Structure-activity relationships of the phosphonate antibiotic dehydrophos

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Supporting information

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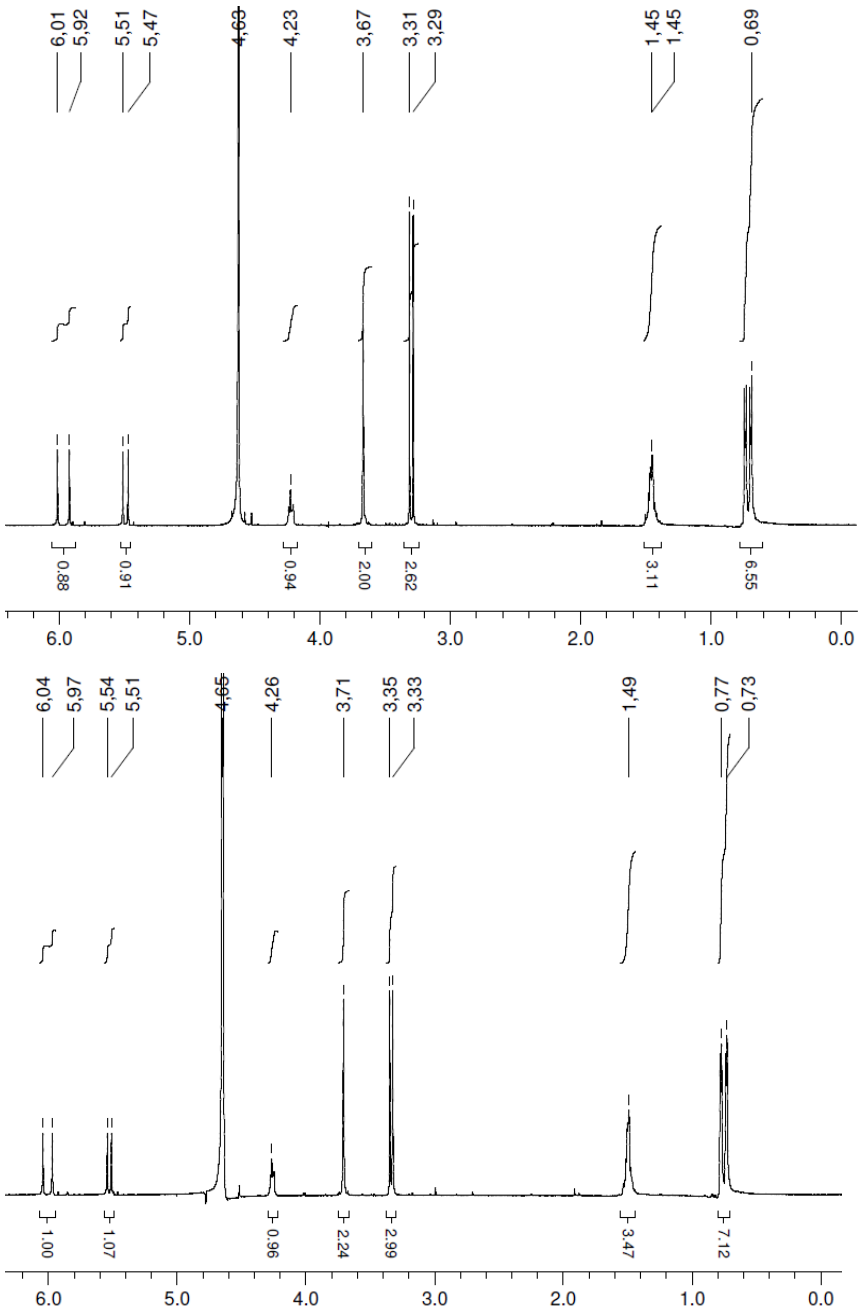
General aspects

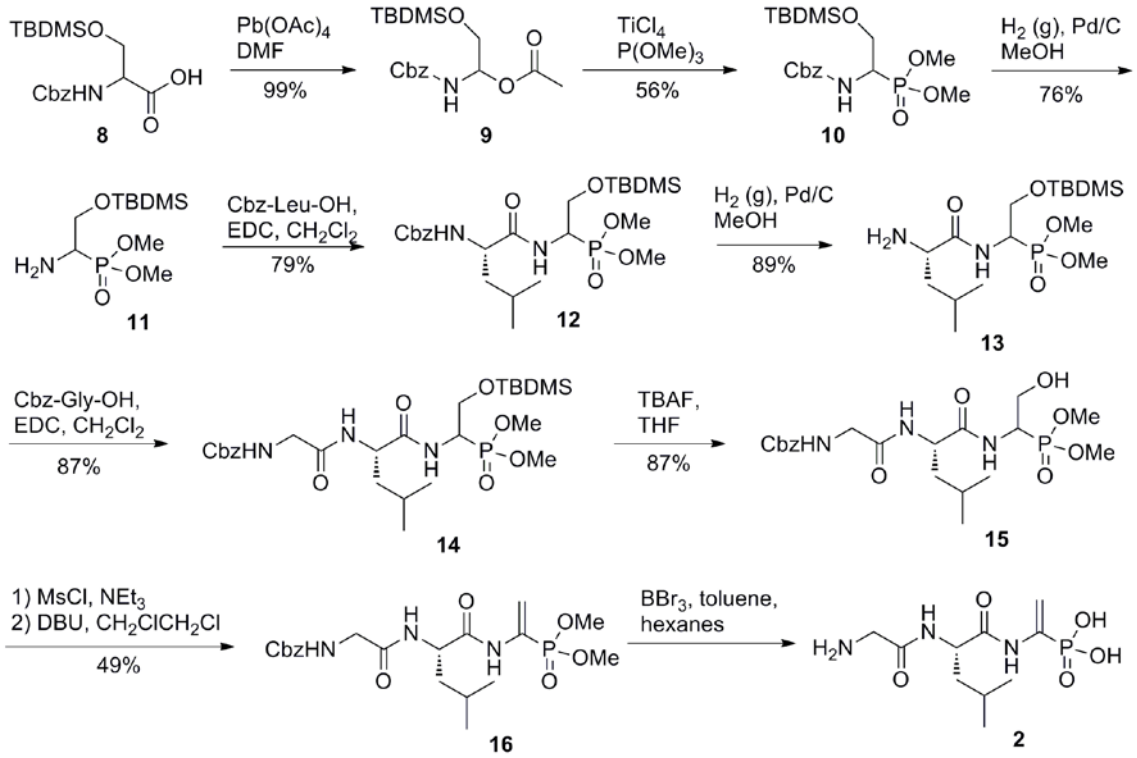
Materials and reagents were of the highest commercially available grade and used without further purification. Reactions were monitored by thin layer chromatography using Merck silica gel 60 F_{254} glass plates. Compounds were visualised by UV, ninhydrin or $KMnO_4$. Flash chromatography was performed 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 μ 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 μ 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.[1] 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

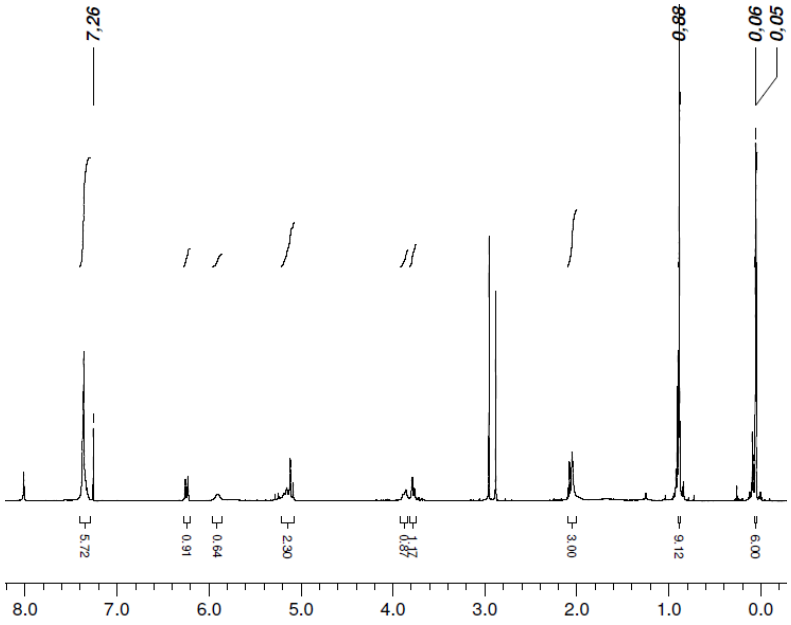


Compound **8** was synthesised from Cbz-Ser-OH according to Luo et al.[2]

*Synthesis of compound **9**[3]*

$Pb(OAc)_4$ (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_2SO_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, $CDCl_3$): δ/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; $Si(CH_3)$), 0.05 (s, 3H; $Si(CH_3)$). MS(ESI): m/z= 390.3 $[M+Na]^+$, 757.2 $[2M+Na]^+$ calculated for $C_{18}H_{29}NO_5Si$.

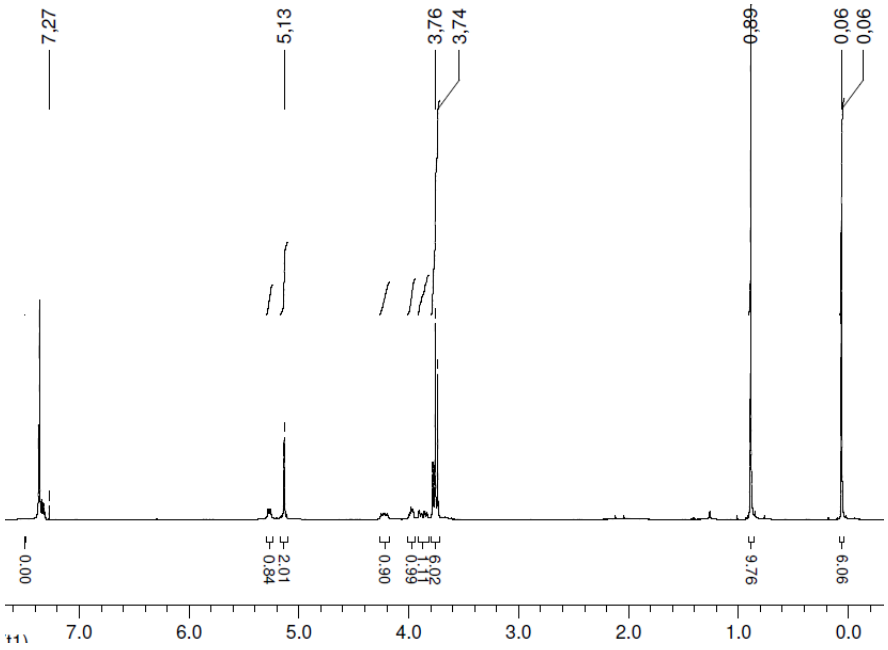
NMR spectra:

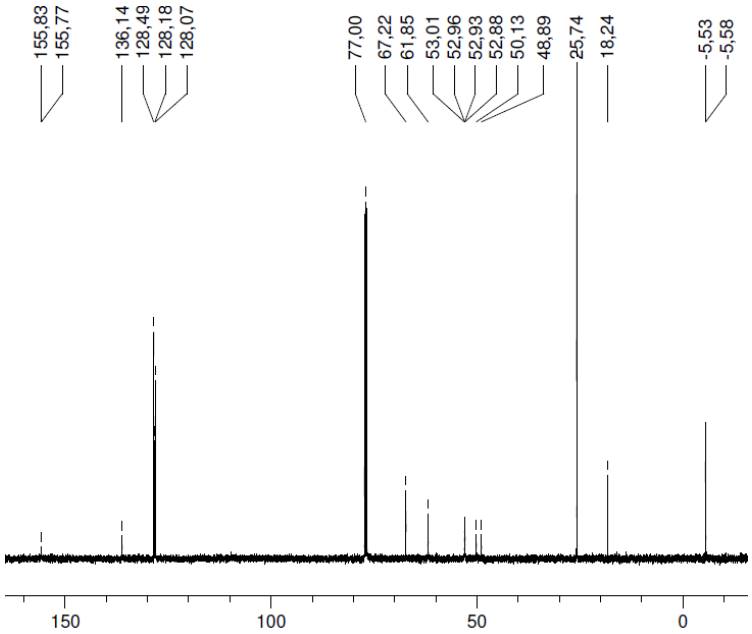


Synthesis of compound ***10***[4]

N,O actetal **9** (4.75, 12.9 mmol) was dissolved in 36 ml CH_2Cl_2. $P(OMe)_3$ (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 $TiCl_4$ in CH_2Cl_2 (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 Na_2CO_3 (2.4 g, 22.6 mmol) in CH_2Cl_2 (62 ml) and H_2O (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). ^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 7.38-7.29 (m, 5H; arom), 5.27 (d, *J* = 9.7 Hz, 1H; N*H*), 5.13 (d, *J* = 13 Hz, 1H, Bn CH_2), 5.10 (d, *J* = 13 Hz, 1H; Bn CH_2), 4.21 (ddt, *J* = 17.7 Hz, 9.9 Hz, 3.7 Hz, 1H; *H*α), 3.96 (m, 1H; *H*β), 3.86 (ddd, *J* = 24.4 Hz, 10.5 Hz, 3.8 Hz, 1H; *H*β), 3.76 (d, *J* = 11 Hz, 3H, OCH_3), 3.73 (d, *J* = 10.7 Hz, 3H, OCH_3), 0.88 (s, 9H; C(CH_3)$_3$), 0.05 (s, 3H; SiCH_3), 0.05 (s, 3H; SiCH_3). ^{13}C NMR (126 MHz, $CDCl_3$): 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. ^{31}P-NMR (202 MHz, $CDCl_3$): δ/ppm = 26.7. MS(ESI): m/z= 418.3 $[M+H]^+$ calculated for $C_{18}H_{32}NO_6PSi$.

NMR spectra:



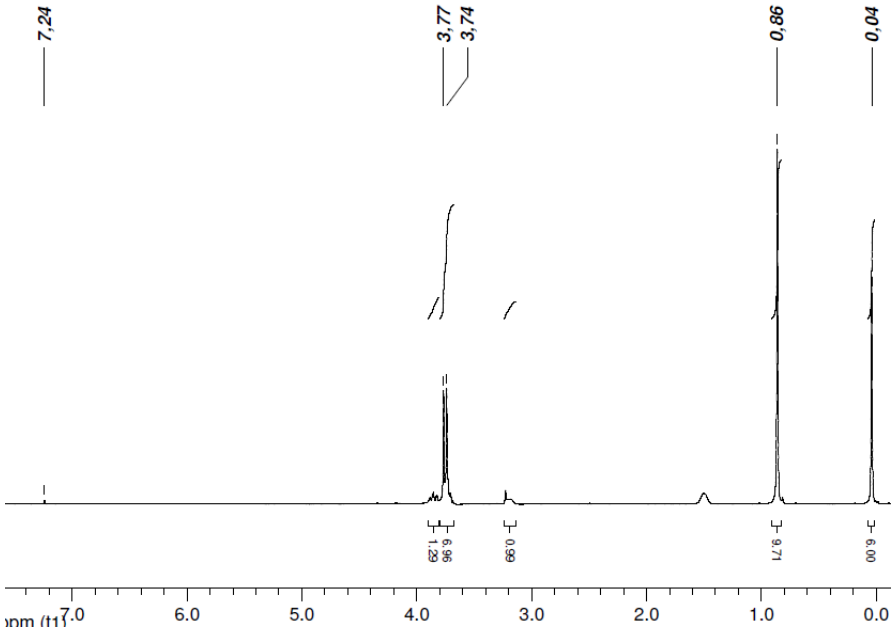


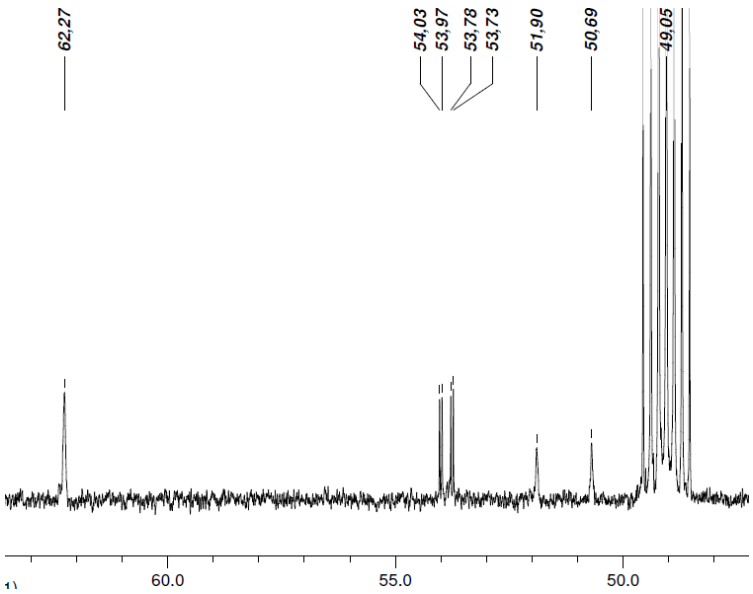
*Synthesis of compound **11***

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; $C(CH_3)_3$)), 0.04 (s, 6H; $Si(CH_3)$). ^{13}C NMR (126 MHz, $CDCl_3$): δ/ppm = 62.3, 54.0 (d, J = 6.9 Hz), 53.7 (d, J = 7.0 Hz), 51.3 (d, J = 151 Hz).^{31}P-NMR (162 MHz, $CDCl_3$): δ/ppm = 29.9. ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{10}H_{26}NO_4PSi$.

NMR Spectra:

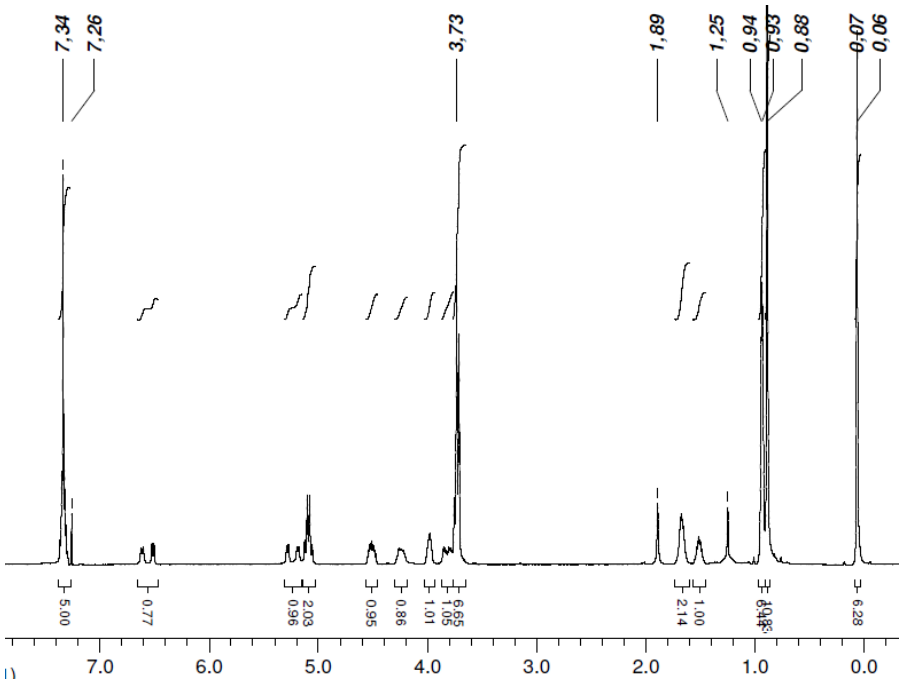


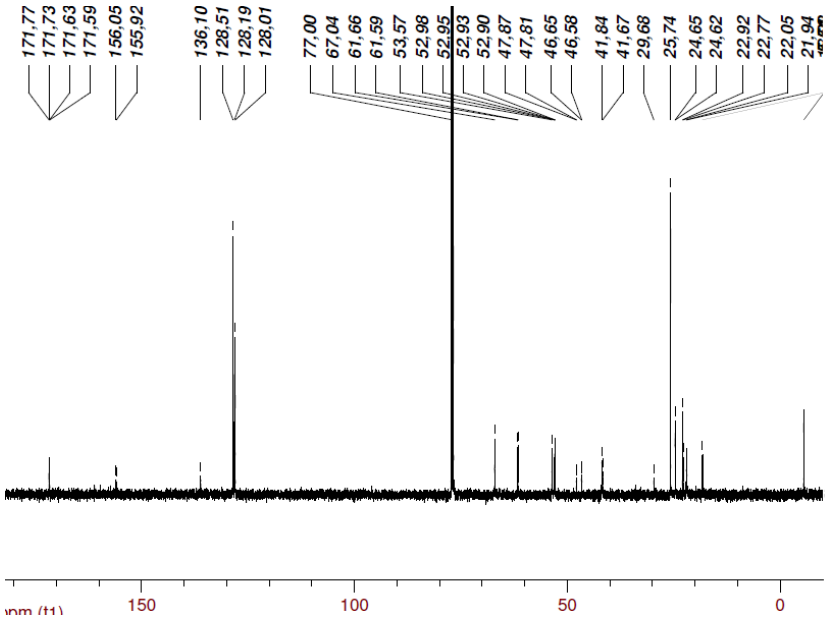


Synthesis of compound ***12***

Amine **11** (185 mg, 653 µmol) and Cbz-Leu-OH (260 mg, 0.98 mmol) were dissolved in CH_2Cl_2 (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 CH_2Cl_2 and washed with aqueous 5% citric acid and saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, 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. ^{1}H NMR (500 MHz, $CDCl_3$): 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; CH_2-Bn), 5.07 (d, *J* = 12.1 Hz, 1H; CH_2-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; $C(CH_3)_3$), 0.07 and 0.06 (s, 6H; $Si(CH_3)$). ^{31}P-NMR (202 MHz, $CDCl_3$): δ/ppm = 26.4 and 26.3. ^{13}C NMR (126 MHz, $CDCl_3$): 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, 128.2 128.0, 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: R_f=0.5 (EtOAc/hexane 3:1). MS(ESI): m/z= 531.3 $[M+H]^+$ calculated for $C_{24}H_{43}N_2O_7PSi$.

NMR spectra

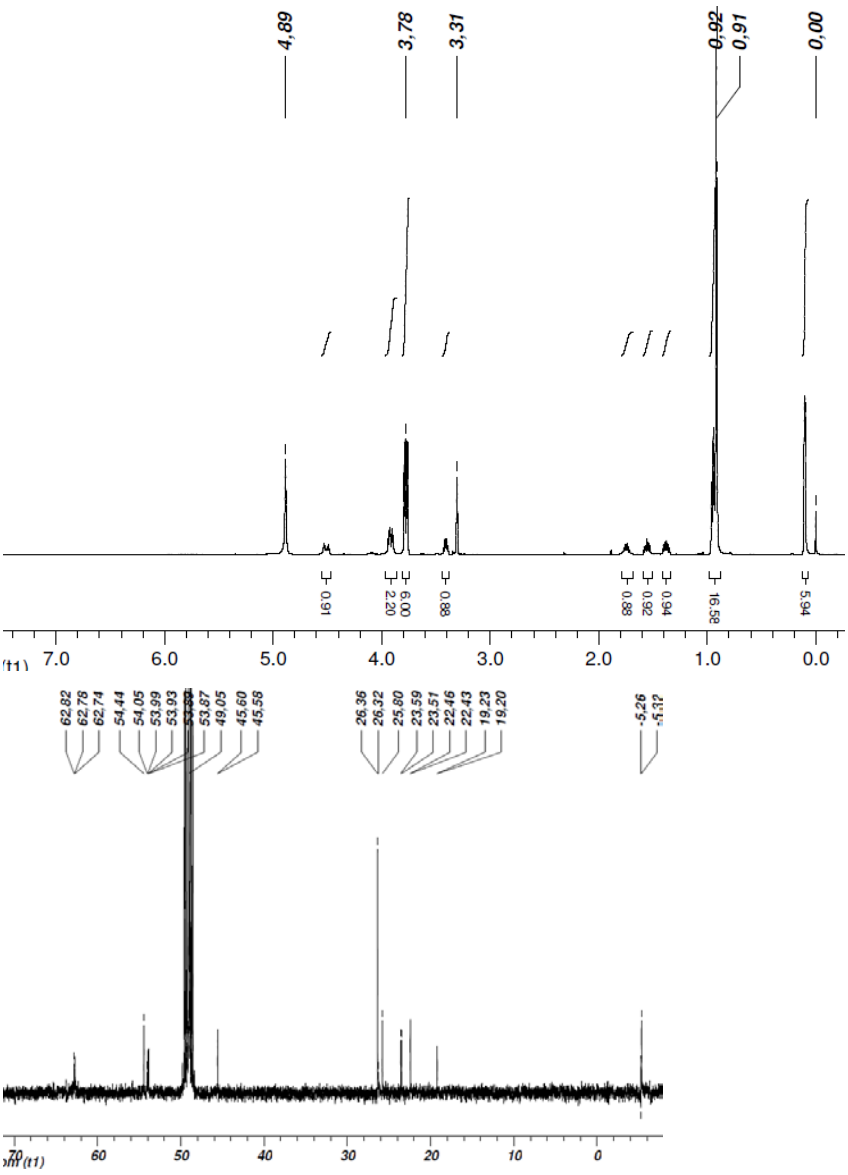




*Synthesis of compound **13***

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_3OD): 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) 1.55 (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_3OD): 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_3OD): δ/ppm = 26.8 and 26.6. MS(ESI): m/z= 397.4 $[M+H]^+$ calculated for $C_{16}H_{37}N_2O_5PSi$.

NMR spectra:

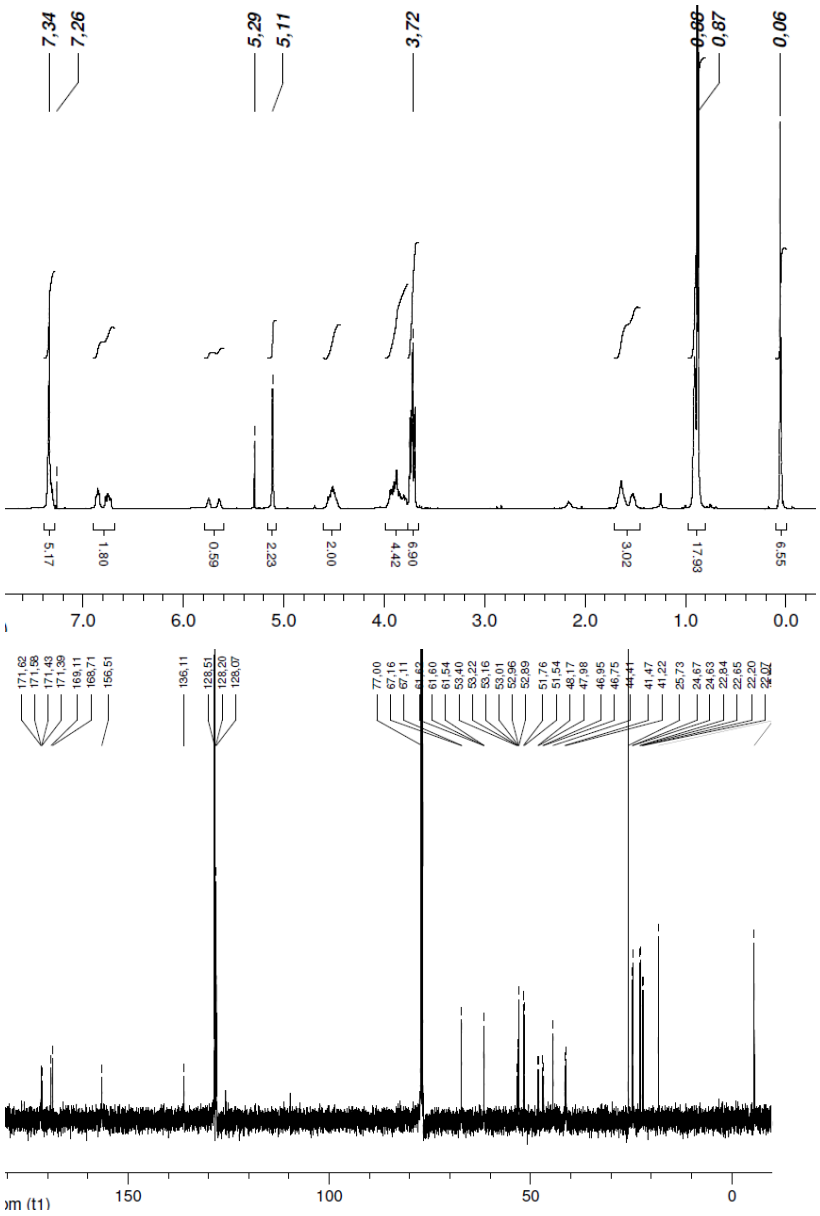


Synthesis of compound ***14***

Amine **13** (62 mg, 156 µmol) and Cbz-Gly-OH (50 mg, 239 µmol) were dissolved in CH_2Cl_2 (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 CH_2Cl_2 and washed with aqueous 5% citric acid, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (4% MeOH in CH_2Cl_2) to yield the protected tripeptide **14** as clear oil (80 mg, 136 µmol, 87% yield). NMR spectra show a mixture of the two diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$): 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; CH_2-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; $C(CH_3)_3$), 0.06 and 0.05 (s, 6H; $Si(CH_3)$). ^{31}P-NMR (202 MHz, $CDCl_3$): δ/ppm = 26.4 and 26.0. ^{13}C NMR (126 MHz, $CDCl_3$): 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: R_f=0.4 (5% MeOH in CH_2Cl_2). MS(ESI): m/z= 588.4 $[M+H]^+$, 610.4 $[M+Na]^+$, 626.3 $[M+K]^+$, calculated for $C_{26}H_{46}N_3O_8PSi$.

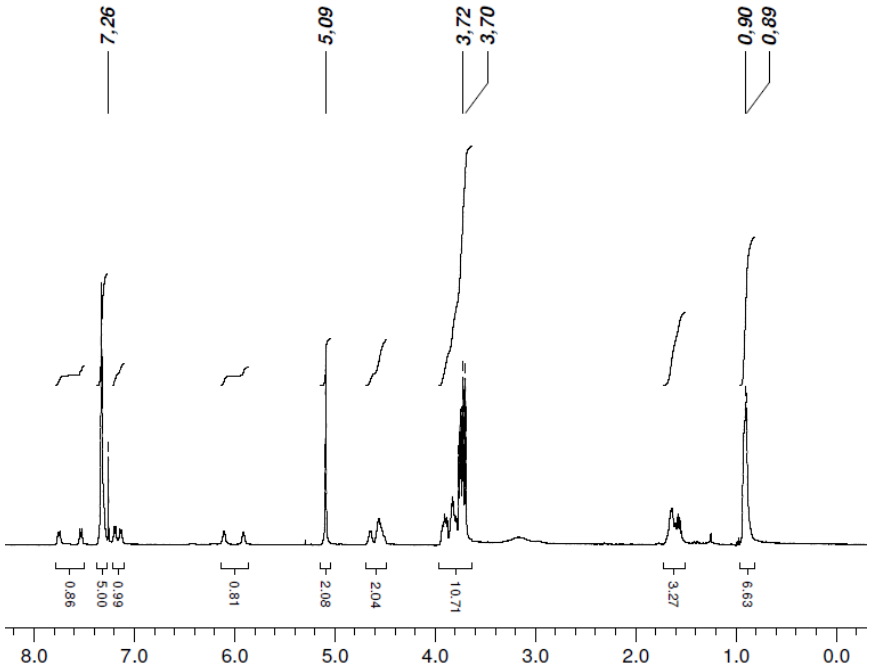
NMR spectra:

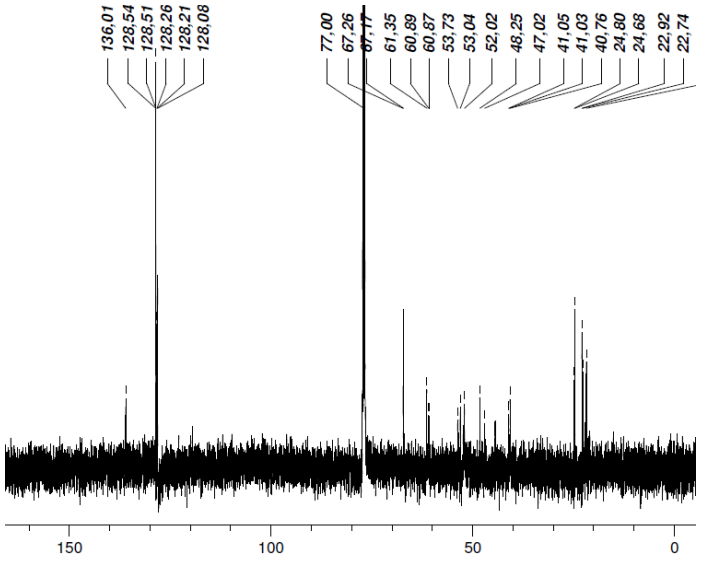


*Synthesis of compound **15***

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 CH_2Cl_2 (20 ml) and washed with a 0.1 M aqueous HCl solution. The organic fraction was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was subjected to column chromatography (3% MeOH to 5% MeOH in CH_2Cl_2) to yield **15** (148 mg, 313 µmol, 73% yield) as a slightly yellow solid. NMR spectra show a mixture (~1:1) of diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$): 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; CH_2-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). ^{13}C NMR (126 MHz, $CDCl_3$) 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. ^{31}P-NMR (202 MHz, $CDCl_3$): δ/ppm = 26.3 and 26.2. MS(ESI): m/z= 474.3 $[M+H]^+$ calculated for $C_{20}H_{32}N_3O_8P$.

NMR spectra:





*Synthesis of compound **16***

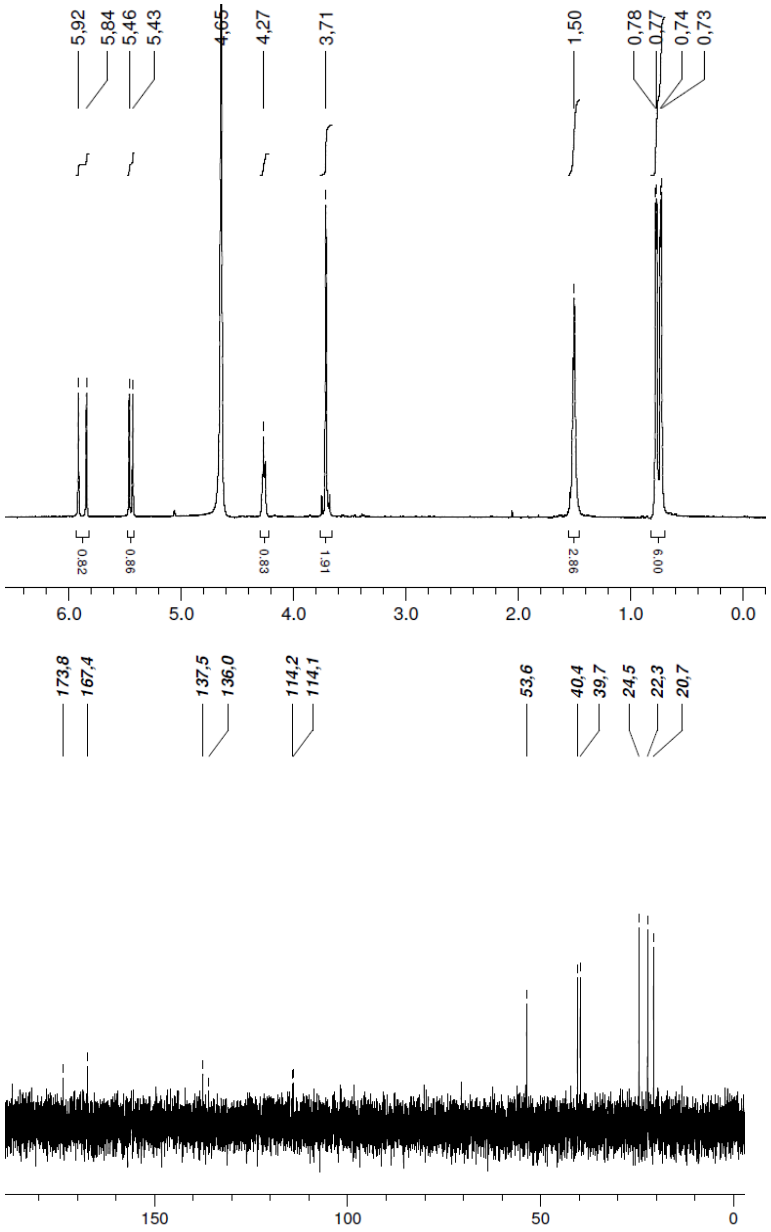
The alcohol **15** (111 mg, 234 µmol) was dissolved in dry CH_2Cl_2 (1 ml). NEt_3 (65 µl, 466 µmol) and MsCl (37 µl, 478 µmol) were added at 0 °C and the solution was stirred at 25 °C for 45 min. The solvent was removed under reduced pressure and the mesylated alcohol was dissolved in 1,2-dichloroethane (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 CH_2Cl_2 and washed with 0.1 M aqueous HCl (5 ml). The combined organic phases were dried over Na_2SO_4, 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***

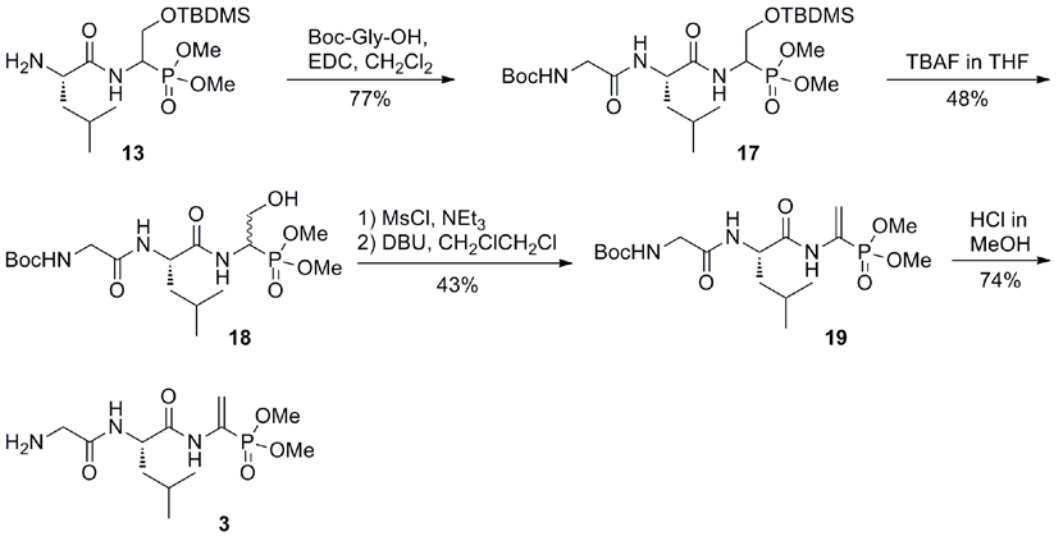
The protected precursor **16** (52 mg, 114 µmol) was dissolved in toluene (1.8 ml) and cooled to 0 °C. A 1 M solution of BBr_3 in hexanes (342 µl, 342 µmol) was added drop wise whereupon a yellow precipitate was formed. The mixture was kept at 70 °C for 4 h. At 25 °C MeOH (2 ml) was added forming a yellow solution. All volatiles were removed under reduced pressure and the residue was taken up in H_2O (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% CH_3CN in 20 min, the other solvent being 0.1% formic acid in H_2O) to yield **2** (t_R =

17 min) as a white solid (3 mg) after lyophilisation. ^{1}H NMR (500 MHz, D_2O): 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_2O): δ/ppm = 6.8. ^{13}C NMR (126 MHz, D_2O): 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 $C_{10}H_{21}N_3O_5P^+$, found: 294.1212.

^{1}H NMR spectra:



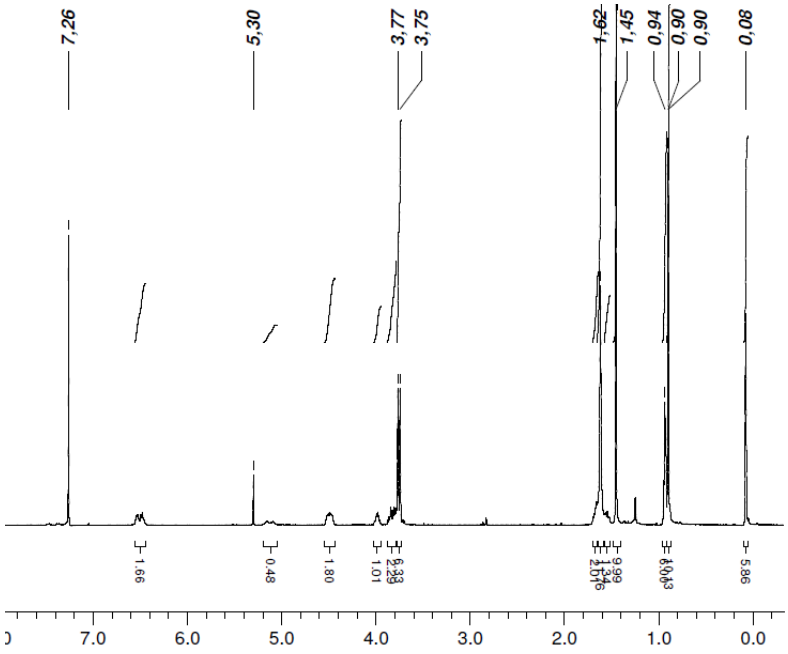
Synthesis of dimethyldehydrophos 3



Synthesis of compound 17

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_2Cl_2 (2 ml). The solution was stirred at 25 °C over night, diluted with CH_2Cl_2 and washed with aqueous 5% citric acid, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (3% MeOH in CH_2Cl_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; Hδ 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 $C_{23}H_{48}N_3O_8PSi$

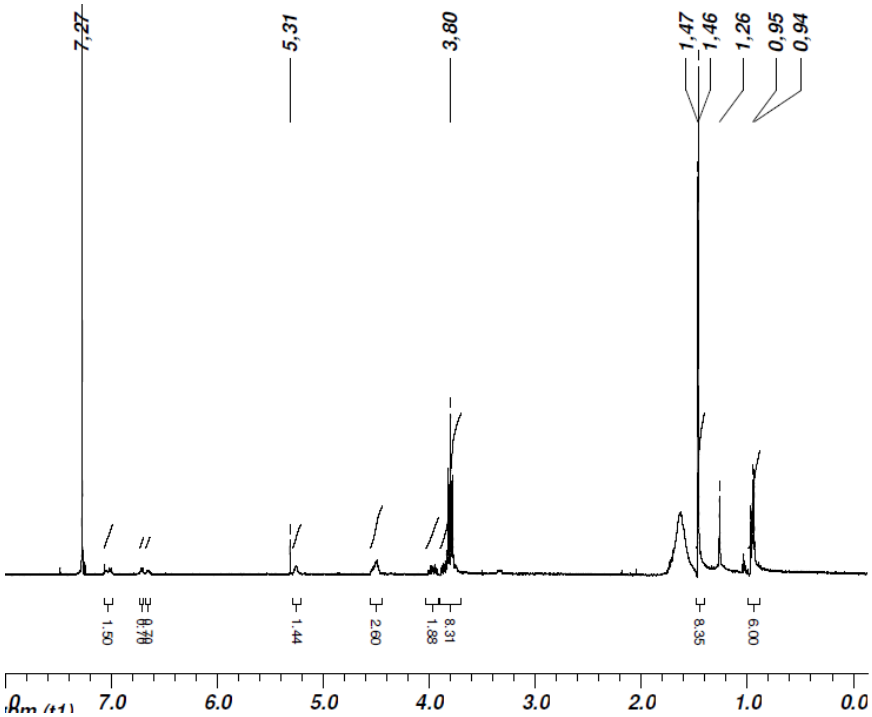
NMR spectra:



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 CH_2Cl_2 and washed with 0.1 M aqueous HCl. The organic fraction was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The remaining oil was purified by column chromatography (4% MeOH in CH_2Cl_2) to yield **18** (61 mg, 140 µmol, 48% yield) as a mixture of diasteromers (~1:1). ^{1}H NMR (500 MHz, $CDCl_3$): 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). ^{31}P-NMR (202 MHz, $CDCl_3$): δ/ppm = 26.5 and 26.4. MS(ESI): m/z= 440.4 $[M+H]^+$ calculated for $C_{17}H_{34}N_3O_8P$.

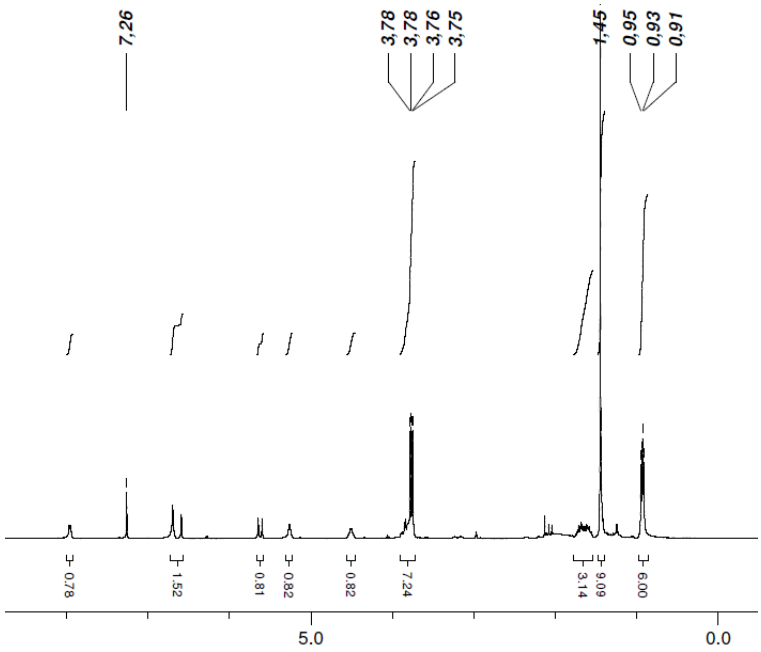
NMR spectra:



*Synthesis of compound **19***

The alcohol **18** (60 mg, 137 µmol) was dissolved in dry CH_2Cl_2. NEt_3 (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 CH_2Cl_2 and washed with water. The organic phase was dried over Na_2SO_4, 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 CH_2Cl_2 and washed with 0.2 M aqueous HCl. The organic phase was dried over Na_2SO_4, 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. ^{1}H NMR (400 MHz, $CDCl_3$): 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; $=CH_2$), 5.63 (d, *J* = 19.1 Hz, 1H; $=CH_2$), 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). ^{31}P-NMR (162 MHz, $CDCl_3$): δ/ppm = 15.7. MS(ESI): m/z = 422.4 $[M+H]^+$ calculated for $C_{17}H_{32}N_3O_7P$.

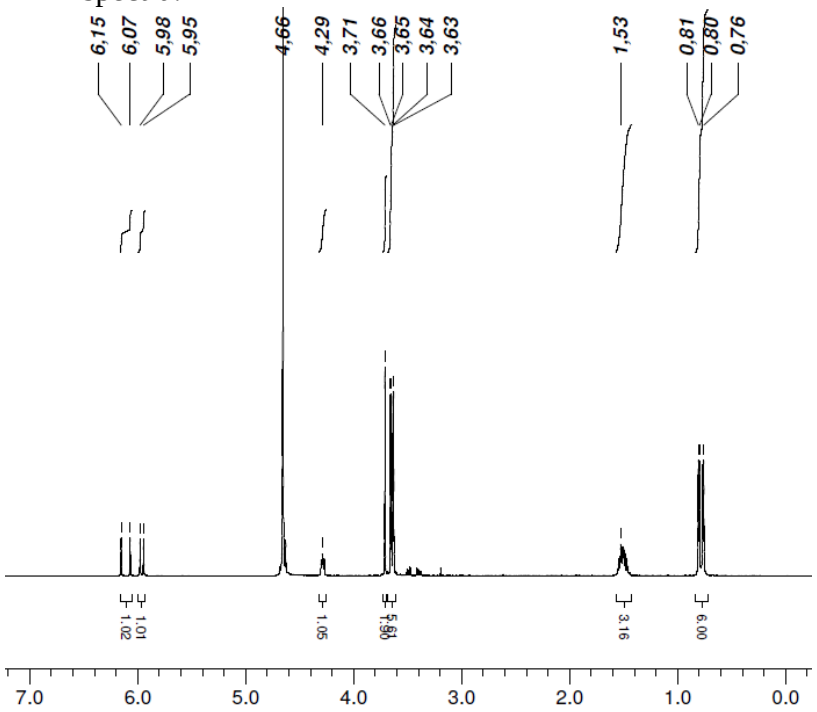
NMR spectra:



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 H_2O) to yield **3** as an oil (t_R = 15 min, 5 mg) after lyophilisation. ^{1}H NMR (500 MHz, D_2O): δ/ppm = 6.11 (d, *J* = 41.3 Hz, 1H; $=CH_2$), 5.96 (d, *J* = 16.1 Hz, 1H; $=CH_2$), 4.28 (dd, *J* = 8.7 Hz, 5.6 Hz, 1H; Hα Leu), 3.71 (s, 2H; Hα Gly), 3.65 (d, *J* = 11.3 Hz, 3H; OCH_3), 3.64 (d, *J* = 11.3 Hz, 3H; OCH_3), 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). ^{31}P-NMR (202 MHz, D_2O): δ/ppm = 17.0. ^{13}C NMR (126 MHz, D_2O): 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): t_R = 16.6 min (linear gradient with 0% MeOH to 30% MeOH in 20 minutes, the other solvent being 0.1% formic acid in D_2O). HRMS (ESI): m/z = 322.1526 calc for $C_{12}H_{25}N_3O_5P^+$, found: 322.1525.

NMR spectra: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0,80
0,76
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1.01
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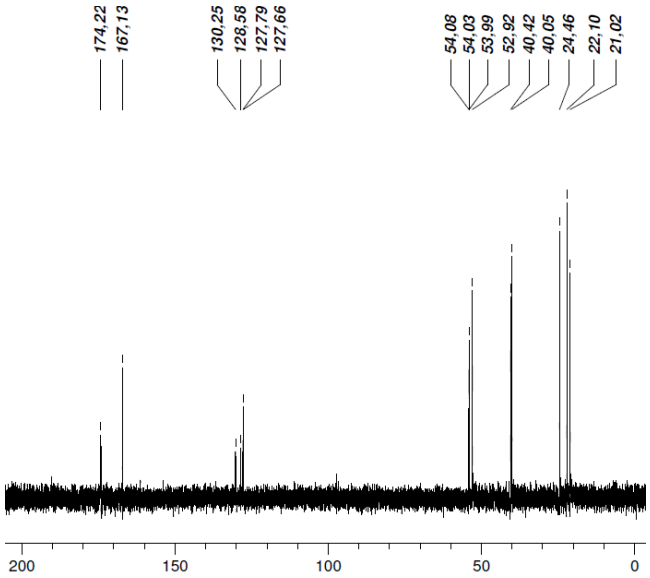
5.0
4.0
3.0
2.0
1.0
0.0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54,03
53,99
52,92
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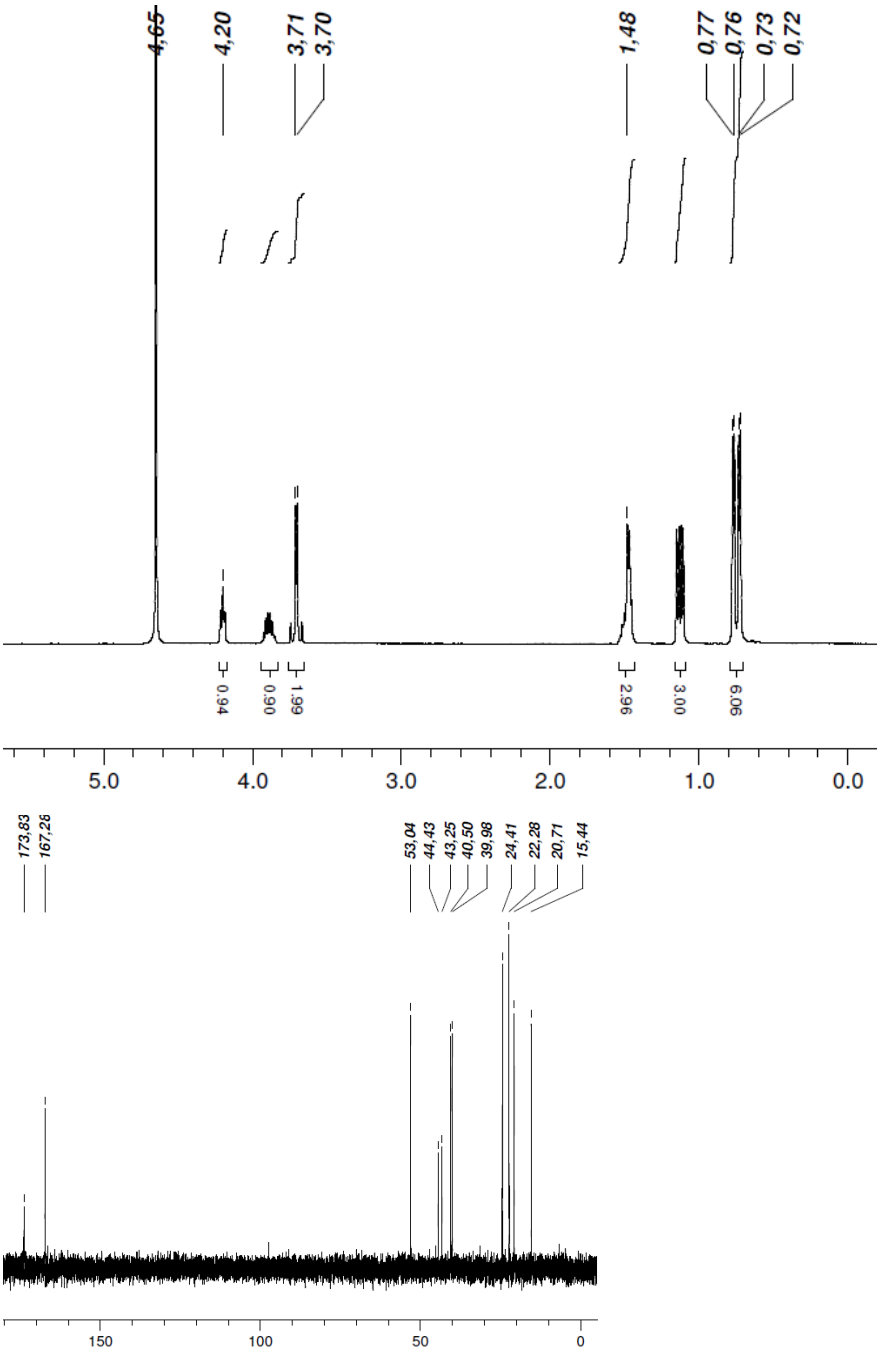
22,10
21,02
200
150
100
50
0

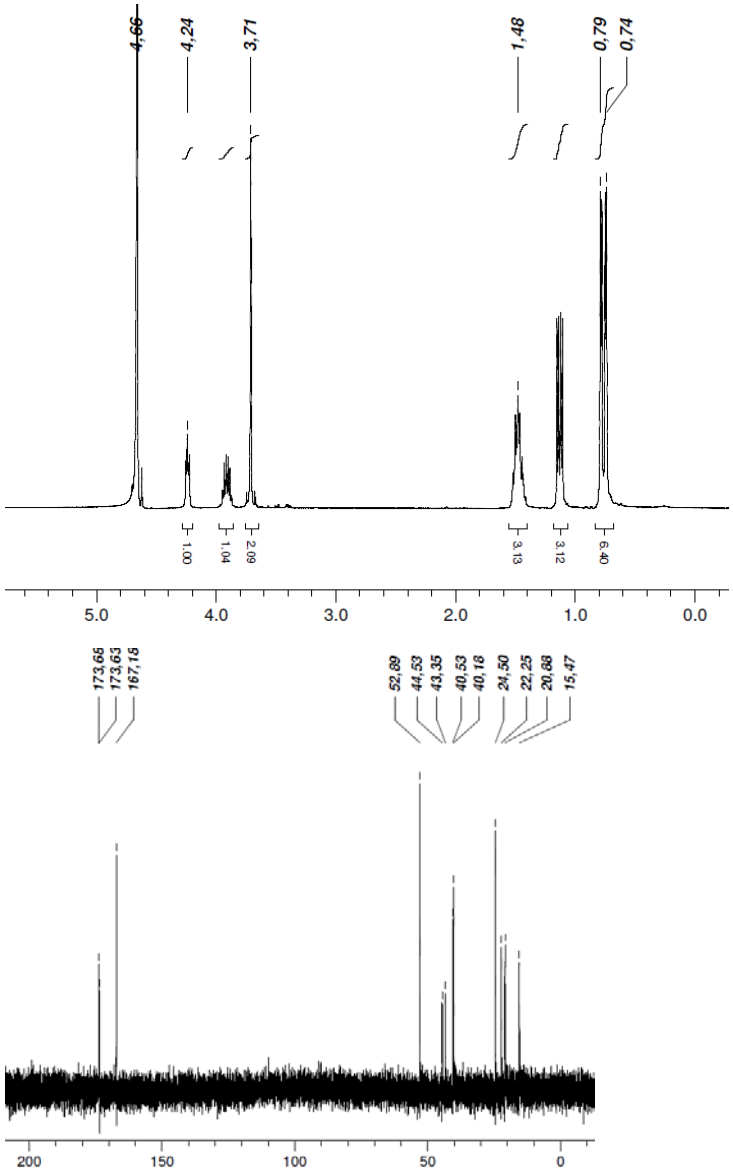
Synthesis of dehydrophos derivatives 4, 4b, 5, 5b, ent-5 and ent-5b

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.[5]

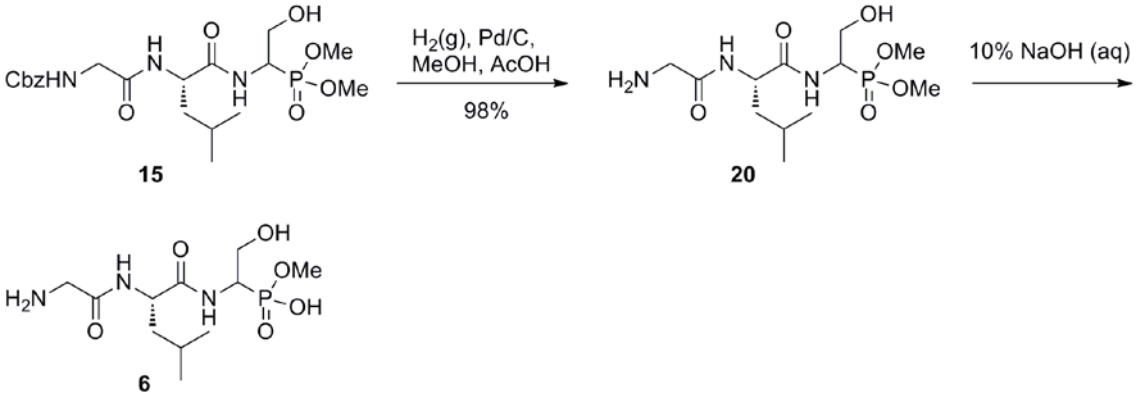
NMR spectra of **ent-5**:



NMR spectra of **ent-5b**:



Synthesis of serine derivates 6a and 6b (epimers at the carbon alpha to the methyl phosphonate)



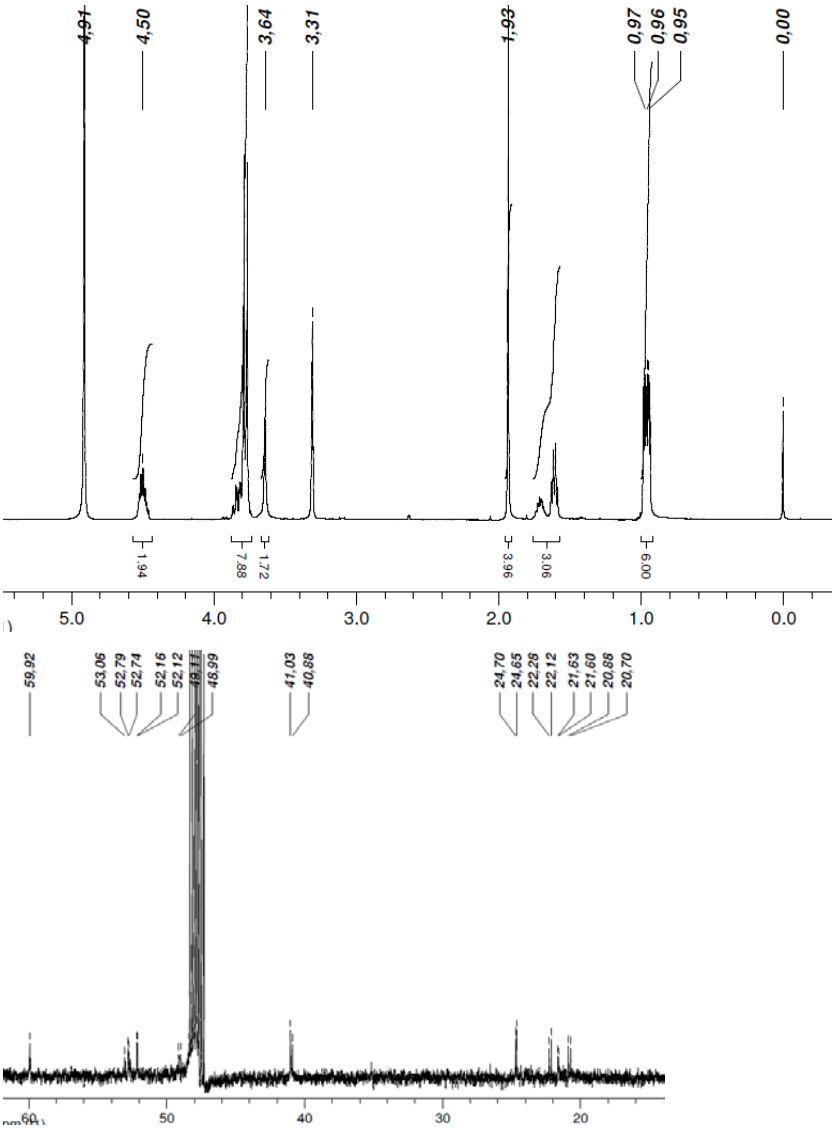
15 20

6

*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_3OD): δ/ppm = 4.54-4.44 (m, 2H), 3.87-3.75 (m, 8H), 3.65 (s, 2H), 1.93 (s, 3H, CH_3COOH), 1.75-1.57 (m, 3H; Hγ/β Leu), 0.99-0.93 (m, 6H; Hδ Leu). ^{13}C NMR (126 MHz, CD_3OD) 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_3OD): δ/ppm = 26.8 and 26.3. MS(ESI): m/z= 340.3 $[M+H]^+$ calculated for $C_{12}H_{26}N_3O_6P$.

NMR spectra:

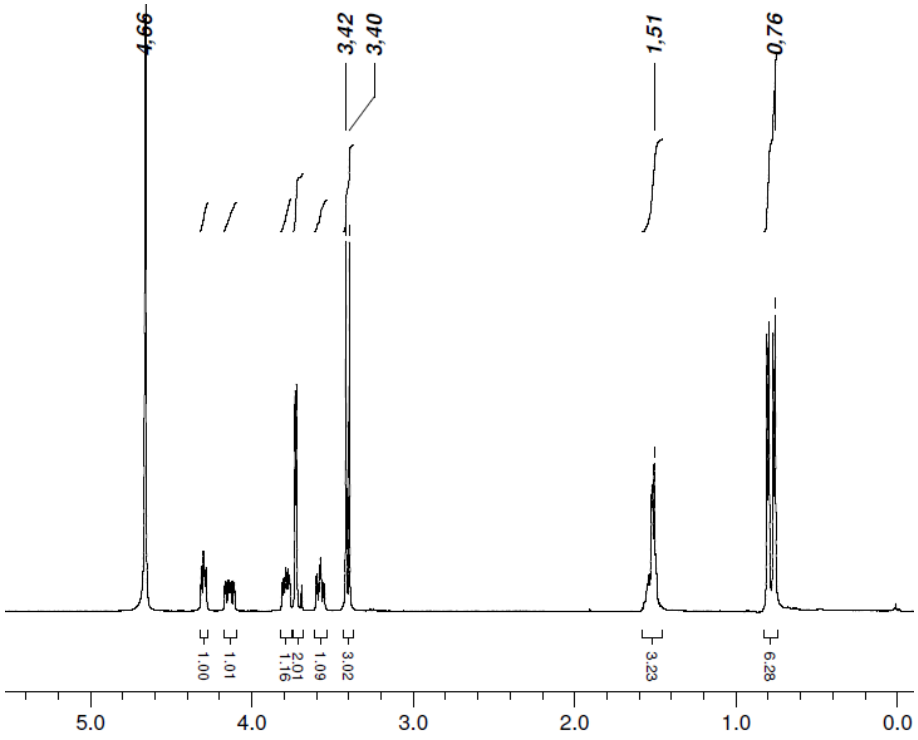


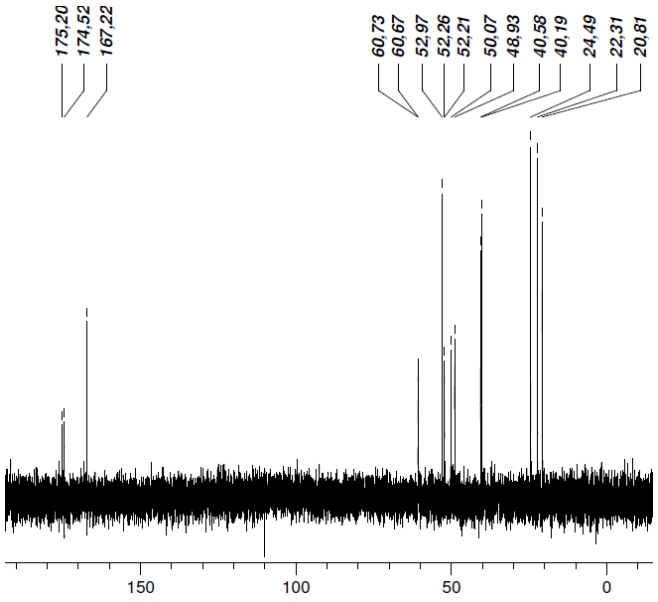
Synthesis of compound ***6***

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 H_2O (3 ml) and purified by preparative reversed phase HPLC (column C: isocratic 0.1% formic acid in H_2O). The two diastereomers of **6** (**6a** t_R = 14 min, **6b** t_R = 18 min) were separated and after lyophilisation isolated as white solids (9 mg and 7 mg, respectively). **6a:** ^{1}H NMR (500 MHz, D_2O): 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 Ser^P), 3.79 (ddd, *J* = 11.9 Hz, 6.1 Hz, 3.6 Hz, 1H; Hβ Ser^P), 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 Ser^P), 3.41 (d, *J*= 10.5 Hz, 3H; OCH_3), 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

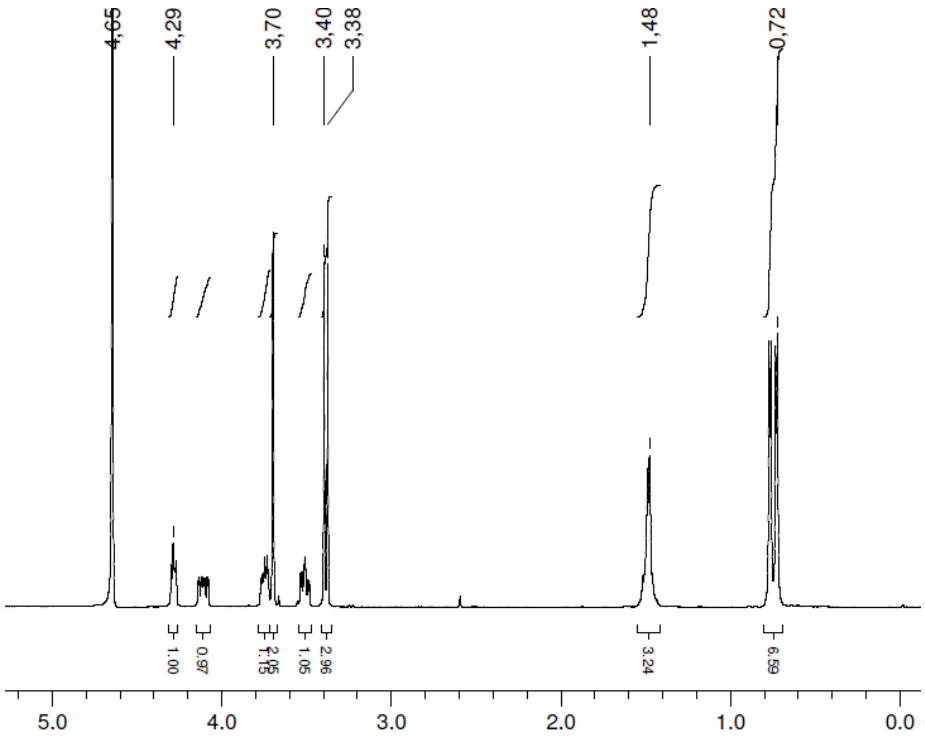
Leu). ^{13}C NMR (126 MHz, D_2O): δ/ppm = 174.5, 167.2, 60.7 (d, *J* = 8.3 Hz), 53.0, 52.2 (d, *J* = 5.8 Hz), 49.5 (d, *J* = 143 Hz), 40.6, 40.2, 24.5, 22.3, 20.8. ^{31}P-NMR (202 MHz, D_2O): δ/ppm = 18.5. LC-MS (column A): t_R = 8.8 min (isocratic 0.1% formic acid in H_2O). HRMS (ESI): m/z = 326.1481 calculated for $C_{11}H_{26}N_3O_6P^+$, found: 326.1469. **6b:** ^{1}H NMR (500 MHz, D_2O): δ/ppm = 4.28 (dd, *J* = 9.0 Hz, 5.1 Hz, 1H; Hα Leu), 4.11 (ddd, *J* = 16.9 Hz, 9.3 Hz, 3.5 Hz, 1H; Hα Ser^P), 3.75 (ddd, *J* = 11.6 Hz, 6.5 Hz, 3.6 Hz, 1H; Hβ Ser^P), 3.71 (d, *J* = 16.7 Hz, 1H; Hα Gly), 3.68 (d, *J* = 16.7 Hz, 1H; Hα Gly), 3.51 (ddd, *J* = 12.4 Hz, 9.6 Hz, 3.9 Hz, 1H, Hβ Ser^P), 3.37 (d, *J*= 10.4 Hz, 3H; OCH_3), 1.55-1.42 (m, 3H; Hγ/β Leu), 0.77 (d, *J* = 5.7 Hz, 3H; Hδ Leu), 0.73 (d, *J* = 5.6 Hz, 3H; Hδ Leu). ^{13}C NMR (126 MHz, D_2O): δ/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_2O): δ/ppm = 18.7. RP-HPLC column B: t_R = 10.2 min (isocratic 0.1% formic acid in H_2O until 10 min, then linear gradient up to 10% CH_3CN in the next 5 minutes). HRMS (ESI): m/z = 326.1481 calculated for $C_{11}H_{26}N_3O_6P^+$, found: 326.1479.

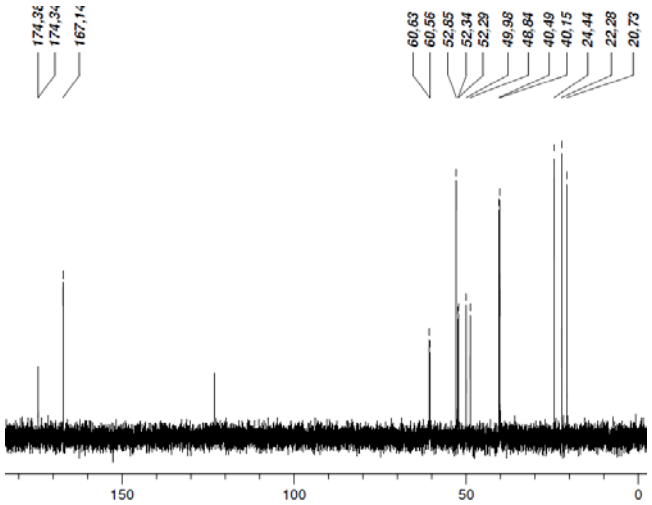
NMR spectra of **6a**:





NMR spectra of **6b**:

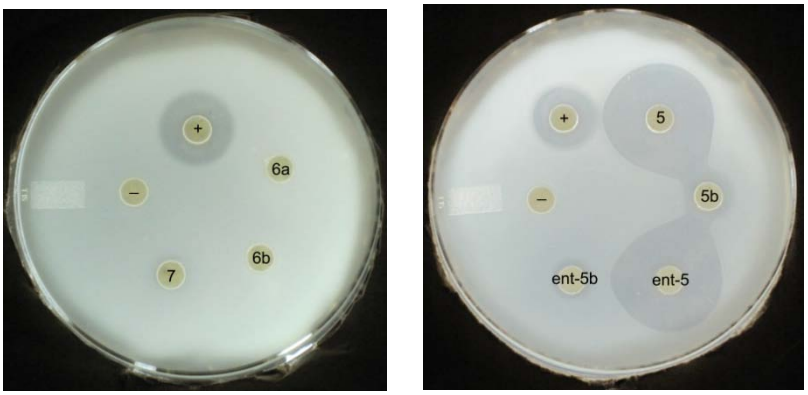




Agar diffusion bioassays

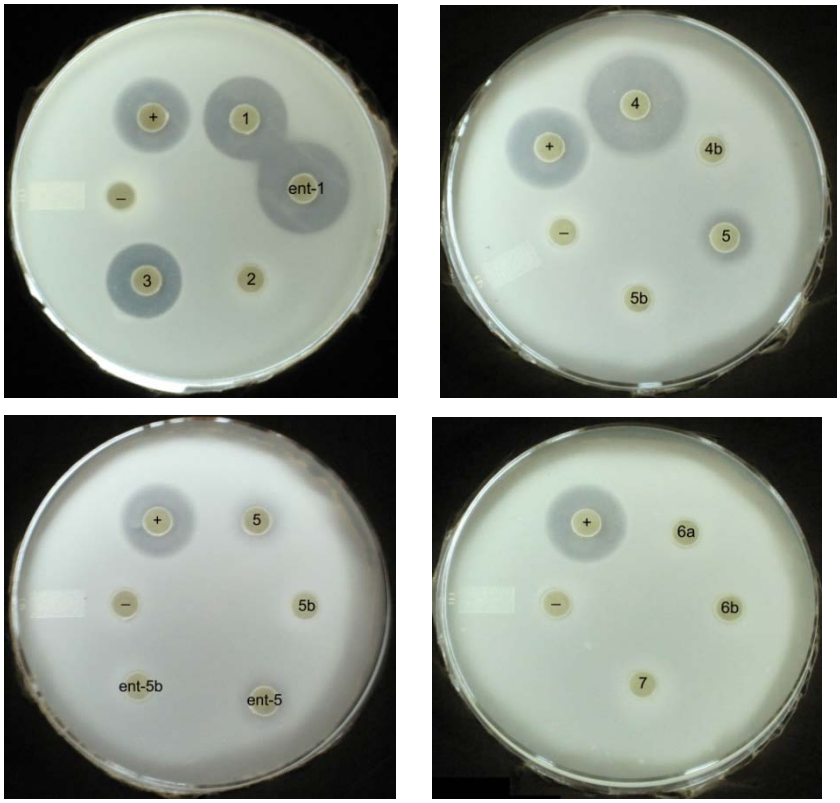
Solid agar diffusion assays were used to assess antimicrobial activity. The bacterial cultures were grown overnight in LB media at 30 °C (*B. subtilis*) or 37 °C (*E. coli*). Cultures of *B. subtilis* were diluted with M9 media to an OD600 value close to 0.3. Cultures of *E. coli* were centrifuged. The 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:

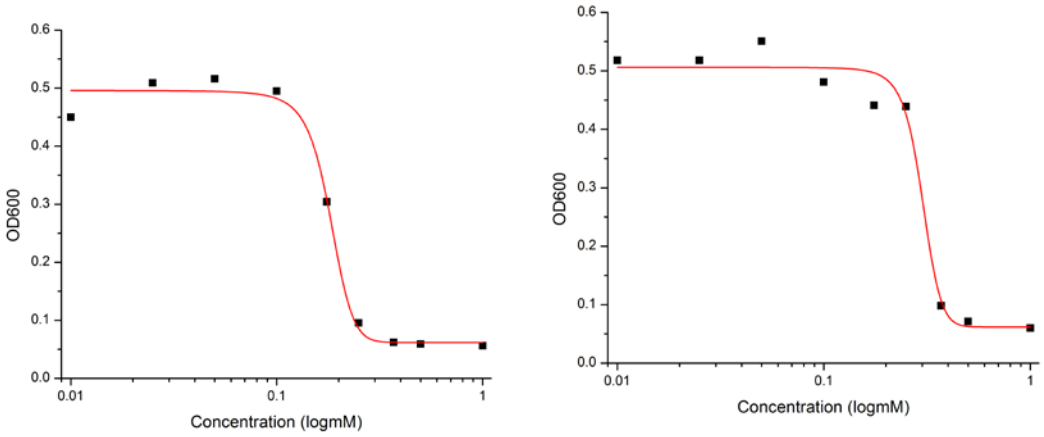


(-) negative control (water), (+) positive control (erythromycin), compounds **1**, **ent-1**, **2**, **3**, **4**, **4b**, **5**, **5b**, **ent-5**, **ent-5b**, **6a**, **6b**, **7** (methyl acetylphosphonate),.

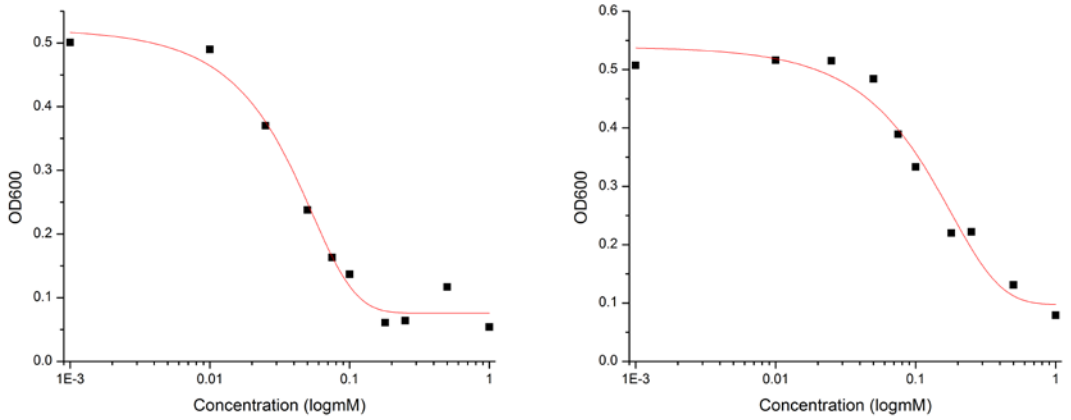
Liquid broth growth inhibition assays

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**. IC_{50} 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).

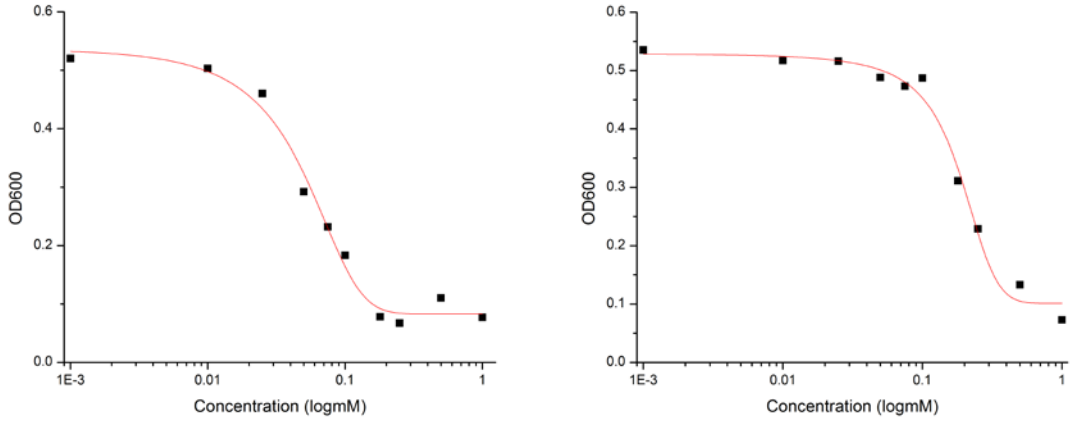
Fit for compound **1** (left, IC_{50} = 180 μM) and **ent-1** (right, IC_{50} = 300 μM):



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):



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6. J. S. Parkinson, *Methods Enzymol.*, 2007, **423**, 432-435.