Convenient Synthesis and Diversification of Dehydroalaninyl Phosphinic Peptide Analogues

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Supporting Information

General: All of the compounds, for which analytical and spectroscopic data are quoted, were homogenous by TLC. TLC analyses were performed using silica gel plates (E. Merck silica gel 60 F-254) and components were visualized by the following methods: ultraviolet light absorbance, charring after spraying with a solution of $(NH_4)HSO_4$ and ninhydrin spray. The solvent systems used for TLC developments were (a) 1-butanol-acetic acid-water (4:1:1), (b) chloroform-methanol-acetic acid (7:2:1), (c) chloroform-methanol-acetic acid (7:0.5:0.5), (d) petroleum ether 40-60^o-ethyl acetate-acetic acid (3:3:0.6). In most solvent systems close, but different, R_f values have been observed for the various diastereoisomers, where present, due to the presence of asymmetric centers. Thus, the R_f values quoted correspond to an average value. Column chromatography was carried out on silica gel (E.Merck, 70-230 mesh), height 42cm, diameter 2.3cm. All the compounds were characterized by ^{1}H, ^{13}C and ^{31}P-NMR spectroscopy. ^{1}H, ^{13}C and ^{31}P-NMR spectra were recorded on a 200 MHz Mercury Varian spectrometer. ^{13}C and ^{31}P-NMR spectra are fully proton decoupled. ^{31}P- chemical shifts are reported on δ scale (in ppm) downfield from 85% H_3PO_4. Melting points provided are those of the diastereoisomeric mixtures-where present-and are not corrected. Phosphinic peptides purity was established by analytical HPLC. Elemental analytical data are also provided. These analyses were obtained from the CNRS, CEA-DIEP, C.E.N., Saclay, Route Departementale 36, Bat 152, Vernaison, 91191, Gif sur Yvette Cedex, France and from Laboratory of Inorganic Chemistry, University of Athens, 15771, Athens, Greece. Electron spray mass spectroscopy (ESMS) was performed on a Micromass Platform II instrument using negative ionization mode by Atheris Laboratories, 314 CH-1233 Bernex, Geneva, Switzerland. *K*i values were determined according to the experimental procedures previously described.[1]

General Procedures: (*R,S*)-(1-(amino)-2-phenylethyl)phosphinic acid was synthesized as described by Baylis et al. Ethyl 2-(acetoxymethyl) acrylate and ethyl 2-(bromomethyl) acrylate were prepared as described by Villieras et al[2] and t-butyl 2-(bromomethyl) acrylate was prepared from 2-(bromomethyl) acrylic acid by acid-catalysed esterification with isobutylene.
Commercially available reagents and starting materials were purchased from Aldrich, Merck, Sigma and Novabiochem.

[1] Vassiliou, S.; Mucha, A.; Cuniasse, P.; Georgiadis, D.; Lucet-Levannier, K.; Beau, F.; Kannan, R.; Murphy, G.; Knauper, V.; Rio, M. C.; Basset, P.; Yiotakis, A.; Dive, V. *J. Med. Chem.*, **1999**, *42*, 2610.
[2] Villieras, J.; Rambaud, M. *Synthesis*, **1982**, 924.

General method for the synthesis of compounds type 1: Method a and c: In an ice cold suspension of phosphinic acid (1 mmol) in CH_2Cl_2 (7 ml) diisopropylethylamine (4.5 mmol, 0.58 g, 0.78 ml) and chlorotrimethylsilane (4.5 mmol, 0.49 g, 0.57 ml) were added, under argon atmosphere. This solution was stirred for 3 h at room temperature. Then, the mixture was cooled at 0°C and the appropriate acrylate was added (1.3 mmol) dropwise. The solution was stirred for 24 h at room temperature. Then, absolute ethanol (0.8 ml) was added dropwise and the mixture was stirred for 20 min. The solvents were evaporated. The residue was dissolved in 5% $NaHCO_3$ (10 ml) and the resulting suspension was extracted with diethylether (2x3 ml). The crude product was precipitated by acidification with 1N HCl to pH 1. Purification by column chromatography using chloroform/methanol/acetic acid, (7:0.4:0.4) as eluent afforded the products as white solids. Method b and d: A suspension of the phosphinic acid (1 mmol) and hexamethyldisilazane (3 mmol, 0.48 g, 0.63 ml) was heated at 110°C for 1h under argon atmosphere. The appropriate acrylate (1.3 mmol) was added at this temperature dropwise for 15 min and the reaction mixture was stirred for 3 h. Then, absolute ethanol (3 ml) was added dropwise. After cooling to room temperature, the mixture was evaporated in vacuo. The residue was dissolved in 5% $NaHCO_3$ (10 ml) and the resulting suspension was extracted with diethylether (2x3 ml). The crude product was precipitated by acidification with 1N HCl to pH 1. Purification by column chromatography using chloroform/methanol/acetic acid, (7:0.4:0.4) as eluent afforded the products as white solids.

(R,S)-2-((1′-(N-benzyloxycarbonyl)amino-2′-phenylethyl)-hydroxyphosphinyl)methyl-prop-2-enoic acid, ethyl ester (1a): TLC R_f(c) 0.38, R_f(d) 0.27; m.p. 90-93 °C; ^{1}H NMR (200 MHz, $CDCl_3$/d_1-TFA=99.5/0.5) δ 1.27 (t, $^3J_{HH}$=7.3Hz, 3H, CH_2CH_3), 2.78-3.20 (m, 3H, PCH_2, PCHH), 3.20-3.35 (m, 1H, PCHH), 4.15-4.46 (m, 3H, CH_2CH_3, PCH), 4.82-5.07 (br s, 2H, OCH_2Ph), 5.70-5.92 (m, 2H, NH, C=CHH), 6.35 (s, 1H, C=CHH), 7.05-7.34 (m, 10H, aryl); ^{13}C-NMR (50 MHz, $CDCl_3$/d_1-TFA=99.5/0.5) δ 13.9 (CH_2CH_3), 29.7 (d, $^1J_{PC}$=86.9Hz, PCH_2), 33.7 (CH_2Ph), 50.5 (d, $^1J_{PC}$=104.8Hz, PCH), 61.4 (CH_2CH_3), 66.7 (OCH_2Ph), 126.5, 126.9, 127.6, 127.8, 128.2, 128.3, 129.1, 129.5, 130.6, 130.7, 136.3, 136.6, 136.8 (aryl, vinyl), 156.1 (OCONH), 166.5 (COOEt); ^{31}P-NMR (81 MHz, $CDCl_3$/d_1-TFA=99.5/0.5) δ 48.66; ESMS *m/z* calcd for $C_{22}H_{25}NO_6P$ $(M-H)^-$ 430.4, found 430.2; Anal. Calcd for $C_{22}H_{26}NO_6P$ (431.4); C, 61.25; H, 6.07; N, 3.25. Found: C, 61.59; H, 5.89; N, 3.30.

(R)-2-((1′-(N-benzyloxycarbonyl)amino-2′-phenylethyl)-hydroxyphosphinyl)methyl-prop-2-enoic acid, *tert*-butyl ester (1b): TLC R_f(c) 0.53, R_f(d) 0.37; m.p. 141-145 °C; ^{1}H NMR (200 MHz, $CDCl_3$/d_1-TFA=99.5/0.5) δ 1.47 (s, 9H, $C(CH_3)_3$), 2.60-3.05 (m, 3H, PCH_2, PCHH), 3.15-3.37 (m, 1H, PCHH), 4.16-4.35 (m, 1H, PCH), 4.85-5.06 (br s, 2H, OCH_2Ph), 5.70-5.88 (m, 2H, NH, C=CHH), 6.23 (s, 1H, C=CHH), 7.02-7.32 (m, 10H, aryl); ^{13}C-NMR (50 MHz, $CDCl_3$/d_1-TFA=99.5/0.5) δ 27.8 ($C(CH_3)$), 30.8 (d, $^1J_{PC}$=94.6Hz, PCH_2), 34.3 (CH_2Ph), 51.3 (d, $^1J_{PC}$=107.5Hz, PCH), 66.5 (OCH_2Ph), 81.7 ($C(CH_3)$), 126.1, 127.6, 128.2, 129.1, 133.5, 136.5, 137.8, 138.1 (aryl, vinyl), 156.8 (OCONH), 167.3 (COOBut); ^{31}P-NMR (81 MHz, $CDCl_3$/d_1-TFA=99.5/0.5) δ 49.53; ESMS *m/z* calcd for $C_{24}H_{29}NO_6P$ $(M-H)^-$ 458.5, found 458.5; Anal. Calcd for $C_{24}H_{30}NO_6P$ (459.5); C, 62.74; H, 6.58; N, 3.05. Found: C, 62.59; H, 6.39; N, 2.93.

(R)-2-((1′-(N-benzyloxycarbonyl)amino-2′-phenylethyl)-hydroxyphosphinyl)methyl-prop-2-enoic acid (1d): In a solution of compound **1b** (1mmol, 0.46g) in CH_2Cl_2 (5ml) TFA (5ml) was added. The reaction mixture was stirred for 1 h at room temperature and then evaporated to

dryness. The residue was treated with CH_2Cl_2 and a solid precipitated. Filtration afforded the product quantitatively (0.4 g) as a white solid.
TLC R_f(a) 0.74, R_f(b) 0.57; m.p. 179-181 ^oC; ^1H NMR (200 MHz, d_6-DMSO) δ 2.45-2.86 (m, 3H, PC*H*$_2$, PhC*H*H), 3.03-3.18 (m, 1H, PhCH*H*), 3.85-4.04 (m, 1H, PC*H*), 4.82-5.01 (br s, 2H, OC*H*$_2$Ph), 5.75-5.82 (m, 2H, N*H*, C=CH*H*), 6.16 (s, 1H, C=C*H*H), 7.12-7.30 (m, 10H, aryl); ^{13}C-NMR (50 MHz, d_6-DMSO) δ 29.3 (d, $^1J_{PC}$=85.2Hz, P*C*H$_2$), 32.9 (*C*H$_2$Ph), 51.8 (d, $^1J_{PC}$=105.1Hz, P*C*H), 65.1 (O*C*H$_2$Ph), 126.2, 127.1, 127.6, 128.2, 129.0, 132.8, 137.3, 138.3, 138.6 (aryl, vinyl), 156.0 (d, $^3J_{PC}$=4.2Hz, O*C*ONH), 167.6 (d, $^3J_{PC}$=4.1Hz, *C*OOH); ^{31}P-NMR (81 MHz, d_6-DMSO) δ 42.38; ESMS *m/z* calcd for $C_{20}H_{21}NO_6P$ $(M-H)^-$ 402.4, found 402.1; Anal. Calcd for $C_{20}H_{22}NO_6P$ (403.4); C, 59.55; H, 5.50; N, 3.47. Found: C, 59.29; H, 5.52; N, 3.60

[(R,S),(R,S)]-2-benzylthio-2-((1´-(N-benzyloxycarbonyl)amino-2´-phenylethyl)-hydroxyphosphinyl)methyl-propanoic acid, ethyl ester (2a): In a solution of compound **1a** (1 mmol, 0.43 g) in CH_2Cl_2 (20 ml) triethylamine (3 mmol, 0.30 g, 0.41 ml) and benzyl mercaptan (3 mmol, 0.37 g, 0.35 ml) were added. The reaction mixture was stirred for 48 h at room temperature and then evaporated to dryness. The residue was dissolved in 5% $NaHCO_3$ (15 ml) and the resulting suspension was extracted with diethylether (2x5 ml). The aqueous solution was acidified with 1N HCl to pH 1 and extracted with AcOEt (2x15 ml). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography using chloroform/methanol/acetic acid, (7:0.3:0.2) as eluent afforded the product (0.51 g, 93%) as a white solid.
Warning! All reactions involving thiols, which are very malodorous, should be performed in an efficient hood.
TLC R_f(c) 0.52, R_f(d) 0.47; ^1H NMR (200 MHz, $CDCl_3$) δ 1.20 (t, $^3J_{HH}$=6.8Hz, 3H, CH_2CH_3), 1.92-2.35 (m, 2H, PC*H*$_2$), 2.36-3.30 (m, 5H, PhCH$_2$SC*H*$_2$, PhCH$_2$SCH$_2$C*H*, PhC*H*$_2$,), 3.64 (s, 2H, PhC*H*$_2$SCH$_2$), 3.95-4.32 (m, 3H, C*H*$_2$CH$_3$, PC*H*), 4.68-5.11 (br s, 2H, OC*H*$_2$Ph), 5.52-5.75 (m, 1H, N*H*), 7.05-7.45 (m, 15H, aryl); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 14.4 (CH_2CH_3), 28.3 (d, $^1J_{PC}$=75.5Hz, P*C*H$_2$), 33.2 (*C*H$_2$Ph), 34.7 (PhCH$_2$S*C*H$_2$), 36.0 (PhCH$_2$SCH$_2$), 39.4 (PCH$_2$*C*H), 51.9 (d, $^1J_{PC}$=103.6Hz, P*C*H), 61.7 (CH_2CH_3), 67.8 (O*C*H$_2$Ph), 126.5, 126.8, 127.4, 128.2, 129.1, 129.5, 136.3, 136.6, 137.1 (aryl), 157.8 (O*C*ONH), 173.6 (*C*OOEt); ^{31}P-NMR (81 MHz, $CDCl_3$) δ 50.43, 50.84; ESMS *m/z* calcd for $C_{29}H_{33}NO_6PS$ $(M-H)^-$ 554.6, found 554.1; Anal. Calcd for $C_{29}H_{34}NO_6PS$ (555.6); C, 62.69; H, 6.17; N, 2.52. Found: C, 62.59; H, 5.89; N, 2.30.

[(R),(S)]-2-((1´-(N-benzyloxycarbonyl)amino-2´-phenylethyl)-hydroxyphosphinyl)methyl-prop-2-enoyl-(L)-phenylalanine, *tert*-butyl ester (3a): The experimental procedure followed is similar to the one described for compound **3c** except that L-phenylalanine *tert*-butyl ester hydrochloride and 1eq diisopropylethylamine were used instead of L-tryptophan amide.
TLC R_f(c) 0.54, R_f(d) 0.29; m.p. 130-134 ^oC; ^1H NMR (200 MHz, d_6-DMSO) δ 1.28 (s, 9H, C(C*H*$_3$)$_3$), 2.28-3.21 (m, 6H, PhC*H*$_2$CHCOOBut, PC*H*$_2$, PhC*H*$_2$), 3.83-4.15 (m, 1H, PC*H*), 4.28-4.47 (m, 1H, PhCH$_2$C*H*COOBut), 4.69-5.15 (br s, 2H, OC*H*$_2$Ph), 5.38-5.65 (m, 2H, N*H*, C=CH*H*), 5.83 (s, 1H, C=C*H*H), 6.95-7.58 (m, 15H, aryl); ^{13}C-NMR (50 MHz, d_6-DMSO) δ 27.5 (C(*C*H$_3$)), 30.9 (d, $^1J_{PC}$=89.5Hz, P*C*H$_2$), 33.8 (*C*H$_2$Ph), 36.9 (PhCH$_2$CHCOOBut), 51.5 (d, $^1J_{PC}$=110.0Hz, P*C*H), 54.9 (PhCH$_2$*C*HCOOBut), 65.1 (O*C*H$_2$Ph), 80.6 (*C*(CH$_3$)$_3$), 126.1, 126.5, 127.1, 127.6, 128.1, 128.3, 129.0, 129.3, 137.5 (aryl, vinyl), 155.8 (O*C*ONH), 167.9 (CH$_2$=C*C*ONH), 170.6 (*C*OOBut); ^{31}P-NMR (81 MHz, d_6-DMSO) δ 28.92; ESMS *m/z* calcd for

$C_{33}H_{38}N_2O_7P$ $(M-H)^-$ 605.6, found 605.3; Anal. Calcd for $C_{33}H_{39}N_2O_7P$ (606.6); C, 65.34; H, 6.48; N, 4.62. Found: C, 65.29; H, 6.52; N, 4.60

[(R),(S)]-2-((1′-(N-benzyloxycarbonyl)amino-2′-phenylethyl)-hydroxyphosphinyl)methyl-prop-2-enoyl-(L)-tryptophanylamide (3c): In a suspension of compound **1d**, (1 mmol, 0.40 g) in CH_2Cl_2 (20 ml) diisopropylethylamine, (0.94 mmol, 0.12 g, 0.16 ml), L-tryptophan amide (1 mmol, 0.20 g), a solution of N-hydroxybenzotriazole monohydrate (HOBt), (0.98 mmol, 0.13 g) in THF (2 ml), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDCHCl), (4 mmol, 0.77 g) and finally another 0.94 mmol of diisopropylethylamine were added. The reaction mixture was stirred for 1 h at room temperature. It was then diluted with CH_2Cl_2 (60 ml) and washed with a solution of HCl 1N (3x5 ml), a saturated solution of NH_4HCO_3 (3x1 ml), HCl 1N to pH 1 and brine (10 ml). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography using chloroform/methanol/acetic acid, (7:1:0.4) as eluent afforded the product (0.41 g, 69%) as a white solid.
TLC R_f(a) 0.73, R_f(b) 0.55; m.p. 176-180 °C; ^{1}H NMR (200 MHz, d_6-DMSO) δ 2.20-3.42 (m, 6H, indolylCH_2, PCH_2, PhCH_2), 3.73-4.02 (m, 1H, PC*H*), 4.33-4.56 (m, 1H, indolylCH_2C*H*), 4.75-5.05 (br s, 2H, OCH_2Ph), 5.17 (s, 1H, C=C*H*H), 5.30-5.56 (m, 2H, N*H*, C=CH*H*), 6.92-7.68 (m, 16H, aryl); ^{13}C-NMR (50 MHz, d_6-DMSO) δ 27.9 (d, $^1J_{PC}$=78.6Hz, P*C*H$_2$), 33.6 (*C*H$_2$Ph), 50.5 (d, $^1J_{PC}$=103.9Hz, P*C*H), 54.7 (indolylCH$_2$*C*H), 64.9 (O*C*H$_2$Ph), 111.1, 118.3, 121.1, 123.9, 126.6, 127.4, 128.1, 129.3, 136.1, 137.5, (aryl, vinyl), 139.9, 155.9 (O*C*ONH), 168.6 (CH$_2$=C*C*ONH), 174.6 (*C*ONH$_2$); ^{31}P-NMR (81 MHz, d_6-DMSO) δ 29.37; ESMS *m/z* calcd for $C_{31}H_{32}N_4O_6P$ $(M-H)^-$ 587.6, found 588.0; Anal. Calcd for $C_{31}H_{33}N_4O_6P$ (588.6); C, 63.26; H, 5.65; N, 9.52. Found: C, 62.99; H, 5.52; N, 9.43

[(R),(R,S),(S)]-2-benzylthio-2-((1′-(N-benzyloxycarbonyl)amino-2′-phenylethyl)-hydroxyphosphinyl)methyl-propanoyl-(L)-tryptophanylamide (4a): In a solution of compound **3c** (0.5 mmol, 0.29 g) in freshly distilled THF (30 ml), cyclohexyl mercaptan (2 mmol, 0.24 g, 0.25 ml) and 1M solution of EtONa/EtOH (1 mmol, 1 ml) were added. The reaction mixture was stirred for 1 h at room temperature and then evaporated to dryness. After the addition of some drops of H_2O in the residue, $CHCl_3$ (50 ml) was added and the resulting solution was washed with a solution of HCl 1N to pH 1. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Dissolution of the mixture in the minimum volume of $CHCl_3$, precipitation with petroleum ether 40-60°, filtration and washings of the residue with petroleum ether 40-60° afforded the pure product (0.34 g, 96%) as a white solid. Further purification of the product can be achieved by column chromatography using chloroform/methanol/acetic acid, (7:0.7:0.4) as eluent system.
TLC R_f(a) 0.82/0.85, R_f(b) 0.71/0.80; m.p. 105-110 °C (dec.); ^{1}H NMR (200 MHz, d_6-DMSO) δ 0.78-2.08 (m, 10H, CH_2 of cyclohexyl), 2.10-3.42 (m, 10H, C*H*SCH$_2$, CHSCH_2, PCH$_2$C*H*, PCH_2, PhCH_2, indolylCH_2), 3.80-4.16 (m, 1H, PC*H*) 4.28-4.56 (m, 1H, indolylCH$_2$C*H*), 4.85-5.05 (br s, 2H, OCH_2Ph), 6.80-7.78 (m, 16H, aryl); ^{13}C-NMR (50 MHz, d_6-DMSO) δ 25.5 (*C*H$_2$ of cyclohexyl), 27.7 (d, $^1J_{PC}$=77.9Hz, P*C*H$_2$), 33.1 (*C*H$_2$Ph), 42.5 (PCH$_2$*C*H), 51.3 (d, $^1J_{PC}$=102.9Hz, P*C*H), 53.8 (indolylCH$_2$*C*H), 65.1 (O*C*H$_2$Ph), 111.1, 118.1, 121.0, 123.3, 123.6, 126.6, 127.4, 128.2, 128.9, 136.2, 137.3, 139.9, (aryl), 156.1 (O*C*ONH), 173.3 (CHC*C*ONH), 174.3 (*C*ONH$_2$); ^{31}P-NMR (81 MHz, d_6-DMSO) δ 45.94, 46.85; ESMS *m/z* calcd for $C_{37}H_{44}N_4O_6PS$ $(M-H)^-$ 703.8, found 704.0; Anal. Calcd for $C_{37}H_{45}N_4O_6PS$ (704.8); C, 63.05; H, 6.44; N, 7.95. Found: C, 62.99; H, 6.52; N, 7.93