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Enantioselective synthesis of phosphonate analogues of (*R*)- and (*S*)-homoserine

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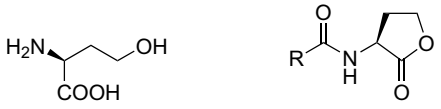
Abstract—The enantioselective synthesis of (*R*)- and (*S*)-1-amino-3-hydroxypropylphosphonic acid, the phosphonate analogues of (*S*)- and (*R*)-homoserine, has been accomplished in four steps and good overall yield starting from diethyl (3*R*,5*R*)- or (3*S*,5*S*)-5-(hydroxymethyl)-2-[(*S*)-1-phenylethyl]isoxazolidinyl-3-phosphonate, respectively.

1. Introduction

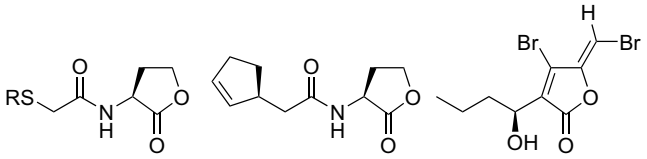
Homoserine **1** and homoserine lactone derivatives **2** have received considerable attention due to their biological activity (Fig. 1). While homoserine is an important amino acid involved in physiologically relevant transformations such as the biosynthesis of methionine and (*S*)-adenosylmethionine, its N-acylated lactones **2** (AHLs) play role as quorum-sensing signal molecules used by many Gram-negative bacteria to control their population density.[1–5]



1 **2**

Figure 1. (*S*)-Homoserine **1** and *N*-acyl (*S*)-homoserine lactones **2**.

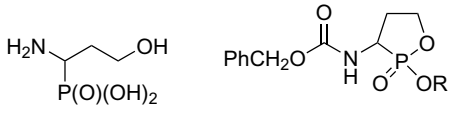
Since chemical signalling processes used by bacteria have been recognised, the design and synthesis of new compounds which would be capable of inhibiting quorum-sensing activity and gene expression have become a challenging task. The inhibitory activity has been found, among others, for several derivatives of homoserine lactones and their structural analogues (Fig. 2).[6–8]



3 **4** **5**

Figure 2. Known QS inhibitors **3**, **4** and **5**.

So far, only a few syntheses of the racemic and enantiomerically pure phosphonate analogues of homoserine **6**[9–12] as well as oxaphospholanes **7a**–**7b** have been reported.[13,14] Vasella employed *N*-glycosyl-*C*-dialkoxyphosphorylated nitrone in the highly selective multistep synthesis of (*R*)-phosphohomoserine (*R*)-**6**.[9] However, this approach led to the formation of (*R*)-**6** only. Moreover, this procedure suffers from several limitations when scaled-up. Roumestant et al. have obtained (*S*)-phosphohomoserine (*S*)-**6** by the asymmetric alkylation of a Schiff base prepared from (1*S*,2*S*,5*S*)-2-hydroxypinan-3-one and diethyl aminomethylphosphonate.[11] Later, the same authors transformed the *N*-Cbz derivative of (*S*)-**6** into the lactone (*S*)-**7** using KF in the presence of 18-crown-6.[14]



6 **7a** R=Me **7b** R=Et

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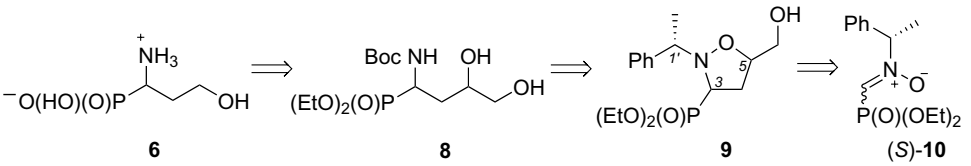


6 8 9 (*S*)-**10**

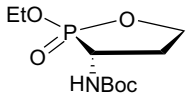
Scheme 1. Retrosynthesis of phosphohomoserine **6**.

Recently, the cycloaddition of (*R*)- and (*S*)-*N*-(1-phenylethyl)-*C*-phosphorylated nitrones **10** to allyl alcohol and further transformations of cycloadducts **9** into enantiomerically pure phosphonate analogues of 4-hydroxyprolines has been described.[15] Since isoxazolidines (3*R*,5*R*,1′*S*)- and (3*S*,5*S*,1′*S*)-**9** are easily available, we noticed their usefulness in the synthesis of both enantiomers of phosphohomoserine **6** (Scheme 1). Hydrogenolyses of isoxazolidines **9** in the presence Boc_2O would lead to *N*-Boc-aminodiols **8**, which could easily be transformed into (*R*)- and (*S*)-**6** via the oxidative cleavage of the terminal diol moiety followed by immediate $NaBH_4$ reduction of the aldehyde functional and hydrolysis of the resulted 1-amino-3-hydroxyphosphonates.

2. Results and discussion

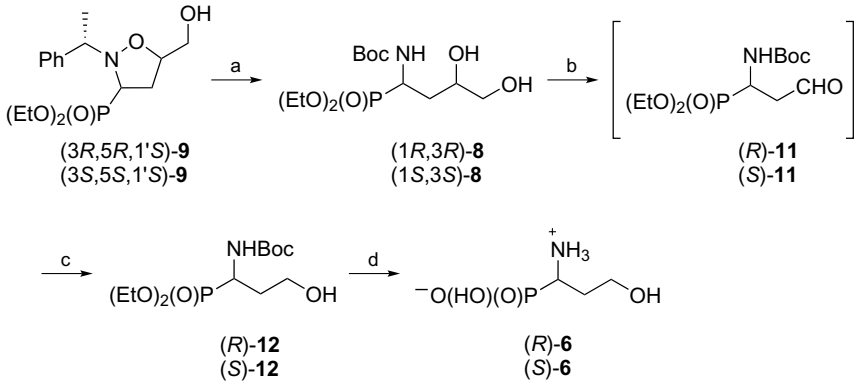
Isoxazolidines (3*R*,5*R*,1′*S*)- and (3*S*,5*S*,1′*S*)-**9** were synthesised according to the described procedure.[15] They were subjected to catalytic hydrogenation performed in the presence of Boc_2O to give *N*-Boc-aminodiols (1*R*,3*R*)- and (1*S*,3*S*)-**8** in good yields (74% and 75%) after chromatographic purification (Scheme 2). Under these conditions, the cleavage of the N–O bond and simultaneous removal of the 1-phenylethyl substituent followed by the protection of the amino group were accomplished in a one-pot procedure. The ^{31}P NMR spectra of **8** showed two signals in a 93:7 ratio due to the presence of the *tert*-butoxycarbonyl group. Treatment of diols (1*R*,3*R*)- and (1*S*,3*S*)-**8** with sodium metaperiodate afforded aldehydes (*R*)- and (*S*)-**11**, which were immediately reduced with $NaBH_4$ to alcohols (*R*)- and (*S*)-**12**. The enantiomeric purities of (*R*)- and (*S*)-**12** were confirmed by ^{31}P NMR analysis using quinine as an enantiodifferentiating agent.[16,17]

The crude alcohols were contaminated (less then 10%) with the respective oxaphospholanes (2*R*,3*R*)- and (2*S*,3*S*)-**13**, due to partial cyclisation of the γ-hydroxypropylphosphonates **12** under basic conditions. Fortunately, oxaphospholanes **13** were easily removed chromatographically, although for the purpose of the syntheses of (*R*)- and (*S*)-**6** their separation from the alcohol (*R*)- or (*S*)-**12** is not necessary. The structure of (2*R*,3*R*)-**13** was established on the basis of the ^{1}H, ^{13}C and ^{31}P NMR spectra. This was further supported by the independent synthesis of (2*S*,3*S*)-**13** from (2*S*,3*S*)-2-ethoxy-2-oxo-3-{*N*-hydroxy-*N*-[(*S*)-1-phenylethyl]amino}-1,2-oxaphospholane.[18] Finally, phosphonates (*R*)- and (*S*)-**12** were hydrolysed under acidic conditions and subsequently treated with propane oxide to give free α-aminophosphonic acids (*R*)- and (*S*)-**6** in 95% and 94% yields, respectively.



(2*R*,3*R*)-**13**

In assigning the absolute configurations of *N*-Boc-aminodiols (1*R*,3*R*)- and (1*S*,3*S*)-**8**, we took advantage of the known configurations of starting isoxazolidines (3*R*,5*R*,1′*S*)- and (3*S*,5*S*,1′*S*)-**9**,[15] since the transformations of (3*R*,5*R*,1′*S*)- and (3*S*,5*S*,1′*S*)-**9** into the respective (1*R*,3*R*)- and (1*S*,3*S*)-**8** did not involve any reactions at the stereogenic centres. From the diol (1*R*,3*R*)-**8** levorotatory phosphohomoserine (*R*)-**6** was obtained, which agrees



(3*R*,5*R*,1′*S*)-**9** (3*S*,5*S*,1′*S*)-**9** (1*R*,3*R*)-**8** (1*S*,3*S*)-**8** (*R*)-**11** (*S*)-**11** (*R*)-**12** (*S*)-**12** (*R*)-**6** (*S*)-**6**

Scheme 2. Reagents and conditions: (a) Boc_2O, $H_2/Pd(OH)_2$, EtOH, rt, 24 h; (b) $NaIO_4$, CH_2Cl_2–H_2O, rt, 2 h; (c) $NaBH_4$, EtOH, 30 min; (d) 6 M HCl, 65 °C, 6 h.

with the specific rotation determined previously for (*R*)-**6** by Vasella.

3. Conclusions

A short and efficient synthesis of enantiomerically pure (*R*)- and (*S*)-phosphohomoserine **6**, from isoxazolidines (3*R*,5*R*,1′*S*)- and (3*S*,5*S*,1′*S*)-**9** has been reported. Hydrogenolysis of (3*R*,5*R*,1′*S*)- and (3*S*,5*S*,1′*S*)-**9** in the presence of Boc_2O gave the respective *N*-substituted 1-amino-3,5-dihydroxybutylphosphonates, (1*R*,3*R*)- and (1*S*,3*S*)-**8**, which were subsequently transformed into (*R*)- and (*S*)-**6** via reaction sequence consisted of standard $NaIO_4$ oxidation, $NaBH_4$ reduction and acid hydrolysis.

4. Experimental

^{1}H NMR spectra were recorded with a Varian Mercury-300 spectrometer; chemical shifts δ in ppm with respect to TMS; coupling constants J in Hz. ^{13}C and ^{31}P NMR spectra were recorded on a Mercury-300 machine at 75.5 and 121.5 MHz, respectively. IR spectra data were measured on a Infinity MI-60 FT-IR spectrometer. Melting points were determined in a capillary on a Electrothermal apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory of this Faculty on a Perkin Elmer PE 2400 CHNS analyser. Polarimetric measurements were conducted on a Perkin Elmer 241 MC apparatus. The following absorbents were used: column chromatography, Merck silica gel 60 (70–230 mesh); analytical TLC plastic sheets silica gel 60 F_{254}.

4.1. *tert*-Butyl (1*R*,3*R*)- and (1*S*,3*S*)-1-(diethoxyphosphono)-3,4-dihydroxybutylcarbamates (1*R*,3*R*)- and (1*S*,3*S*)-8

4.1.1. *tert*-Butyl (1*R*,3*R*)-1-(diethoxyphosphono)-3,4-dihydroxybutylcarbamate (1*R*,3*R*)-8. A solution of isoxazolidine (3*R*,5*R*,1′*S*)-**9** (0.400 g, 1.16 mmol) and Boc_2O (0.254 g, 1.16 mmol) in ethanol (2 mL) was kept under an atmospheric pressure of hydrogen over 20% $Pd(OH)_2$–C (15 mg) at room temperature for 24 h. The suspension was filtrated through a layer of Celite. The solution was concentrated and the residue was chromatographed on a silica gel column with chloroform–methanol (first 100:1, 50:1 and later 10:1, v/v) to give (1*R*,3*R*)-**8** (0.294 g, 74%) as a colourless oil. $[\alpha]_D^{20} = -2.7$ (*c* 1.3, $CHCl_3$). IR (film): $\nu = 3363, 2980, 2933, 1709, 1529, 1367, 1301, 1224, 1170, 1031, 973\ cm^{-1}$. ^{1}H NMR ($CDCl_3$): $\delta = 5.07$ (br d, $J = 9.0$ Hz, 1H, N*H*), 4.25–4.10 (m, 4H), 3.95–3.86 (very br m, 1H), 3.83 (br s, 1H), 3.64 (br dd, $J = 10.8$, 3.0 Hz, 1H), 3.52 (dd, $J = 10.8$, 6.3 Hz, 1H), 2.62 (br s, 1H), 2.08–1.90 (m, 1H), 1.90–1.72 (m, 2H), 1.45 (s, 9H), 1.34 (t, $J = 6.9$ Hz, 6H). ^{13}C NMR ($CDCl_3$): $\delta = 155.0$ (d, $J = 6.6$ Hz), 8.39, 69.40 (d, $J = 10.6$ Hz), 65.96, 63.30 (d, $J = 6.8$ Hz), 63.00 (d, $J = 6.8$ Hz), 44.64 (d, $J = 156.3$ Hz), 33.81 (d, $J = 3.8$ Hz), 28.53, 16.67 (d, $J = 7.5$ Hz), 16.58 (d, $J = 7.5$ Hz). ^{31}P NMR ($CDCl_3$): $\delta = 26.22$ (93%) and 25.63 (7%). Anal. Calcd for $C_{13}H_{28}NO_7P$: C, 45.74; H, 8.26; N, 4.10. Found: C, 46.01; H, 8.48; N, 4.36.

4.1.2. *tert*-Butyl (1*S*,3*S*)-1-(diethoxyphosphono)-3,4-dihydroxybutylcarbamate (1*S*,3*S*)-8. Isoxazolidine (3*S*,5*S*,1′*S*)-**9** (0.479 g, 1.39 mmol) was hydrogenated as described in Section 4.1.1 to give (1*S*,3*S*)-**8** (0.355 g, 75%) as a colourless oil. $[\alpha]_D^{20} = +2.8$ (*c* 1.2, $CHCl_3$). Anal. Calcd for $C_{13}H_{28}NO_7P$: C, 45.74; H, 8.26; N, 4.10. Found: C, 45.53; H, 8.55; N, 4.26.

4.2. *tert*-Butyl (*R*)- and (*S*)-1-(diethoxyphosphono)-3-hydroxypropylcarbamates (*R*)- and (*S*)-12

4.2.1. Synthesis of (*R*)-12. A mixture of *N*-Boc-aminodiol (1*R*,3*R*)-**8** (0.128 g, 0.375 mmol), $NaIO_4$ (0.096 g, 0.450 mmol), water (2 mL) and methylene chloride (4 mL) was stirred at room temperature for 2 h. The organic layer was separated and the water phase was extracted with methylene chloride (3 × 5 mL). The organic extracts were collected, dried over $MgSO_4$ and evaporated in vacuo to afford crude (*R*)-**11** (0.116 g, 100%), which was used immediately in the next step without purification. ^{1}H NMR ($CDCl_3$): $\delta = 9.74$ (dd, $J = 2.7$, 1.2 Hz, 1H, C*H*O), 4.96 (br d, $J = 9.6$ Hz, 1H, N*H*), 4.66–4.50 (m 1H, P–C*H*), 4.23–4.10 (m, 4H, 2 × P–O–CH_2), 2.90 (dddAB, $J_{AB} = 16.8$ Hz, $J = 12.0$, 4.8, 1.2 Hz, 1H, *H*CH), 2.70 (dddAB, $J_{AB} = 16.8$ Hz, $J = 9.3$, 8.4, 2.7 Hz, 1H, HC*H*), 1.44 (s, 9H, (CH_3)$_3$C), 1.34 (t, $J = 6.9$ Hz, 3H, P–O–CH_2CH_3), 1.33 (t, $J = 6.9$ Hz, 3H, P–O–CH_2CH_3). ^{31}P NMR ($CDCl_3$): $\delta = 24.30$.

The crude aldehyde (*R*)-**11** (0.116 g, 0.375 mmol) was dissolved in ethanol (3 mL), cooled to 0 °C and $NaBH_4$ (0.028 g, 0.750 mmol) was added. After 30 min, the reaction mixture was concentrated, the residue was dissolved in chloroform (10 mL) and $MgSO_4$ (0.5 g) was added. The solids were removed, and the residue was evaporated and chromatographed on a silica gel column with chloroform–methanol (100:1, v/v). A less polar fraction (0.012 g, 10%) contained an impure (2*R*,3*R*)-**13**. In more polar fractions *N*-Boc-aminoalcohol (*R*)-**12** (0.100 g, 85%) was collected as a colourless oil.

4.2.1.1. *tert*-Butyl (*R*)-1-(diethoxyphosphono)-3-hydroxypropylcarbamate (*R*)-12. $[\alpha]_D^{20} = -15.3$ (*c* 1.0, $CHCl_3$). IR (film): $\nu = 3364, 2980, 2932, 1706, 1530, 1367, 1231, 1169, 1050, 1028\ cm^{-1}$. ^{1}H NMR ($CDCl_3$): $\delta = 4.95$ (br dd, $J = 10.2$, 4.2 Hz, 1H, N*H*), 4.25–4.15 (m, 4H, 2 × P–O–CH_2), 3.80–3.60 (br m, 2H, CH_2OH), 3.34 (dd, $J = 8.7$, 5.2 Hz, 1H, O*H*), 2.20–2.00 (m, 1H, P–C*H*), 1.80–1.60 (m, 2H, CH_2CH_2OH), 1.45 (s, 9H, (CH_3)$_3$C), 1.34 (t, $J = 7.1$ Hz, 6H, 2 × P–O–CH_2CH_3). ^{13}C NMR ($CDCl_3$): $\delta = 156.59$ (d, $J = 9.4$ Hz), 80.87, 63.14 (d, $J = 6.9$ Hz), 62.81 (d, $J = 6.6$ Hz), 58.16 (d, $J = 12.6$ Hz), 44.18 (d, $J = 157.5$ Hz), 33.46, 28.49, 16.72 (d, $J = 5.2$ Hz), 16.65 (d, $J = 5.4$ Hz). ^{31}P NMR ($CDCl_3$): $\delta = 26.74$. Anal. Calcd for $C_{12}H_{26}NO_6P$: C, 46.30; H, 8.42; N, 4.50. Found: C, 46.05; H, 8.68; N, 4.40.

4.2.1.2. *tert*-Butyl (2*R*,3*R*)-2-ethoxy-2-oxo-1,2-oxaphospholan-3-ylcarbamate (2*R*,3*R*)-13. Further purification of the less polar fraction on a silica gel column with chloroform–methanol (200:1, v/v) gave (2*R*,3*R*)-**13** (0.010 g, 9%) as a colourless oil. $[\alpha]_D^{20} = +32.6$ (*c* 0.7, $CHCl_3$).

IR (film): $\nu = 3268$, 2980, 2933, 1741, 1708, 1530, 1455, 1367, 1256, 1167, 1044, 1006, 969, 830 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 5.15$ (br s, 1H, N*H*), 4.29 (dddd, $J = 13.8$, 9.6, 6.8, 4.5 Hz, 1H), 4.20 (dq, $J = 8.7$, 7.2 Hz, 2H), 4.07 (dddd, $J = 9.6$, 8.4, 7.5, 5.7 Hz, 1H), 3.98 (br q, $J = 6.9$ Hz, 1H), 2.58 (ddddd, $J = 24.9$, 13.2, 7.5, 5.7, 4.5 Hz, 1H), 2.22 (ddq, $J = 13.2$, 8.4, 6.9 Hz, 1H), 1.45 (s, 9H), 1.37 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR ($CDCl_3$): $\delta = 155.65$ (d, $J = 11.8$ Hz), 80.55, 65.11 (d, $J = 7.8$ Hz), 63.85 (d, $J = 6.6$ Hz), 42.81 (d, $J = 136.9$ Hz), 32.69 (d, $J = 10.9$ Hz), 28.53, 16.69 (d, $J = 5.7$ Hz). ^{13}P NMR ($CDCl_3$): $\delta = 41.01$. Anal. Calcd for $C_{10}H_{20}NO_5P$: C, 45.28; H, 7.60; N, 5.28. Found: C, 45.40; H, 7.85; N, 5.04.

4.2.2. *tert*-Butyl (*S*)-1-(diethoxyphosphono)-3-hydroxypropylcarbamate (*S*)-12. Following the procedure described in Section 4.2.1, from *N*-Boc-aminodiol (1*S*,3*S*)-**8** (0.150 g, 0.440 mmol) *N*-Boc-aminoalcohol (*S*)-**12** (0.109 g, 80%) was obtained. $[\alpha]_D^{20} = +15.2$ (*c* 1.1, $CHCl_3$). Anal. Calcd for $C_{12}H_{26}NO_6P$: C, 46.30; H, 8.42; N, 4.50. Found: C, 46.29; H, 8.21; N, 4.33.

4.3. (*R*)- and (*S*)-1-Amino-3-hydroxypropylphosphonic acids (*R*)- and (*S*)-6

4.3.1. (*R*)-1-Amino-3-hydroxypropylphosphonic acid (*R*)-6. A solution of *N*-Boc-aminoalcohol (*R*)-**12** (0.060 g, 0.19 mmol) in 6 M HCl was refluxed for 6 h. The solvent was removed under reduced pressure, and the residue was dissolved in ethanol (1 mL) and neutralised with propylene oxide. The solvent was withdrawn with a pipette. The solid was washed with anhydrous ethanol and dried to afford (*R*)-**6** (0.029 g, 95%) as a white amorphous solid. Mp: 215–216 °C (lit.[9] mp 214–217 °C). $[\alpha]_D^{20} = -6.6$ (*c* 1.1, H_2O) {lit.[9] $[\alpha]_D^{25} = -6.2$ (*c* 1, H_2O)}. ^1H NMR (D_2O): $\delta = 3.82$ (t, $J = 6.6$ Hz, 2H, CH_2OH), 3.40 (ddd, $J = 13.8$, 8.7, 4.2 Hz, 1H, P–C*H*), 2.24–2.07 (m, 1H, P–CH–*H*CH), 2.05–1.85 (m, 1H, P–CH–HC*H*). ^{31}P NMR (D_2O): $\delta = 14.01$. Anal. Calcd for $C_3H_{10}NO_4P$: C, 23.23; H, 6.50; N, 9.03. Found: C, 22.96; H, 6.31; N, 8.73.

4.3.2. (*S*)-1-Amino-3-hydroxypropylphosphonic acid (*S*)-6. Following the procedure described in Section 4.3.1, from *N*-Boc-aminoalcohol (*S*)-**6** (0.060 g, 0.19 mmol) the acid (*S*)-**12** (0.028 g, 94%) was obtained. Mp: 215–215.5 °C (lit.[11] mp 214 °C). $[\alpha]_D^{20} = +6.9$ (*c* 1.8, H_2O) {lit.[11] $[\alpha]_D = +7.3$ (*c* 1, H_2O)}. Anal. Calcd for $C_3H_{10}NO_4P$: C, 23.23; H, 6.50; N, 9.03. Found: C, 22.93; H, 6.45; N, 8.91.

4.4. Estimation of ee's of (*R*)-8 and (*S*)-8

A solution of (*R*)-**8** (0.014 g, 0.040 mmol) in $CDCl_3$ (0.7 mL) containing quinine (0.075 g, 0.23 mmol) was analysed by ^{31}P NMR spectroscopy. ^{31}P NMR ($CDCl_3$): $\delta = 26.79$.

In a similar manner a solution of (*S*)-**8** and quinine in $CDCl_3$ was analysed. ^{31}P NMR ($CDCl_3$): $\delta = 26.59$.

Both solutions of (*R*)-**8** and (*S*)-**8** containing quinine used above were combined and the ^{31}P NMR spectrum showed two signals at δ $^{31}P = 26.79$ [(*R*)-**8**], 26.59 [(*S*)-**8**].

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