Structure-activity relationships of the phosphonate antibiotic dehydrophos

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General aspects

Materials and reagents were of the highest commercially available grade and used without further purification. Reactions were monitored by thin layer chromatography using Merck silica gel 60 F_{254} glass plates. Compounds were visualised by UV, ninhydrin or KMnO₄. Flash chromatography was performed using Silicycle SilicaFlash® P60, particle size 40 - 63 μm. NMR spectra were recorded on a Varian Unity 500, Varian Unity Inova 500 or on a Varian Unit 400 spectrometer. Phosphorus shifts are reported relative to an external standard of 85% phosphoric acid (0.00 ppm).

Data are represented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), integration, assignment. Mass spectrometry was performed by the University of Illinois Mass Spectrometry Center. Reversed phase HPLC analysis was performed on an Agilent 1200 series quad pump system equipped with a diode array detector and a G1956B mass spectrometer with a multimode-electrospray/atmospheric pressure chemical ionization (MM-ES+APCI) source. The flow rate was 0.5 ml/min. The following columns were used: column A: Synergi 4 μm Fusion-RP 80A column (150 x 4.6 mm, 4 μm, Phenomenex Torrance, CA) and column B: Eclipse XDB-C18 5 μ column (4.6 x 150 mm, 5 μm, Agilent). Reversed phase HPLC purification was carried out on a Agilent 1200 series system equipped with a UV absorbance detector (220 nm or 210 nm). The flow rate was 4 ml/min. The following two columns were used: column C: Synergi 4 μm Fusion-RP 80A column (250 x 10 mm, 4 μm, Phenomenex Torrance, CA) and column D: Eclipse XDB-C18 5 μ column (9.4 x 250 mm, 5 μm, Agilent).
Synthesis of dehydrophos (1) and its enantiomer (ent-1)

The synthesis of 1 (13 mg) and ent-1 (38 mg, using H-D-Leu-OMe) was performed according to Whitteck et al. Analytical data are in accordance with the reported data. $^1$H NMR spectra are shown below (top 1, bottom ent-1):
Synthesis of desmethyl dehydrophos 2

Compound 8 was synthesised from Cbz-Ser-OH according to Luo et al.2

Synthesis of compound 9

Pb(OAc)$_4$ (6.88 g, 15.6 mmol) was suspended in DMF (18 ml) and cooled with an ice bath. Then a solution of Cbz-Ser(OTBDMS)-OH (8, 4.57 g, 12.9 mmol) in dry DMF (18 ml) was added. After 30 min the cooling bath was removed and the brown suspension turned into a yellow solution. After 3.5 h stirring at 25 °C the reaction was quenched by the addition of saturated aqueous NaHCO$_3$ (150 ml) and immediately extracted with EtOAc (4 times 110 ml). The combined organic fractions were washed with sat. aqueous NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure to obtain 9 as a colourless oil (4.75 g, 12.9 mmol, quantitative yield). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$/ppm = 7.40-7.30 (m, 5H; arom), 6.24 (dt, $J =$ 9.9 Hz, 2.7 Hz, 1H; CH), 5.89 (d, $J = 8.5$ Hz, 1H; NH), 5.20-5.08 (m, 2H; CH$_2$-Bn), 3.87 (bd, $J =$ 10.5 Hz, 1H; CH$_2$), 3.78 (dd, $J = 11.6$ Hz, 2.8 Hz, 1H; CH$_2$), 2.95 (s, 3H; Ac), 0.89 (s, 9H; C(CH$_3$)$_3$), 0.06 (s, 3H; Si(CH$_3$)$_3$), 0.05 (s, 3H; Si(CH$_3$)). MS(ESI): m/z= 390.3 [M+Na]$^+$, 757.2 [2M+Na]$^+$ calculated for C$_{18}$H$_{20}$NO$_5$Si.
Synthesis of compound 10′

N,O actetal 9 (4.75, 12.9 mmol) was dissolved in 36 ml CH₂Cl₂. P(OMe)₃ (2.3 ml, 19.5 mmol) was added and the solution was cooled to -78 °C. After the addition of a 1 M solution of TiCl₄ in CH₂Cl₂ (14.3 ml, 14.3 mmol) the yellow solution was stirred for 2 h at -87 °C and then allowed to warm to 25 °C over night. The solution was poured into a suspension of Na₂CO₃ (2.4 g, 22.6 mmol) in CH₂Cl₂ (62 ml) and H₂O (4.0 ml, 22.6 mmol) and the mixture was stirred at 25 °C for 30 min and then filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc 1:1) to yield racemic 10 as a white solid (3.02 g, 7.24 mmol, 56% yield).

¹H NMR (500 MHz, CDCl₃): δ/ppm = 7.38-7.29 (m, 5H; arom), 5.27 (d, J = 9.7 Hz, 1H; NH), 5.13 (d, J = 13 Hz, 1H, Bn CH₂), 5.10 (d, J = 13 Hz, 1H; Bn CH₂), 4.21 (ddt, J = 17.7 Hz, 9.9 Hz, 3.7 Hz, 1H; Hα), 3.96 (m, 1H; Hβ), 3.86 (ddd, J = 24.4 Hz, 10.5 Hz, 3.8 Hz, 1H; Hβ), 3.76 (d, J = 11 Hz, 3H, OCH₃), 3.73 (d, J = 10.7 Hz, 3H, OCH₃), 0.88 (s, 9H; C(CH₃)₃), 0.05 (s, 3H; SiCH₃), 0.05 (s, 3H; SiCH₃). ¹³C NMR (126 MHz, CDCl₃): δ/ppm = 155.7, 136.1, 128.5, 128.2, 128.1, 67.2, 61.8, 53.0 (d, J = 6.5 Hz), 53.9 (d, J = 6.3 Hz), 49.5 (d, J = 154 Hz), 25.7, 18.2, -5.5, -5.6. ³¹P-NMR (202 MHz, CDCl₃): δ/ppm = 26.7. MS(ESI): m/z= 418.3 [M+H]⁺ calculated for C₁₈H₃₂NO₆PSi.
Synthesis of compound 11

Compound 10 (358 mg, 858 µmol) was dissolved in MeOH (20 ml). Pd/C (60 mg, 5% Pd) was added and the suspension was vigorously stirred under a hydrogen atmosphere for 3 h at 25 °C. The mixture was filtrated over celite and rinsed with MeOH. The solvent was removed under reduced pressure to yield 11 as a clear oil (185 mg, 653 µmol, 76% yield). $^1$H NMR (400 MHz, CDCl$_3$): δ/ppm = 3.86 (ddd, $J$ = 13.5 Hz, 10.3 Hz, 3.6 Hz,
1H), 3.75 (d, J = 10.5 Hz, 6H; OMe), 3.71 (m, 1H), 3.19 (m, 1H), 0.86 (s, 9H; C(CH₃)₃), 0.04 (s, 6H; Si(CH₃)). ¹³C NMR (126 MHz, CDCl₃): δ/ppm = 62.3, 54.0 (d, J = 6.9 Hz), 53.7 (d, J = 7.0 Hz), 51.3 (d, J = 151 Hz). ³¹P-NMR (162 MHz, CDCl₃): δ/ppm = 29.9. ¹³C NMR (100 MHz, CDCl₃): δ/ppm = 63.5, 52.9 (d, J = 7.0 Hz), 52.8 (d, J = 6.9), 51.1 (d, J = 152 Hz), 25.8, 18.3, -5.4. TLC: R_f = 0.5 (EtOAc/hexane 3:1). MS(ESI): m/z= 284.3 [M+H]^+, calculated for C₁₀H₂₆NO₄PSi.

NMR Spectra:

Synthesis of compound 12
Amine 11 (185 mg, 653 µmol) and Cbz-Leu-OH (260 mg, 0.98 mmol) were dissolved in CH₂Cl₂ (1 ml). EDC (188 mg, 984 µmol) was added and the solution was stirred for 5 h at 25 °C. The solution was diluted with CH₂Cl₂ and washed with aqueous 5% citric acid and saturated aqueous NaHCO₃. The organic layer was dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (EtOAc:hexanes 3:1) to yield the protected dipeptide 12 as a clear oil (275 mg, 518 µmol, 79% yield). NMR spectra show a mixture of the two diastereoisomers. ¹H NMR (500 MHz, CDCl₃): δ/ppm = 7.38-7.28 (m, 5H; arom), 6.62 and 6.51 (d, J = 9.2 Hz/9.4 Hz, 1H; NH), 5.28 and 5.19 (d, J = 8.2 Hz/8.0 Hz, 1H; NH), 5.11 (d, J = 12.0 Hz, 1H; CH₂-Bn), 5.07 (d, J = 12.1 Hz, 1H; CH₂-Bn), 4.56-4.45 (m, 1H), 4.29-4.20 (m, 1H), 4.02-3.94 (m, 1H), 3.87-3.76 (m, 1H), 3.72 (d, J = 10.8 Hz, 6H; OMe), 1.71-1.62 (m, 2H), 1.55-1.47 (m, 1H), 0.93 (m, 6H; Hδ Leu), 0.88 (s, 9H; C(CH₃)₃), 0.07 and 0.06 (s, 6H; Si(CH₃)). ³¹P-NMR (202 MHz, CDCl₃): δ/ppm = 26.4 and 26.3. ¹³C NMR (126 MHz, CDCl₃): δ/ppm = 171.7 (d, J = 4.7 Hz) and 171.6 (d, J = 4.8 Hz), 156.0 and 155.9, 136.1, 128.5, 128.0, 128.0, 67.0, 61.6 and 61.6, 53.6, 52.9 (d, J = 6.3 Hz), 52.9 (d, J = 6.2 Hz), 47.3 (d, J = 153 Hz) and 47.2 (d, J = 154 Hz), 41.8 and 41.7, 29.7, 25.7, 24.7 and 24.6, 22.9 and 22.8, 22.0 and 21.9, 18.2, -5.5 and -5.6. TLC: Rf = 0.5 (EtOAc/hexane 3:1). MS(ESI): m/z = 531.3 [M+H]⁺ calculated for C₂₄H₄₃N₂O₇PSi.

NMR spectra
Synthesis of compound 13

Compound 12 (93 mg, 175 µmol) was dissolved in MeOH (6 ml). Pd/C (20 mg, 5% Pd) was added and the suspension was vigorously stirred under a hydrogen atmosphere for 4 h at 25 °C. The mixture was filtrated over celite and rinsed with MeOH. The solvent was removed under reduced pressure to yield 13 as a clear oil (62 mg, 156 µmol, 89% yield). NMR spectra show a mixture of the two diastereoisomers. $^1$H NMR (500 MHz, CD$_3$OD): δ/ppm = 4.54-4.74 (m, 1H), 3.95-3.88 (m, 2H), 3.78 (d, J = 10.8 Hz, 3H; OMe), 3.77 (d, J = 10.8 Hz, 3H; OMe), 3.44-3.38 (m, 1H), 1.75 (m, 1H) 1.38 (m, 1H), 0.93-0.97 (m, 6H; Hδ Leu), 0.92 and 0.91 (s, 9H; C(CH$_3$)$_3$), 0.11 and 0.10 (s, 6H; Si(CH$_3$)). $^{13}$C NMR (126 MHz, CD$_3$OD): signal-to-noise insufficient to observe the carbonyl resonance δ/ppm = 62.8, 54.4, 53.9 (d), 52.8 (d), ~, 45.6, 26.3, 25.7, 23.6 and 23.5, 22.5 and 22.4, 19.2 and 19.2, -5.3. $^{31}$P-NMR (202 MHz, CD$_3$OD): δ/ppm = 26.8 and 26.6. MS(ESI): m/z = 397.4 [M+H]$^+$ calculated for C$_{16}$H$_{37}$N$_2$O$_5$PSi.

NMR spectra:
Synthesis of compound 14

Amine 13 (62 mg, 156 µmol) and Cbz-Gly-OH (50 mg, 239 µmol) were dissolved in CH₂Cl₂ (0.5 ml). EDC (45 mg, 235 µmol) was added and the solution was stirred over night at 25 °C. The solution was diluted with CH₂Cl₂ and washed with aqueous 5% citric acid, saturated aqueous NaHCO₃ and brine. The organic layer was dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (4% MeOH in CH₂Cl₂) to yield the protected tripeptide 14 as clear oil (80 mg, 136 µmol, 87% yield). NMR spectra show a mixture of the two diastereoisomers. ¹H NMR (500 MHz, CDCl₃): δ/ppm = 7.38-7.27 (m, 5H; arom), 6.85 (m, 1H; NH), 6.74 (m, 1H; NH), 5.74 and 5.64 (m, 1H; NH), 5.11 (s, 2H; CH₂-Bn), 4.58-
4-44 (m, 2H), 3.97-3.67 (m, 10H), 1.69-1.47 (m, 3H), 0.95-0.83 (m, 6H, Hδ Leu), 0.88 and 0.87 (s, 9H; C(CH₃)₃), 0.06 and 0.05 (s, 6H; Si(CH₃)). ³¹P-NMR (202 MHz, CDCl₃): δ/ppm = 26.4 and 26.0. ¹³C NMR (126 MHz, CDCl₃): δ/ppm = 171.6 (d, J = 4.7 Hz) and 171.4 (d, J = 4.9 Hz), 169.1 and 168.7, 156.0, 136.1, 128.5, 128.2, 128.1, 67.2 and 67.1, 61.6 and 61.5, 53.4, 53.2 (d, J = 6.7 Hz), 53.0 (d, J = 6.6 Hz), 51.8 and 51.5, 47.5 (d, J = 154 Hz) and 47.3 (d, J = 154 Hz), 44.2, 41.4 and 41.2, 25.7, 24.7 and 24.6, 22.8 and 22.7, 22.2 and 22.1, 18.2, -5.6 and -5.6. TLC: Rf=0.4 (5% MeOH in CH₂Cl₂). MS(ESI): m/z=588.4 [M+H]⁺, 610.4 [M+Na]⁺, 626.3 [M+K]⁺, calculated for C₂₆H₄₆N₃O₈PSi.

NMR spectra:
Synthesis of compound 15

The TBDMS protected precursor 14 (251 mg, 427 μmol) was dissolved in a 1 M solution of TBAF in THF (900 μl, 900 μmol). The yellow solution was stirred at 25 °C for 30 min, diluted with CH₂Cl₂ (20 ml) and washed with a 0.1 M aqueous HCl solution. The organic fraction was dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was subjected to column chromatography (3% MeOH to 5% MeOH in CH₂Cl₂) to yield 15 (148 mg, 313 μmol, 73% yield) as a slightly yellow solid. NMR spectra show a mixture (~1:1) of diastereoisomers. ¹H NMR (500 MHz, CDCl₃):  δ/ppm = 7.75 (d,  J = 9.2 Hz, ½ H; NH), 7.53 (d,  J = 9.4 Hz, ½ H; NH), 7.36-7.26 (m, 5H; Ph), 7.18 (d,  J = 7.9 Hz, ½ H; NH), 7.13 (d,  J = 7.4 Hz, ½ H; NH), 6.11 (s, ½ H; NH), 5.91 (s, ½ H; NH), 5.09 (s, 2H; CH₂-Bn), 4.68-4.47 (m, 2H), 3.95-3.65 (m, 10H), 1.70-1.51 (m, 3H; Hγ/β Leu), 0.95-0.82 (m, 6H; Hδ Leu). ¹³C NMR (126 MHz, CDCl₃) signal-to-noise insufficient to observe the carbonyl resonances:  δ/ppm = 136.0, 128.5, 128.2, 128.1, 67.3/67.2, 61.4/60.9, 53.7, 53.0, 52.0, 47.6 (d,  J = 154 Hz), 44.3/44.3 41.0/41.7, 24.8/24.7, 22.9/22.7, 21.8. ³¹P-NMR (202 MHz, CDCl₃):  δ/ppm = 26.3 and 26.2. MS(ESI): m/z= 474.3 [M+H]+ calculated for C₂₀H₃₂N₃O₈P.
Synthesis of compound 16

The alcohol 15 (111 mg, 234 μmol) was dissolved in dry CH₂Cl₂ (1 ml). NEt₃ (65 μl, 466 μmol) and MsCl (37 μl, 478 μmol) were added at 0 °C and the solution was stirred at 25 °C for 45 min. The solvent was removed under reduced pressure and the mesylated alcohol was dissolved in 1,2-dichloroethane (3 ml) without purification. DBU (89 μl, 596 μmol) was added and the solution was heated to reflux for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in CH₂Cl₂ and washed with 0.1 M aqueous HCl (5 ml). The combined organic phases were dried over Na₂SO₄, filtered, concentrated in vacuo and purified by column chromatography (EtOAc) to yield 16 (52 mg, 114 μmol, 49% yield) as an oil. The analytical data are in agreement with the corresponding intermediate in the original synthesis of dehydrophos.¹

Synthesis of compound 2

The protected precursor 16 (52 mg, 114 μmol) was dissolved in toluene (1.8 ml) and cooled to 0 °C. A 1 M solution of BBr₃ in hexanes (342 μl, 342 μmol) was added drop wise whereupon a yellow precipitate was formed. The mixture was kept at 70 °C for 4 h. At 25 °C MeOH (2 ml) was added forming a yellow solution. All volatiles were removed under reduced pressure and the residue was taken up in H₂O (4 ml) and washed twice with EtOAc (3 ml). The aqueous phase was lyophilized. The resulting brown solid was purified by preparative reversed phase HPLC (column C: linear gradient of 0% MeOH to 5% CH₃CN in 20 min, the other solvent being 0.1% formic acid in H₂O) to yield 2 (tᵣ =

¹ Supplementary Material (ESI) for Chemical Communications
17 min) as a white solid (3 mg) after lyophilisation. $^1$H NMR (500 MHz, D$_2$O): δ/ppm = 5.88 (d, $J = 35.7$ Hz, 1H; =CH$_2$), 5.44 (d, $J = 16.1$ Hz, 1H; =CH$_2$), 4.26 (m, 1H; Hα Leu), 3.73 (d, $J = 16.7$ Hz, 1H; Hα Gly), 3.69 (d, $J = 16.6$ Hz, 1H; Hα Gly), 1.55-1.46 (m, 3H; Hγ/β Leu), 0.77 (d, $J = 4.9$ Hz, 3H; Hδ Leu), 0.73 (d, $J = 5.0$ Hz, 3H; Hδ Leu). $^{31}$P-NMR (202 MHz, D$_2$O): δ/ppm = 6.8. $^{13}$C NMR (126 MHz, D$_2$O): δ/ppm = 173.7, 167.4, 136.7 (d, $J = 129$ Hz), 114.1 (d, $J = 12$ Hz), 53.6, 40.4, 39.7, 24.5, 22.3, 20.7. LC-MS (column A): t$_R = 12.8$ min (isocratic 0.1% formic acid). HRMS (ESI): m/z = 294.1213 calculated for C$_{10}$H$_{21}$N$_3$O$_5$P$,^+$, found: 294.1212.

$^1$H NMR spectra:
Synthesis of dimethyldehydrophos 3

EDC (108 mg, 563 μmol) was added to a mixture of amine 13 (148 mg, 374 μmol), Boc-Gly-OH (98 mg, 560 μmol) and CH$_2$Cl$_2$ (2 ml). The solution was stirred at 25 °C overnight, diluted with CH$_2$Cl$_2$ and washed with aqueous 5% citric acid, saturated aqueous NaHCO$_3$ and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (3% MeOH in CH$_2$Cl$_2$) to yield 17 as an oil (160 mg, 289 μmol, 77% yield). $^1$H NMR (500 MHz, CDCl$_3$): δ/ppm = 6.53 (d, J = 7.0 Hz, 1 H; NH), 6.48 (d, J = 7.9 Hz, 1 H; NH), 5.15 and 5.09 (m, 1 H; NH), 4.54-4.44 (m, 2H), 4.02-3.95 (m, 1H), 3.88-3.73 (m, 9H), 1.71-1.52 (m, 3H), 1.45 (s, 9H; Boc), 0.96-0.91 (m, 6H; H OFFSET Leu), 0.90 and 0.90 (s, 9H), 0.08 (s, 6H). $^{31}$P-NMR (202 MHz, CDCl$_3$): δ/ppm = 26.4 and 26.3. MS(ESI): m/z= 554.4 [M+H]$^+$ calculated for C$_{23}$H$_{48}$N$_3$O$_8$PSi

NMR spectra:
Synthesis of compound 18

Compound 17 (160 mg, 289 μmol) was dissolved in a 1 M solution of TBAF in THF (0.6 ml, 600 μmol). The solution was stirred at 25 °C for 1 h. The solution was diluted with CH₂Cl₂ and washed with 0.1 M aqueous HCl. The organic fraction was dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The remaining oil was purified by column chromatography (4% MeOH in CH₂Cl₂) to yield 18 (61 mg, 140 μmol, 48% yield) as a mixture of diasteromers (~1:1). ¹H NMR (500 MHz, CDCl₃): δ/ppm = 7.07-6.98 (m, 1 H; NH), 6.73-6.61 (m, 1 H; NH), 5.28-5.23 (m, 1 H; NH), 4.55-4.46 (m, 2H), 4.02-3.91 (m, 1H), 3.89-3.77 (m, 9H), 1.76-1.56 (m, 3H), 1.47 and 1.46 (s, 9H; Boc), 0.97-0.92 (m, 6H; Hδ Leu). ³¹P-NMR (202 MHz, CDCl₃): δ/ppm = 26.5 and 26.4. MS(ESI): m/z = 440.4 [M+H]⁺ calculated for C₁₇H₃₄N₃O₈P.
The alcohol 18 (60 mg, 137 μmol) was dissolved in dry CH₂Cl₂. NEt₃ (38 μl, 272 μmol) and MsCl (21 μl, 271 μmol) were added at 0 °C. The solution was stirred at 25 °C for 40 min. Then the solution was diluted with CH₂Cl₂ and washed with water. The organic phase was dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was dissolved in 1,2-dichloroethane (0.5 ml) treated with DBU (51 μl, 342 μmol). After heating to reflux for 1 h, the solution was diluted with CH₂Cl₂ and washed with 0.2 M aqueous HCl. The organic phase was dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (EtOAc) to yield 19 (25 mg, 59 μmol, 43% yield) as an oil. 

1H NMR (400 MHz, CDCl₃): δ/ppm = 7.96 (d, J = 6.9 Hz, 1 H; NH), 6.69 (s, 1 H; NH), 6.64 (d, J = 41.8 Hz, 1H; =CH₂), 5.63 (d, J = 19.1 Hz, 1H; =CH₂), 5.26 (t, J = 5.2 Hz, 1 H; NH), 4.55-4.46 (m, 1H; Hα Leu), 3.90-3.76 (m, 2H; Hα Gly), 3.77 (d, J = 11.2 Hz, 3H; OMe), 3.76 (d, J = 11.2 Hz, 3H; OMe), 1.75-1.54 (m, 3H; Hγ/β Leu), 1.45 (s, 9H; Boc), 0.96-0.89 (m, 6H; Hδ Leu). 31P-NMR (162 MHz, CDCl₃): δ/ppm = 15.7. MS(ESI): m/z = 422.4 [M+H]+ calculated for C₁₇H₃₂N₃O₇P.

Synthesis of compound 19

NMR spectra:

![NMR spectra image]

S17
**Synthesis of compound 3**

Compound 19 (19 mg, 45 μmol) was dissolved in a 1.25 M solution of HCl in MeOH (200 μl, 250 μmol) and stirred under reflux for 30 min. The solvent was removed under reduced pressure and the resulting HCl salt (12 mg) was purified by preparative reversed phase HPLC (column C: linear gradient of 0% MeOH to 80% MeOH in 50 min, the other solvent being 0.1% formic acid in H₂O) to yield 3 as an oil (tᵣ = 15 min, 5 mg) after lyophilisation. ¹H NMR (500 MHz, D₂O): δ/ppm = 6.11 (d, J = 41.3 Hz, 1H; =CH₂), 5.96 (d, J = 16.1 Hz, 1H; =CH₂), 4.28 (dd, J = 8.7 Hz, 5.6 Hz, 1H; H₁α Leu), 3.71 (s, 2H; Hα Gly), 3.65 (d, J = 11.3 Hz, 3H; OCH₃), 3.64 (d, J = 11.3 Hz, 3H; OCH₃), 1.57-1.43 (m, 3H; Hβ/γ Leu), 0.79 (d, J = 5.7 Hz, 3H; Hδ Leu), 0.76 (d, J = 5.8 Hz, 3H; Hδ Leu). ³¹P-NMR (202 MHz, D₂O): δ/ppm = 17.0. ¹³C NMR (126 MHz, D₂O): δ/ppm = 174.2, 167.1, 129.4 (d, J = 209.3 Hz), 127.7 (d, J = 17.3 Hz), 54.0 (d, J = 5.3 Hz), 54.0 (d, J = 4.8 Hz), 52.9, 40.4, 40.0, 24.5, 22.1, 21.0. LC-MS (column A): tᵣ = 16.6 min (linear gradient with 0% MeOH to 30% MeOH in 20 minutes, the other solvent being 0.1% formic acid in D₂O). HRMS (ESI): m/z = 322.1526 calc for C₁₂H₂₅N₃O₅P⁺, found: 322.1525.
NMR spectra:
Synthesis of dehydrophos derivatives 4, 4b, 5, 5b, ent-5 and ent-5b

The synthesis of 4, 4b, 5, 5b, ent-5 and ent-5b was performed according to Lee et al. Analytical data for 4, 4b, 5 and 5b are presented there.\textsuperscript{5}

NMR spectra of ent-5:
NMR spectra of ent-5b:
Synthesis of serine derivate 6a and 6b (epimers at the carbon alpha to the methyl phosphonate)

\[
\text{CbzHN} \quad \begin{array}{c}
\text{O} \\
\text{N} \\
\text{O} \\
\text{OH} \\
\text{OMe} \\
\text{H}_2 \text{N} \end{array} \quad \begin{array}{c}
\text{N} \\
\text{O} \\
\text{P} \\
\text{OMe} \\
\text{OMe} \\
\end{array} \quad \begin{array}{c}
\text{H}_2 \text{N} \\
\text{N} \\
\text{O} \\
\text{POMe} \\
\text{OMe} \\
\end{array} \quad \begin{array}{c}
\text{H}_2 \text{N} \\
\text{N} \\
\text{O} \\
\text{P} \\
\text{OMe} \\
\end{array}
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Synthesis of compound 20

The Cbz protected compound 15 (100 mg, 211 μmol) was dissolved in MeOH (4 ml). Pd/C (20 mg, 5% Pd) and AcOH (24 μl, 420 μmol) were added and the suspension was vigorously stirred under a hydrogen atmosphere for 3 h at 25 °C. The mixture was filtered over celite and rinsed with MeOH. The solvent was removed under reduced pressure to yield the acetate salt of 20 as an oil (83 mg, 208 μmol, 98% yield). NMR spectra showed a mixture (~1:1) of diastereomers. \(^1\)H NMR (500 MHz, CD\(_3\)OD): δ/ppm = 4.54-4.44 (m, 2H), 3.87-3.75 (m, 8H), 3.65 (s, 2H), 1.93 (s, 3H, CH\(_3\)COOH), 1.75-1.57 (m, 3H; Hγ/β Leu), 0.99-0.93 (m, 6H; Hδ Leu). \(^13\)C NMR (126 MHz, CD\(_3\)OD) signal-to-noise insufficient to observe the carbonyl resonances: δ/ppm = 59.9, 53.1, 52.8, 52.2, 49.0, 41.0, 40.8, 24.7/24.7, 22.3/22.1, 21.6/21.6, 20.8/20.7. \(^31\)P-NMR (202 MHz, CD\(_3\)OD): δ/ppm = 26.8 and 26.3. MS(ESI): m/z= 340.3 [M+H]\(^+\) calculated for C\(_{12}\)H\(_{26}\)N\(_3\)O\(_6\)P.

NMR spectra:
Synthesis of compound 6

The dimethyl ester 20 (82 mg, 205 μmol) was dissolved in an aqueous solution of NaOH (10% NaOH, 1 ml, 250 μmol). The solution was stirred at 25 °C over night, diluted with H₂O (3 ml) and purified by preparative reversed phase HPLC (column C: isocratic 0.1% formic acid in H₂O). The two diastereomers of 6 (6a tᵣ = 14 min, 6b tᵣ = 18 min) were separated and after lyophilisation isolated as white solids (9 mg and 7 mg, respectively).

6a: ¹H NMR (500 MHz, D₂O): δ/ppm = 4.30 (dd, J = 8.4 Hz, 6.1 Hz, 1H; Hα Leu), 4.11 (ddd, J = 16.7 Hz, 9.1 Hz, 3.5 Hz, 1H; Hα Ser₂), 3.79 (ddd, J = 11.9 Hz, 6.1 Hz, 3.6 Hz, 1H; Hβ Ser₂), 3.75 (d, J = 16.4 Hz, 1H; Hα Gly), 3.71 (d, J = 16.2 Hz, 1H; Hα Gly), 3.56 (ddd, J = 12.4 Hz, 9.6 Hz, 3.9 Hz, 1H, Hβ Ser₂), 3.41 (d, J = 10.5 Hz, 3H; OCH₃), 1.57-1.48 (m, 3H; Hγ/β Leu), 0.80 (d, J = 6.0 Hz, 3H; Hδ Leu), 0.76 (d, J = 5.9 Hz, 3H; Hδ
Leu). $^{13}$C NMR (126 MHz, D$_2$O): $\delta$/ppm = 174.5, 167.2, 60.7 (d, $J = 8.3$ Hz), 53.0, 52.2 (d, $J = 5.8$ Hz), 49.5 (d, $J = 143$ Hz), 40.6, 40.2, 24.5, 22.3, 20.8. $^{31}$P-NMR (202 MHz, D$_2$O): $\delta$/ppm = 18.5. LC-MS (column A): $t_R = 8.8$ min (isocratic 0.1% formic acid in H$_2$O). HRMS (ESI): m/z = 326.1481 calculated for C$_{11}$H$_{26}$N$_3$O$_6$P$^+$, found: 326.1469. 6b: 

$^1$H NMR (500 MHz, D$_2$O): $\delta$/ppm = 4.28 (dd, $J = 9.0$ Hz, 5.1 Hz, 1H; H$_\alpha$ Leu), 4.11 (ddd, $J = 16.9$ Hz, 9.3 Hz, 3.5 Hz, 1H; H$_\alpha$ Ser$^\beta$), 3.75 (ddd, $J = 11.6$ Hz, 6.5 Hz, 3.6 Hz, 1H; H$_\beta$ Ser$^\beta$), 3.71 (d, $J = 16.7$ Hz, 1H; H$_\alpha$ Gly), 3.68 (d, $J = 16.7$ Hz, 1H; H$_\alpha$ Gly), 3.51 (ddd, $J = 12.4$ Hz, 9.6 Hz, 3.9 Hz, 1H, H$_\beta$ Ser$^\beta$), 3.37 (d, $J = 10.4$ Hz, 3H; OCH$_3$), 1.55-1.42 (m, 3H; H$_\gamma$/H$_\beta$ Leu), 0.77 (d, $J = 5.7$ Hz, 3H; H$_\delta$ Leu), 0.73 (d, $J = 5.6$ Hz, 3H; H$_\delta$ Leu). $^{13}$C NMR (126 MHz, D$_2$O): $\delta$/ppm = 174.4 (d, $J = 4.9$ Hz), 167.1, 60.7 (d, $J = 8.1$ Hz), 52.9, 52.3 (d, $J = 5.9$ Hz), 49.4 (d, $J = 143$ Hz), 40.5, 40.2, 24.4, 22.3, 20.7. $^{31}$P-NMR (202 MHz, D$_2$O): $\delta$/ppm = 18.7. RP-HPLC column B: $t_R = 10.2$ min (isocratic 0.1% formic acid in H$_2$O until 10 min, then linear gradient up to 10% CH$_3$CN in the next 5 minutes). HRMS (ESI): m/z = 326.1481 calculated for C$_{11}$H$_{26}$N$_3$O$_6$P$^+$, found: 326.1479.

NMR spectra of 6a:
NMR spectra of 6b:
Agar diffusion bioassays

Solid agar diffusion assays were used to assess antimicrobial activity. The bacterial cultures were grown overnight in LB media at 30 °C (B. subtilis) or 37 °C (E. coli). Cultures of B. subtilis were diluted with M9 media to an OD600 value close to 0.3. Cultures of E. coli were centrifuged. The cells were resuspended in M9 media to reach an OD600 value of 0.3. 400 µl of these mixtures were mixed with 5 ml of molten M9 minimal media containing 0.5% agar. This mixture was placed onto solidified M9 minimal media, containing 1.5% agar, on plates (9 cm diameter). Aqueous solutions of the investigated substances (5 - 10 µl, 10 mM) were absorbed on a filter disc. These were placed on the plate and the plates were incubated at 30 °C (B. subtilis) or 37 °C (E. coli) for 12 hours. Pictures were taken using a “Bucket of Light”.6

Additional agar diffusion assays for E. coli:

(-) negative control (water), (+) positive control (ampicillin), compounds 6a, 6b, 7 (methyl acetylphosphonate), 5, 5b, ent-5, ent-5b.
**Agar diffusion assays for B. subtilis:**

(-) negative control (water), (+) positive control (erythromycin), compounds 1, ent-1, 2, 3, 4, 4b, 5, 5b, ent-5, ent-5b, 6a, 6b, 7 (methyl acetylphosphonate).

**Liquid broth growth inhibition assays**

Liquid broth growth inhibition assays were performed in 48-well plates. An overnight culture of *E. coli* in LB media was diluted with M9 and LB media to an OD600 value of 0.1. The culture containing 10% LB and 90% M9 were incubated for 5 h with different amounts of the compounds 1-5. IC₅₀ values were defined as the concentration required to reduce the growth to 50% of the control. The growth was followed by measuring absorbance at 600 nm (OD600).
Fit for compound 1 (left, IC$_{50}$ = 180 μM) and ent-1 (right, IC$_{50}$ = 300 μM):

Fit for compound 5 (left, IC$_{50}$ = 20 μM) and 5b (right, IC$_{50}$ = 120 μM):

Fit for compound ent-5 (left, IC$_{50}$ = 30 μM) and ent-5b (right, IC$_{50}$ = 180 μM):
References