table 1). Figure 3 illustrates packing of

molecules of **2** within the crystal and the associated network of hydrogen bonds. The ammonium and phosphonate groups adopt similar hydrogen bond-forming roles to those seen in crystals of compounds **1** and **8** - **11**. All three N-H groups are hydrogen bond donors, with bifurcation at H4. The three oxygen atoms of the phosphonate group are all hydrogen bond acceptors, with O2 and O3 each linking to two H atoms in neighboring molecules. Additionally, the phosphonate O1-H7 and the alcohol O4-H8 groups act as both hydrogen bond donors and acceptors.

To conclude, we have established the crystal structure of enantiomerically pure **2**, prepared according to our previously outlined method, for which experimental details are now provided. Notably, this highly polar substance crystallizes from aqueous ethanol without retaining solvent. In common with other amino phosphonic acids, **2** is zwitterionic. All the functional groups of **2** contribute to intermolecular hydrogen bonding within the crystal.

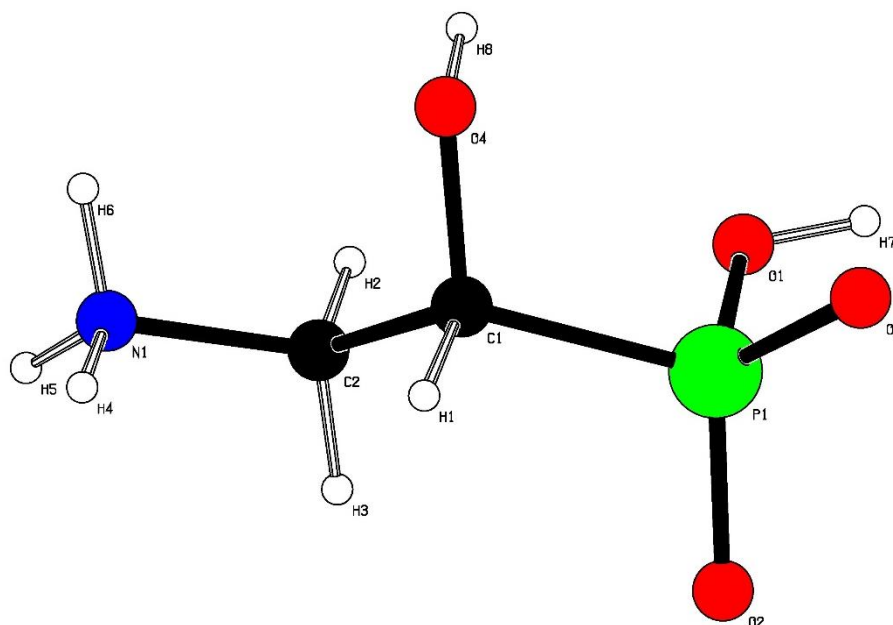


Figure 2. Ball and stick representation of the X-ray crystal structure of the acid **2** showing the atom numbering scheme.

Table 1. Hydrogen bond distances (Å) and angles (°) for acid **2**.

	Donor-H	Acceptor-H	Donor-Acceptor	Angle
N1-H4...O2	0.91	2.23	2.951(2)	135.5
N1-H4...O4	0.91	2.20	2.945(1)	139.1
N1-H5...O2	0.91	1.84	2.730(1)	165.6
N1-H6...O3	0.91	1.90	2.795(1)	165.9
O1-H7...O3	0.84	1.69	2.503(1)	163.6
O4-H8...O1	0.84	2.12	2.880(1)	150.1

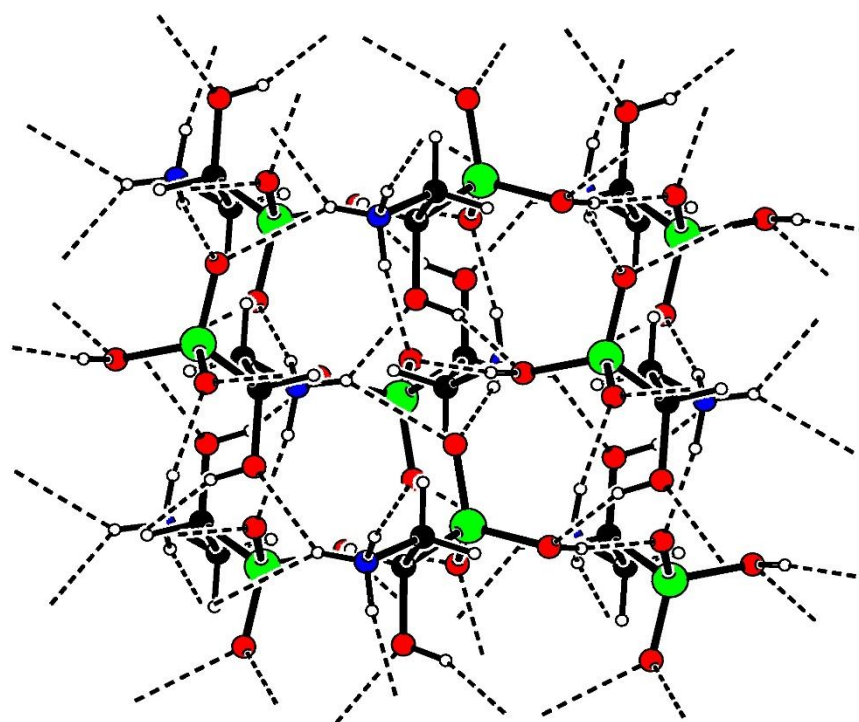


Figure 3. Ball and stick representation showing hydrogen bonding (dashed lines) within the crystal structure of acid **2**. Key: C black, H white, N blue, O red, P green.

3. Experimental

3.1. General Experimental Details

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Melting points were measured using a hot-stage microscope (Reichert). IR spectra were obtained by attenuated total reflection (ATR) using a Perkin-Elmer Spectrum 65 FT-IR spectrometer. NMR spectra were recorded using Bruker AM250, Jeol JNM-EX270 and Bruker AVIII 400 spectrometers. Elemental microanalysis was performed by Medac Ltd., Chobham, Surrey, UK. Mass spectra were obtained by the EPSRC NMSF, Swansea.

3.2.(*R*)-Diethyl oxiran-2-ylphosphonate (*R*)-5

AcOH (7 μ L, 0.12 mmol) was added to a solution of (*R,R*)-(-)-*N,N'*-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) **6** (38.0 mg, 0.06 mmol) in dry toluene (1.5 mL) and stirred in air at 20 $^{\circ}$ C for 1 h. The acetic acid was evaporated under vacuum and *rac*-diethyl oxiran-2-ylphosphonate **5** [15] (1.261 g, 7.00 mmol) was added, followed by water (101 μ L, 5.60 mmol). Stirring was continued for 4 d, after which purification by flash chromatography (CH_2Cl_2 -EtOAc, 3:1) gave (*R*)-diethyl oxiran-2-ylphosphonate (*R*)-**5** (520.1 mg, 2.71 mmol) [ee > 95%, based on ^{31}P integration of the product of epoxide opening with neat (*S*)- α -methylbenzylamine (5 equivalents, 16 h, 20 $^{\circ}$ C)] [4].

3.3.(*R*)-Diethyl (2-benzylamino-1-hydroxyethyl)phosphonate **7**

A mixture of (*R*)-diethyl oxiran-2-ylphosphonate **5** (455.3 mg, 2.53 mmol) and benzylamine (1.35 g, 12.6 mmol) was kept at 20 $^{\circ}$ C for 16 h, after which the excess benzylamine was evaporated in vacuo (70 $^{\circ}$ C, 0.5 mmHg, 1 h). The residue was subjected to flash chromatography (gradient from EtOAc- CH_2Cl_2 1:1 to EtOH-EtOAc 3:7). Appropriate fractions were pooled, evaporated and the residue was

taken up in Et₂O, filtered through celite to remove silica gel residues, and re-evaporated to give (*R*)-diethyl (2-benzylamino-1-hydroxyethyl)phosphonate **7** (557.5 mg, 77%) as a liquid [α]_D³⁰ –7.6 (*c* = 1.67, MeOH); δ_{H} (270 MHz, CDCl₃) 7.37-7.20 (5H, m), 4.22-4.09 (4H, m), 4.00 (1H, dd, *J* 11.2, 6.8 Hz), 3.80 (2H, s), 3.38 (2H, br s), 3.03-2.86 (2H, m), 1.35-1.28 (6H, m).

3.4. (*R*)-2-Amino-1-hydroxyethylphosphonic Acid **2**

(*R*)-Diethyl (2-benzylamino-1-hydroxyethyl)phosphonate **7** (272.4 mg, 0.95 mmol) was refluxed gently for 16 h with 12 M hydrochloric acid (20 mL) then evaporated in vacuo. Water (16 mL), acetic acid (8 mL) and palladium black (120 mg) were added and the mixture was stirred under a balloon of hydrogen at room temperature for 48 h. The mixture was filtered through Celite and the solvent was evaporated in vacuo. The residue was re-evaporated from water, then redissolved in water and applied to a column of Amberlite IR400 (OH), which was eluted first with water and then with 10% aqueous AcOH. The ninhydrin-positive fractions were combined and evaporated. The residue was recrystallized from water-ethanol to give the title compound **2** (109.3 mg, 82%) as colorless needles, mp 264-265 °C (lit. [6] 244-246 °C); [α]_D²⁷ –31.9 (*c* = 0.525, H₂O) (lit. [6] [α]_D²⁰ –30.5). Found: C, 17.10; H, 5.73; N, 9.57 calcd for C₂H₈NO₄P: C, 17.03; H, 5.72; N, 9.92; ν_{max} /cm^{–1} (KBr) 3458, 3157, 2936, 2640, 1663, 1539, 1259, 1159, 1096, 937, 781; δ_{H} (400 MHz, D₂O) 3.90 (1H, td, *J* = 9.9, 2.7 Hz), 3.30 (1H, ddd, *J* = 13.2, 6.4, 3.2 Hz), 3.11 (1H, ddd, *J* = 13.3, 9.8, 6.2 Hz); δ_{C} (62.9 MHz, D₂O) 62.7 (d, ¹*J*_{CP} = 155 Hz), 44.4 (d, ²*J*_{CP} = 8 Hz); δ_{P} (162 MHz, D₂O) 15.1; *m/z* (ESI) found: 142.0262; calcd for C₂H₉NO₄P (*M*+H⁺): 142.0264.

3.5. X-ray Structure Determination of **2**

Single-crystal X-ray diffraction was carried out at the Queen Mary University of London using the KAPPA APEX ii DUO diffractometer, with MoK α radiation (λ = 0.71073 Å). X-ray crystal structures were solved and refined using the Bruker SHELXTL software package.

A translucent colorless block-like specimen of C₂H₈NO₄P, approximate dimensions 0.100 mm × 0.120 mm × 0.200 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured. A total of 2253 frames were collected. The total exposure time was 6.26 h. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using an orthorhombic unit cell yielded a total of 11622 reflections to a maximum θ angle of 30.56° (0.70 Å resolution), of which 1582 were independent (average redundancy 7.346, completeness = 98.9%, *R*_{int} = 1.45%) and 1559 (98.55%) were greater than 2 σ (*F*²). The final cell constants of *a* = 6.303(2) Å, *b* = 7.104(2) Å, *c* = 11.627(3) Å, α = β = γ = 90° and volume = 520.6(2) Å³ are based upon the refinement of the XYZ-centroids of 9792 reflections above 20 σ (*I*) with 6.721° < 2 θ < 61.11°. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.927. The calculated minimum and maximum transmission coefficients (based on crystal size) were 0.9150 and 0.9560, respectively.

The final anisotropic full-matrix least-squares refinement on *F*² with 76 variables converged at *R*₁ = 1.61%, for the observed data and *wR*₂ = 4.64% for all data. The goodness of fit was 1.167. The largest peak in the final difference electron density synthesis was 0.400 e[–]/Å³, and the largest hole was –0.159 e[–]/Å³, with an RMS deviation of 0.052 e[–]/Å³. On the basis of the final model, the calculated density was 1.800 g/cm³ and *F*(000), 296 e[–].

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, CIF report for the crystal structure of **2**, Figure S1: ¹H NMR spectrum of **2** (400 MHz, D₂O); Figure S2: ¹H {³¹P} NMR spectrum of **2** (400 MHz, D₂O); Figure S3: ¹³C NMR spectrum of **2** (62.9 MHz, D₂O); Figure S4: ³¹P {¹H} NMR spectrum of **2** (162 MHz, D₂O).

Author Contributions: Conceptualization, P.B.W.; methodology, P.B.W., M.M., P.B. and I.A.; investigation, P.B.W., M.M., P.B., C.E.W., and I.A.; writing—original draft preparation, P.B.W.; writing—review and editing, P.B.W., and I.A.; visualization, P.B.W., M.M., and I.A.; supervision, P.B.W.; project administration, P.B.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Crystallographic data (excluding structure factors) for the acid **2** are available in the Supplementary Materials of this paper and were deposited at the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 2381816. Copies of the data can be obtained, free of charge, upon application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgments: We are grateful to the EPSRC NMSF at Swansea University for mass spectrometry and to the European Union for enabling P.B. to participate in the ERASMUS student exchange programme.

Conflicts of Interest: The authors declare no conflicts of interest.

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