Supporting Information

Enhanced Brain Delivery of (2-(phosphonomethyl)pentanedioic acid) following Intranasal Administration of its γ-substituted Ester Prodrugs

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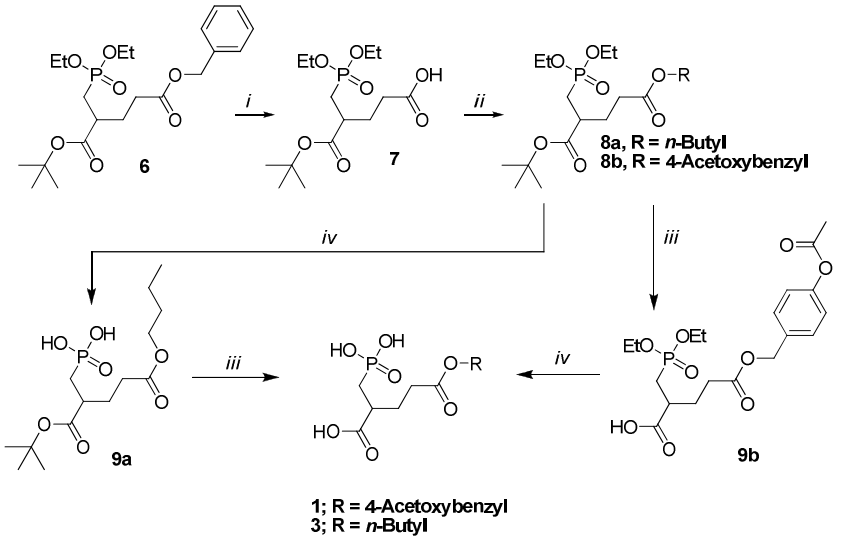
1. Synthesis and characterization of compounds

General.

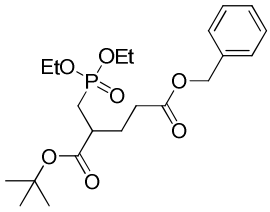
The ^{1}H NMR spectra were measured at 400.13 MHz. The signal of TMS (δ 0.0, $CDCl_3$) was used as internal standard of ^{1}H NMR spectra. The chemical shifts are given in δ-scale, the coupling constants *J* are given in Hz. The IR spectra were measured in $CHCl_3$ on FT-IR spectrometer Bruker Equinox 55. Low and high resolution CI mass spectra were measured using an orthogonal acceleration time-of-flight (OA-TOF) mass spectrometer (GCT premier, Waters) at an ionising voltage of 70 eV, the m/z values are given with their relative intensities (%). The spectra were recorded in positive mode and the source temperature was 150 °C. Methane was present as a reagent gas in the CI source. For exact measurements the spectra were internally calibrated using Heptacosa or 2,4,6-tris(trifluoromethyl)-1,3,5-triazine (Metri). The ESI mass spectra were recorded with a ZQ micromass mass pectrometer (Waters) equipped with an ESCi multi-mode ion source and controlled by MassLynx software. THF was freshly distilled from sodium/benzophenone under nitrogen. The flash chromatography was performed on Silica gel 60 (0.040-0.063 mm, Fluka). All chemicals were purchased from Sigma-Aldrich. The THF was freshly distilled from sodium/benzophenone under nitrogen. Dichloromethane was freshly distilled from calcium hydride under nitrogen. YMC-Pack, ODS-AM column 250 × 20 mm, 5µm was used for RP-HPLC purifications.

Scheme S1. Synthesis of Compounds 1 and 3.



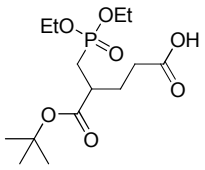
Reagents and Conditions: *i*) Pd/C, H_2 (balloon), THF, rt, 22 h; *ii*) for **8a**: *n*-butanol, DCC, DMAP, DCM, rt, 20 h; for 8b: 4-hydroxymethylphenylacetate, PPh_3, DIAD, 1 h, rt. *iii*) TFA/DCM 1:1, 0 °C to rt, 3 h; *iv*) TMS-Br, DCM, 0 °C to rt, 18 h.

5-Benzyl 1-(*tert*-butyl) 2-((diethoxyphosphoryl)methyl)pentanedioate (6)



The compound was prepared according to the published procedure. ^{1}H and ^{13}C NMR spectra were in agreement with the published data. Majer, P. et al. *J. Med. Chem.*, **2016**, *59*, 2810.

5-(*tert*-Butoxy)-4-((diethoxyphosphoryl)methyl)-5-oxopentanoic acid (7)



Compound **6** (5.90 g, 13.3 mmol) was dissolved in freshly distilled THF (60 mL). 10 % Pd/C (1.42 g, 1.33 mmol, 10 mol%) was added and the flask was filled with hydrogen. The mixture was stirred at rt overnight under hydrogen atmosphere (balloon). After 22 h the mixture was filtered over a pad of celite (3 cm) and washed with further THF (60 mL). The solvent was evaporated and the desired product was obtained in quantitative yield (4.50 g) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.29 (6H, dt, J = 7.1, 1.7 Hz), 1.43 (s, 9H), 1.74 – 2.03 (3H, m), 2.13 – 2.42 (3H, m), 2.60 – 2.75 (1H, m), 4.01 – 4.15 (4H, m).

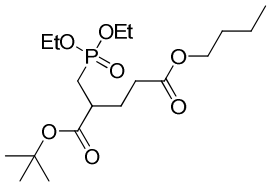
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 16.43, 16.49, 27.52 (d, $J_{C,P}$ = 142.7 Hz), 28.09, 28.46 (d, $J_{C,P}$ = 11.9 Hz), 31.30, 39.91 (d, $J_{C,P}$ = 3.3 Hz), 62.14 (d, $J_{C,P}$ = 4.0 Hz), 62.21 (d, $J_{C,P}$ = 4.0 Hz), 81.38, 173.21 (d, $J_{C,P}$ = 9.5 Hz), 176.40.

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 32.07.

ESI MS: 337 ($[M]^-$).

HR ESI MS: calcd for $C_{14}H_{26}O_7P$: 337.14216; found: 337.14148.

1-(*tert*-Butyl)-5-butyl-2-((diethoxyphosphoryl)methyl)pentanedioate (8a)



Compound **7** (529 mg, 1.56 mmol) was dissolved in dry DCM (10 mL). DCC (355 mg, 1.72 mmol, 1.1 equiv.) and DMAP (19 mg, 0.156 mmol, 10 mol%) were added in one portion. Reaction mixture was stirred for 10 min under inert atmosphere and 1-butanol (128 mg, 157 µL, 1.72 mmol, 1.1 equiv.) was added by syringe. The mixture was then stirred for 24 h. The precipitate was filtered off and washed with DCM (10 mL). The solvent was removed in vacuo and the oily crude product was purified by column chromatography (EtOAc/hexane 2:1 to EtOAc). The desired product was obtained as a colorless oil (530 mg, 86% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.92 (3H, t, J = 7.4 Hz), 1.31 (6H, dt, J = 7.1, 1.7 Hz), 1.33 – 1.42 (2H, m), 1.45 (s, 9H), 1.54 – 1.66 (2H, m), 1.77 (2H, ddd, J = 18.5, 15.5, 5.6 Hz), 1.89 – 2.02 (3H, m), 2.14 – 2.40 (3H, m), 2.62 – 2.74 (1H, m), 4.04 – 4.13 (4H, m).

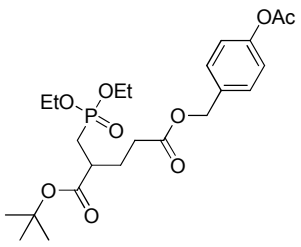
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 13.84, 16.53, 16.59, 19.26, 28.15, 27.80 (d, $J_{C,P}$ = 142.5 Hz), 28.74 (d, $J_{C,P}$ = 12.1 Hz), 30.77, 31.62, 40.11 (d, $J_{C,P}$ = 3.5 Hz), 61.81 (d, $J_{C,P}$ = 5.0 Hz), 61.88 (d, $J_{C,P}$ = 5.0 Hz), 64.55, 81.27, 172.86, 173.28 (d, $J_{C,P}$ = 9.1 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 31.64.

ESI MS: 417 ($[M + Na]^+$).

HR ESI MS: calcd for $C_{18}H_{35}O_7PNa$: 417.20126; found: 417.20123.

5-(4-Acetoxybenzyl)-1-(*tert*-butyl)-2-((diethoxyphosphoryl)methyl) pentanedioate (8b)



Compound **7** (1.01 g, 2.99 mmol), was dissolved in dry THF (12 mL). Triphenylphosphine (979 mg, 3.73 mmol, 1.25 equiv.) and 4-(hydroxymethyl)phenyl acetate (620 mg, 3.73 mmol, 1.25 equiv.) were added in one portion and finally DIAD (755 mg, 735 µl, 3.73 mmol, 1.25 equiv.) was added by syringe over 10 minutes. The yellow reaction mixture was stirred for 1 h at rt. Solvent was evaporated and the

crude product was purified by column chromatography (DCM/EtOAc, 2:1 to EtOAc). The desired product was obtained in quantitative yield, containing triphenylphosphine oxide as an impurity.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.32 (6H, dt, J = 7.0, 2.0 Hz), 1.45 (s, 9H), 1.80 – 2.05 (3H, m), 2.30 (3H, s), 2.22 – 2.48 (3H, m), 2.62 – 2.79 (1H, m), 4.12 (4H, ddd, J = 8.2, 7.0, 0.9 Hz), 5.11 (2H, s), 7.08 (2H, t, J = 8.6 Hz), 7.37 (2H, t, J = 8.6 Hz).

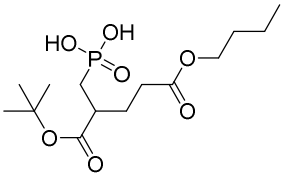
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 16.27, 16.33, 21.15, 27.22 (d, $J_{C,P}$ = 142.6 Hz), 28.01, 28.56 (d, $J_{C,P}$ = 13.0 Hz), 31.38, 39.75 (d, $J_{C,P}$ = 3.6 Hz), 62.80 (d, $J_{C,P}$ = 2.7 Hz), 62.86 (d, $J_{C,P}$ = 2.7 Hz), 65.83, 81.64, 121.81 (2C), 129.59 (2C), 133.51, 150.64, 169.53, 172.39, 172.88 (d, $J_{C,P}$ = 8.6 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 32.10.

ESI MS: 509 ($[M + Na]^+$).

HR ESI MS: calcd for $C_{23}H_{35}O_9PNa$: 509.19109; found: 509.19104.

(5-Butoxy-2-(*tert*-butoxycarbonyl)-5-oxopentyl)phosphonic acid (9a)



Intermediate **8a** (510 mg, 1.29 mmol) was dissolved in freshly distilled DCM (10 mL) and the mixture was cooled down to 0 °C. Bromotrimethylsilane (792 mg, 683 µL, 5.17 mmol, 4 equiv.) was added by syringe over 5 min. The reaction mixture was stirred at 0 °C for 20 h. DCM was evaporated and the rest of TMSBr was removed by co-distillation with dry $PhCH_3$ (3×10 mL). The bis(trimethylsilyl) intermediate was transformed to the desired phosphonic acid by addition of mixture acetonitrile/H_2O (5:1, 12 mL) and solvents were evaporated. The crude product was obtained in a quantitative yield (438 mg) as a light brown oil and for the final step was used without any purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.95 (3H, t, J = 7.4 Hz), 1.35 – 1.44 (2H, m), 1.48 (s, 9H), 1.56 – 1.71 (2H, m), 1.79 – 2.10 (3H, m), 2.15 – 2.53 (3H, m), 2.68 – 2.75 (1H, m), 4.10 (2H, t, J = 7.4 Hz), 10.00 (2H, bs).

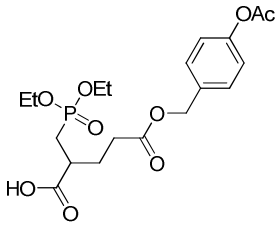
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 13.84, 19.24, 28.05, 28.29 (d, $J_{C,P}$ = 145.2 Hz), 28.53 (d, $J_{C,P}$ = 13.1 Hz), 30.72, 31.65, 40.27 (d, $J_{C,P}$ = 3.2 Hz), 64.82, 81.94, 173.45, 173.90 (d, $J_{C,P}$ = 8.8 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 33.74.

ESI MS: 337 ([M]$^-$).

HR ESI MS: calcd for $C_{14}H_{26}O_7P$: 337.14216; found: 337.14148.

5-((4-Acetoxybenzyl)oxy)-2-((diethoxyphosphoryl)methyl)-5-oxopentanoic acid (9b)



Intermediate **8b** (1.45 g, 2.99 mmol) was dissolved in dry DCM (20 mL). The solution was cooled down to 0 °C and TFA (20 mL) was added by syringe over a period of 15 min. The reaction mixture was slowly heated up to rt and stirred overnight (15 h). DCM and TFA were evaporated in vacuo and the residue was dissolved in $PhCH_3$ (3×30 mL) and co-evaporated to remove traces of TFA. The crude product was purified by column chromatography ($CHCl_3$/MeOH; 20:1 to 10:1) and the product was obtained as a colorless oil (1.19 g, 93% yield over 2 steps).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.29 (6H, dt, J = 7.0, 2.3 Hz), 1.86 – 2.07 (3H, m), 2.29 (3H, s), 2.24 – 2.48 (3H, m), 2.76 – 2.88 (1H, m), 4.04 – 4.17 (4H, m), 5.09 (2H, s), 7.07 (2H, t, J = 8.5 Hz), 7.35 (2H, t, J = 8.5 Hz), 10.68 (1H, s).

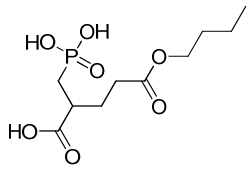
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 16.24 (d, $J_{C,P}$ = 2.5 Hz), 16.30 (d, $J_{C,P}$ = 2.5 Hz), 21.22, 27.37 (d, $J_{C,P}$ = 145.1 Hz), 28.15 (d, $J_{C,P}$ = 13.6 Hz), 31.42, 38.96 (d, $J_{C,P}$ = 3.7 Hz), 63.01 (d, $J_{C,P}$ = 2.5 Hz), 63.07 (d, $J_{C,P}$ = 2.5 Hz), 65.96, 121.86 (2C), 129.67 (2C), 133.48, 150.68, 169.69, 172.46, 178.06 (d, $J_{C,P}$ = 8.0 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 31.68.

ESI MS: 453 ([M + Na]$^+$).

HR ESI MS: calcd for $C_{19}H_{28}O_9PNa$: 453.12849; found: 453.12852.

5-Butoxy-5-oxo-2-(phosphonomethyl)pentanoic acid (3)



Phosphonic acid **9a** (420 mg, 1.24 mmol) was dissolved in dry DCM (5 mL). The solution was cooled down to 0 °C and TFA (5 mL) was added by syringe over a period of 10 min. The reaction mixture was slowly heated up to rt and stirred overnight (18 h). DCM and TFA were evaporated and the crude product was purified by reverse phase HPLC (2 to 50% of B; A = acetonitrile, B = H_2O buffered with 0.1 % aq. TFA) and lyophilized. The final product **3** (280 mg, 80% yield) was obtained as a colorless oil.

^{1}H NMR (400 MHz, d_6-DMSO): δ_H 0.88 (3H, t, J = 7.4 Hz), 1.24 – 1.39 (2H, m), 1.47 – 1.58 (2H, m), 1.53 – 1.68 (1H, m), 1.72 – 1.98 (3H, m), 2.20 – 2.36 (2H, m), 2.52 – 2.59 (1H, m), 4.00 (2H, t, J = 6.6 Hz).

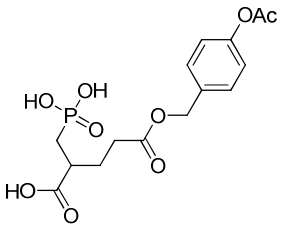
^{13}C NMR (101 MHz, d_6-DMSO): δ_C 13.58, 18.62, 27.61 (d, $J_{C,P}$ = 9.6 Hz), 29.50 (d, $J_{C,P}$ = 135.6 Hz), 30.18, 31.14, 63.55, 172.33, 175.52 (d, $J_{C,P}$ = 10.6 Hz).

^{31}P NMR (162 MHz, d_6-DMSO): δ_P 26.18.

ESI MS: 281 ([M]$^-$).

HR ESI MS: calcd for $C_{10}H_{18}O_7P$: 281.07956; found: 281.07968.

5-((4-Acetoxybenzyl)oxy)-5-oxo-2-(phosphonomethyl)pentanoic acid (1)



Compound **9b** (1.19 g, 2.76 mmol) was dissolved in freshly distilled DCM (22 mL) and the mixture was cooled down to 0 °C. Bromotrimethylsilane (2.54 g, 2.19 mL, 16.6 mmol, 6 equiv.) was added by syringe during 10 min. The reaction mixture was stirred at 0 °C for 1 h, slowly heated up to rt and stirred for further 14 h. DCM was evaporated and the rest of TMSBr was removed by co-distillation with dry $PhCH_3$ (3×20 mL). The bis(trimethylsilyl) intermediate was transformed to the desired phosphonic acid by addition of acetonitrile/H_2O (4:1, 25 mL) and solvents were removed in vacuo. The crude product was purified by reverse phase HPLC (2 to 50% of B; A = acetonitrile, B = H_2O buffered with 0.1% aq. TFA) and compound **1** was obtained as a colorless oil (333 mg, 33% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.62 (dt, 1H, J = 16.3, 6.4 Hz), 1.77 – 2.01 (2H, m), 2.26 (3H, s), 2.29 – 2.45 (3H, m), 2.54 – 2.63 (1H, m), 5.07 (2H, s), 7.12 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 8.5 Hz).

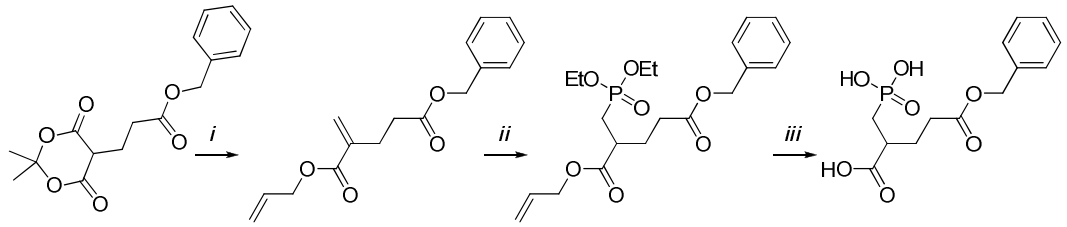
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 21.17, 28.29 (d, $J_{C,P}$ = 15.4 Hz), 28.60 (d, $J_{C,P}$ = 141.2 Hz), 31.46, 39.39, 66.06, 121.81 (2C), 129.56 (2C), 133.43, 150.55, 169.83, 173.46, 178.93 (d, $J_{C,P}$ = 5.0 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 26.04.

ESI MS: 373 ([M]$^-$).

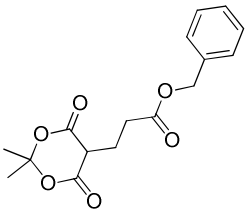
HR ESI MS: calcd for $C_{15}H_{18}O_9P$: 373.06939; found: 373.06891.

Scheme S2. Synthesis of Compound 4.



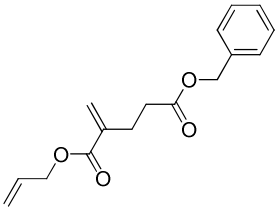
Reagents and Conditions: *i)* Eschenmoser's salt, allyl alcohol, 70 °C, 21 h; *ii)* diethyl phosphite, $Al(CH_3)_3$, DCM, 0 °C to rt, 16 h; *iii) 1)* TMS-Br, DCM, 0 °C to rt, 18 h; *2)* phenylsilane, $Pd(PPh_3)_4$, THF, rt, 1 h.

Benzyl 3-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)propanoate (10)



The compound was prepared according to the published procedure. ^1H and ^{13}C NMR spectra were in agreement with the published data.[29]

1-Allyl-5-benzyl-2-methylenepentanedioate(11)



A dry Schlenk flask was charged with the compound **10** (1.00 g, 3.26 mmol)and *N,N*-dimethylmethyleneiminium iodide (1.51 g, 8.16 mmol, 2.5 equiv.) and it was flushed with argon. Allyl alcohol (10 mL) was added and the mixture was stirred at 70-75 °C for 21 h. The organic solvent was evaporated in vacuo. The residue was dissolved in Et_2O (80 mL) and washed with sat. $NaHCO_3$ (50 mL), 10% $KHSO_4$ (50 mL), 10% $Na_2S_2O_5$ (50 mL), sat. NaCl (50 mL), and then dried over $MgSO_4$. The solvent was evaporated in vacuo. The crude product was purified by column chromatography (hexane/EtOAc, 10:1) to afford the desired product (817 mg, 91% yield) as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.56 – 2.62 (2H, m), 2.64 – 2.73 (2H, m), 4.65 (2H, dt, J = 5.6, 1.5 Hz), 5.12 (2H, s), 5.24 (1H, dq, J = 10.4, 1.5 Hz), 5.33 (1H, dq, J = 17.2, 1.5 Hz), 5.60 (1H,

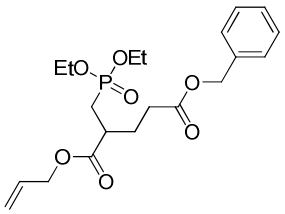
q, J = 1.3 Hz), 5.94 (1H, ddt, J = 17.2, 10.4, 5.6 Hz), 6.21 (1H, d, J = 1.0 Hz), 7.29 – 7.42 (5H, m).

^{13}C NMR (101 MHz, $CDCl_3$): δ_C27.47, 33.24, 65.51, 66.44, 118.28, 126.29, 128.36, 128.38 (2C), 128.67 (2C), 132.20, 136.01, 138.87, 166.35, 172.59.

ESI MS: 297 ($[M + Na]^+$).

HR ESI MS: calcd for $C_{16}H_{18}O_4Na$: 297.10973; found: 297.10980.

1-Allyl 5-benzyl 2-((diethoxyphosphoryl)methyl)pentanedioate (12)



Diethyl phosphite (376 µL, 2.92 mmol) was dissolved in absolute dichloromethane (25 mL) under argon and reaction mixture was cooled down to 0 °C. A solution of trimethyl aluminium (2 M in hexane, 1.46 mL, 2.92 mmol) was added dropwise and the solution was stirred at 0 °C for 30 min. A solution of the compound **11** (800 mg, 2.92 mmol) in dry dichloromethane (7 mL) was added and the cooling bath was removed. The reaction mixture was then stirred at rt overnight. The reaction was quenched with 1M HCl (20 mL), water phase was extracted with diethyl ether (3×50 mL), the combined organic layers were washed with water (50 mL), brine (50 mL) and dried over $MgSO_4$. The evaporation of the solvents afforded an oil, which was filtered through pad of silica gel (hexane/EtOAc, 1:1) to afford the desired product (1.16 g, 96% yield) as an oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H1.29 (3H, t, J = 7.0 Hz), 1.28 (3H, t, J = 7.1 Hz), 1.80 – 1.90 (1H, m), 1.95 – 2.08 (2H, m), 2.20 – 2.30 (1H, m), 2.23 – 2.46 (2H, m), 2.78 – 2.88 (1H, m), 4.02 – 4.11 (4H, m), 4.58 (2H, dt, J = 5.8, 1.4 Hz), 5.10 (2H, s), 5.23 (1H, dq, J = 10.4, 1.3 Hz), 5.32 (1H, dq, J = 17.2, 1.5 Hz), 7.29 – 7.38 (5H, m).

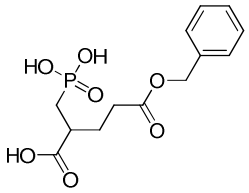
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 16.46 (d, $J_{C,P}$ = 2.0 Hz), 16.52 (d, $J_{C,P}$ = 2.0 Hz), 27.90 (d, $J_{C,P}$ = 142.8 Hz), 28.47 (d, $J_{C,P}$ = 12.5 Hz), 31.57, 39.36 (d, $J_{C,P}$ = 3.7 Hz), 61.92 (d, $J_{C,P}$ = 6.7 Hz), 61.98 (d, $J_{C,P}$ = 6.6 Hz), 65.75, 66.54, 118.77, 128.36 (2C), 128.40, 128.68 (2C), 131.97, 135.91, 172.41, 173.74 (d, $J_{C,P}$ = 8.6 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 31.09.

ESI MS: 435 ($[M + Na]^+$).

HR ESI MS: calcd for $C_{20}H_{29}O_7NaP$: 435.15431; found: 435.15433.

5-(Benzyloxy)-5-oxo-2-(phosphonomethyl)pentanoic acid (4)



The compound **12** (740 mg, 1.79 mmol) was dissolved in absolute dichloromethane (10 mL) under inert and cooled to 0 °C. Bromotrimethylsilane (950 μL, 7.18 mmol, 4 equiv.) was added dropwise and the solution was stirred at 0 °C overnight. The volatiles were removed in vacuo and the residue was diluted with mixture of acetonitrile/water (4:1, 5 mL) and evaporated. The residue was dissolved in absolute THF (8 mL). $Pd(PPh_3)_4$ (60 mg, 52 μmol, 5 mol%) was added and reaction mixture was cooled to 0 °C. Phenylsilane (512 μL, 4.16 mmol, 4 equiv.) was added and the cooling bath was removed. The reaction mixture was stirred at rt for 1 h. The volatiles were evaporated and the crude product was purified by preparative HPLC (acetonitrile/water, 2:98 to 50:50). The desired product (246 mg, 52% yield) was obtained as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.58 – 1.68 (1H, m), 1.77 – 1.88 (1H, m), 1.90 – 2.00 (2H, m), 2.31 – 2.45 (2H, m), 2.55 – 2.63 (1H, m), 5.09 (2H, s), 7.31 – 7.40 (5H, m).

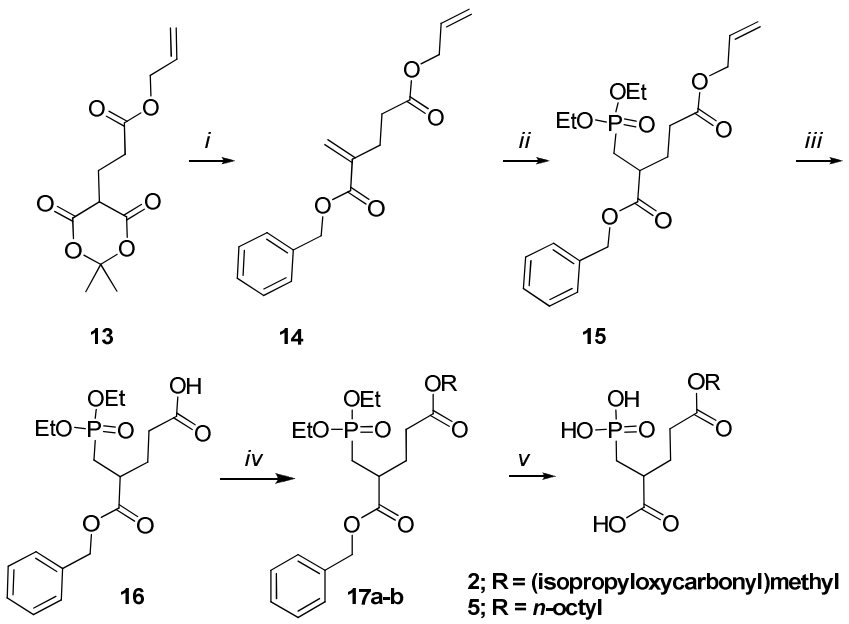
^{13}C NMR (101 MHz, DMSO): δ_C 27.54 (d, $J_{C,P}$ = 9.4 Hz), 29.56 (d, $J_{C,P}$ = 136.8 Hz), 31.13, 65.44, 127.88 (2C), 127.96, 128.43 (2C), 136.20, 172.21, 175.55 (d, $J_{C,P}$ = 10.3 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 26.13.

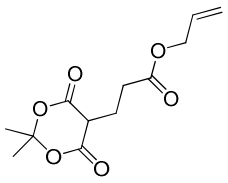
ESI MS: 315 ($[M - H]^-$).

HR ESI MS: calcd for $C_{13}H_{16}O_7P$: 315.06391; found: 315.06334.

Scheme S3. Synthesis of Compounds 2 and 5.



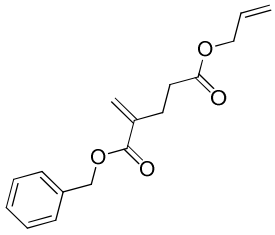
Reagents and Conditions: i) Eschenmoser's salt, BnOH, 70 °C, 21 h; *ii*) diethyl phosphite, $Al(CH_3)_3$, DCM, 0 °C to rt, 16 h; *iii*) phenylsilane, $Pd(PPh_3)_4$, THF, rt, 1 h; *iv*) for **17a**: POC-Cl, NaI, K_2CO_3, acetonitrile, rt to 50 °C, overnight; for **17b**: $C_8H_{17}Br$, NaI, K_2CO_3, acetonitrile, rt to 50 °C, overnight *v*) *1*) TMS-Br, DCM, 0 °C to rt, 18 h; *2*) Pd/C, H_2 (balloon), THF, rt, 22 h.



Allyl-3-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)propanoate (13)

Compound was prepared according to the published procedure. ^1H and ^{13}C NMR spectra were in agreement with published data. WO2016/22827 A1, 2016.

5-Allyl-1-benzyl-2-methylenepentanedioate (**14**)



A dry Schlenk flask was charged with the compound **13** (4.00 g, 15.6 mmol), *N,N*-dimethylmethyleneiminium iodide (7.22 g, 39.0 mmol, 2.5 equiv.) and then it was flushed with argon. Absolute benzyl alcohol (40 mL) was added and the mixture was stirred at 70-75 °C for 21 h. Benzyl alcohol was evaporated in vacuo (80-90 °C). The residue was dissolved in Et_2O (200 mL) and washed with sat. $NaHCO_3$ (150 mL), 10 % $KHSO_4$ (150 mL), 10% $Na_2S_2O_5$ (150 mL), sat. NaCl (150 mL), and dried over $MgSO_4$. The solvent was evaporated in vacuo and the crude product was purified by column chromatography (hexanes/EtOAc, 15:1) to afford the desired product (3.43 g, 80% yield) as a colorless liquid.

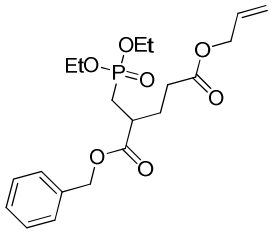
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.52 – 2.61 (2H, m), 2.65 – 2.73 (2H, m), 4.57 (2H, dt, *J* = 5.7, 1.5 Hz), 5.21 (2H, s), 5.22 (1H, dq, *J* = 10.4, 1.5 Hz), 5.30 (1H, dq, *J* = 17.2, 1.5 Hz), 5.63 (1H, q, *J* = 1.3 Hz), 5.90 (1H, ddt, *J* = 17.2, 10.4, 5.7 Hz), 6.25 (1H, d, *J* = 1.3 Hz), 7.30 – 7.42 (5H, m).

^{13}C NMR (101 MHz, $CDCl_3$): δ_C 27.44, 33.16, 65.29, 66.66, 118.39, 126.42, 128.18 (2C), 128.33, 128.69 (2C), 132.26, 136.04, 138.91, 166.55, 172.41.

ESI MS: 297 ($[M + Na]^+$).

HR ESI MS: calcd for $C_{16}H_{18}O_4Na$: 297.10973; found: 297.10983.

5-Allyl-1-benzyl-2-((diethoxyphosphoryl)methyl)pentanedioate (15)



Diethyl phosphite (1.31 g, 9.51 mmol) was dissolved in absolute dichloromethane (26 mL) under argon and cooled down to 0 °C. A solution of trimethyl aluminium (2 M in hexane, 4.76 mL, 9.51 mmol) was added dropwise and the mixture was stirred at 0 °C for 30 min. The solution of the compound **14** (2.61 g, 9.51 mmol) in dichloromethane (9 mL) was added over 10 min at 0°C, the mixture was stirred for further 30 min at 0°C and then heated up to room temperature and stirred overnight. After 15 h the reaction was quenched with 2M HCl (50 mL). The organic layer was separated and a water phase was extracted with DCM (2×50 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and then dried over $MgSO_4$. The crude product was filtered through pad of silica gel (EtOAc) to afford the desired product (3.24 g, 83% yield) as a colourless liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.27 (6H, dt, *J* = 7.1, 4.4 Hz), 1.86 (1H, ddd, *J* = 18.5, 15.5, 5.4 Hz), 1.94 – 2.06 (2H, m), 2.20 – 2.29 (1H, m), 2.30 – 2.36 (2H, m), 2.78 – 2.93 (1H, m), 3.98 – 4.11 (4H, m), 4.54 (2H, dt, *J* = 5.8, 1.4 Hz), 5.12 (2H, s), 5.21 (1H, dq, *J* = 10.4, 1.3 Hz), 5.28 (1H, dq, *J* = 17.2, 1.3 Hz), 5.87 (1H, ddt, *J* = 17.2, 10.4, 5.8 Hz), 7.28 – 7.39 (5H, m).

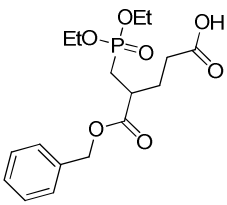
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 16.46 (d, $J_{C,P}$ = 1.8 Hz), 16.52 (d, $J_{C,P}$ = 1.8 Hz), 27.78 (d, $J_{C,P}$ = 142.7 Hz), 28.48 (d, $J_{C,P}$ = 12.4 Hz), 31.42, 39.39 (d, $J_{C,P}$ = 3.7 Hz), 61.86 (d, $J_{C,P}$ = 6.6 Hz), 61.95 (d, $J_{C,P}$ = 6.6 Hz), 65.32, 66.89, 118.41, 128.40 (2C), 128.43, 128.67 (2C), 132.16, 135.71, 172.18, 173.88 (d, $J_{C,P}$ = 8.5 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 30.80.

ESI MS: 435 ([M + Na]$^+$).

HR ESI MS: calcd for $C_{20}H_{29}O_7PNa$: 435.15431; found: 435.15432.

5-(Benzyloxy)-4-((diethoxyphosphoryl)methyl)-5-oxopentanoic acid (16)



Compound **15** (3.20 g, 7.76 mmol) was dissolved in dry THF (45 mL). $Pd(PPh_3)_4$ (179 mg, 0.155 mmol, 2 mol%) was added followed by dropwise addition of diethylamine (1.13 g, 1.60 mL, 15.5 mmol, 2 equiv.). The reaction mixture was stirred at rt for 2 h. The solvent was evaporated and the crude product was purified by column chromatography ($CHCl_3$/MeOH, 12:1). The desired compound was obtained as light yellow oil (2.63 g, 91% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.27 (6H, dt, J = 7.1, 0.7 Hz), 1.85 – 2.07 (3H, m), 2.19 – 2.42 (3H, m), 2.81 – 2.95 (1H, m), 4.02 – 4.11 (4H, m), 5.12 (2H, s), 7.28 – 7.40 (5H, m).

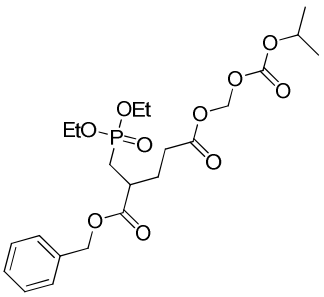
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 16.43, 16.49, 27.66 (d, $J_{C,P}$ = 142.9 Hz), 28.25 (d, $J_{C,P}$ = 12.0 Hz), 31.18, 39.22 (d, $J_{C,P}$ = 3.6 Hz), 62.20 (d, $J_{C,P}$ = 6.8 Hz), 62.28 (d, $J_{C,P}$ = 6.8 Hz), 66.96, 128.44 (2C), 128.48, 128.70 (2C), 135.70, 173.89 (d, $J_{C,P}$ = 9.2 Hz), 176.27.

^{31}P NMR (162 MHz, $CDCl_3$): δ_P31.26.

ESI MS: 371 ([M]$^-$).

HR ESI MS: calcd for $C_{17}H_{24}O_7P$: 371.12651; found: 371.12628.

1-Benzyl 5-(((isopropoxycarbonyl)oxy)methyl)-2-((diethoxyphosphoryl)methyl) pentanedioate (17a)



Compound**16** (500 mg, 1.34 mmol), NaI (402 mg, 2.69 mmol, 2 equiv.) and K_2CO_3 (371 mg, 2.69 mmol, 2 equiv.) were suspended in dry acetonitrile (10 mL), the reaction mixture was stirred at rt for 15 min and then chloromethyl isopropyl carbonate (POC-Cl) (0.36 mL, 2.69 mmol, 2 equiv.) was added dropwise. The reaction mixture was stirred at 50 °C overnight (16 h). The solvent was removed under reduced pressure and the residue was purified on silica gel (hexane/EtOAc, 1:2) to afford the desired product (495 mg, 75% yield) as an oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.25 – 1.29 (6H, m), 1.31 (6H, d, J = 6.3 Hz), 1.80 – 1.90 (1H, m), 1.94 – 2.09 (2H, m), 2.20 – 2.31 (1H, m), 2.32 – 2.42 (2H, m), 2.81 – 2.91 (1H, m), 4.01 –

4.09 (4H, m), 4.91 (1H, hept, J = 6.3 Hz), 5.13 (2H, s), 5.70 (1H, d, J = 5.7 Hz), 5.72 (1H, d, J = 5.7 Hz), 7.30 – 7.39 (5H, m).

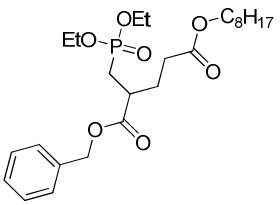
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 16.41 (d, $J_{C,P}$ = 1.5 Hz), 16.47 (d, $J_{C,P}$ = 1.4 Hz), 21.72, 27.81 (d, $J_{C,P}$ = 142.8 Hz), 27.89 (d, $J_{C,P}$ = 12.1 Hz), 31.07, 61.86 (d, $J_{C,P}$ = 6.6 Hz), 61.92 (d, $J_{C,P}$ = 6.5 Hz), 66.91, 73.16, 81.76, 128.40 (2C), 128.43, 128.65 (2C), 135.62, 153.40, 171.09, 173.67 (d, $J_{C,P}$ = 8.9 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 30.87.

ESI MS: 511 ($[M + Na]^+$).

HR ESI MS: calcd for $C_{22}H_{33}O_{10}PNa$: 511.17035; found: 511.17028.

1-Benzyl 5-octyl 2-((diethoxyphosphoryl)methyl)pentanedioate (17b)



Intermediate **16** (500 mg, 1.34 mmol), NaI (402 mg, 2.69 mmol, 2 equiv.) and K_2CO_3 (371 mg, 2.69 mmol, 2 equiv.) were suspended in dry acetonitrile (10 mL) and reaction mixture was stirred at rt for 15 min. 1-Bromooctane (0.46 mL, 2.69 mmol, 2 equiv.) was added dropwise. The reaction mixture was stirred at 50 °C overnight (16 h). The solvent was removed under reduced pressure and the residue was purified on silica gel (hexane/EtOAc, 1:1) to afford the desired product (341 mg, 52% yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.89 (3H, t, J = 6.8 Hz), 1.26 – 1.31 (16H, m), 1.56 – 1.62 (2H, m), 1.82 – 1.92 (1H, m), 1.95 – 2.07 (2H, m), 2.22 – 2.37 (3H, m), 2.81 – 2.92 (1H, m), 4.02 – 4.10 (6H, m), 5.14 (2H, s), 7.30 – 7.40 (5H, m).

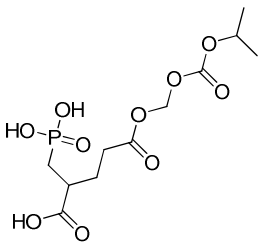
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 14.18, 16.43 (d, $J_{C,P}$ = 1.7 Hz), 16.49 (d, $J_{C,P}$ = 1.7 Hz), 22.72, 25.97, 27.89 (d, $J_{C,P}$ = 147.6 Hz), 28.54 (d, $J_{C,P}$ = 7.6 Hz), 28.66, 29.26, 29.29, 31.48, 31.87, 39.40 (d, $J_{C,P}$ = 3.7 Hz), 61.83 (d, $J_{C,P}$ = 6.6 Hz), 61.91 (d, $J_{C,P}$ = 6.5 Hz), 64.84, 66.84, 128.35 (2C), 128.39, 128.63 (2C), 135.73, 172.61, 173.89 (d, $J_{C,P}$ = 8.3 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 31.08.

ESI MS: 507 ($[M + H]^+$).

HR ESI MS: calcd for $C_{25}H_{41}O_7NaP$: 507.24821; found: 507.24812.

5-(((Isopropoxycarbonyl)oxy)methoxy)-5-oxo-2-(phosphonomethyl)pentanoic acid (2)



Compound **17a** (196 mg, 0.401 mmol) was dissolved in absolute dichloromethane (3 mL) under inert and cooled down to 0 °C. Bromotrimethylsilane (212 µL, 1.61 mmol, 4 equiv.) was added dropwise and the solution was stirred at 0 °C overnight (16 h). The volatiles were removed in vacuo and the residue was diluted with mixture of acetonitrile/water (4:1, 5 mL) and solvents were evaporated. The crude product was dissolved in absolute THF. 10% Pd/C (44 mg, 0.040 mmol, 10 mol%) was added and reaction mixture was bubbled with hydrogen for 10 min. The reaction mixture was stirred at rt overnight (16 h) under hydrogen atmosphere. The precipitate was removed by filtration through cotton and the volatiles were removed in vacuo to afford desired product (130 mg, 95% yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.28 (6H, d, J = 6.3 Hz), 1.89 – 2.09 (3H, m), 2.19 – 2.38 (1H, m), 2.40 – 2.57 (2H, m), 2.72 – 2.90 (1H, m), 4.88 (1H, hept J = 6.2 Hz), 5.71 (2H, s).

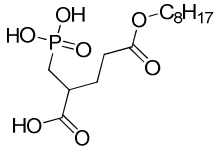
^{13}C NMR (101 MHz, $CDCl_3$): δ_C 21.66 (2C), 27.75, 27.91, 31.08, 39.17, 73.13, 82.04, 153.51, 171.89, 179.12 (d, $J_{C,P}$ = 5.3 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 33.27.

ESI MS: 341 ($[M - H]^-$).

HR ESI MS: calcd for $C_{11}H_{18}O_{10}P$ 341.06321; found 341.06277.

5-(Octyloxy)-5-oxo-2-(phosphonomethyl)pentanoic acid (5)



Compound **17b** (790 mg, 1.63 mmol) was dissolved in absolute dichloromethane (9 mL) under inert and cooled down to 0 °C. Bromotrimethylsilane (860 µL, 6.52 mmol, 4 equiv.) was added dropwise and the solution was stirred at 0 °C overnight (16 h). The volatiles were removed in vacuo and the residue was diluted with a mixture of acetonitrile/water (4:1, 5 mL) and solvents were evaporated. The residue was dissolved in absolute THF, 10% Pd/C (173 mg, 0.163 mmol, 10 mol%) was added and reaction mixture was bubbled with hydrogen for 10 min. The reaction mixture was stirred at rt overnight (16 h) under hydrogen atmosphere (balloon). The crude

reaction mixture was filtered through cotton to remove the precipitates and the volatiles were evaporated to afford desired product (529 mg, 96% yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.85 – 0.92 (3H, m), 1.20 – 1.37 (10H, m), 1.56 – 1.65 (2H, m), 1.90 – 2.05 (3H, m), 2.20 – 2.35 (1H, m), 2.37 – 2.50 (2H, m), 2.77 – 2.80 (1H, m), 4.06 (2H, t, J = 6.8 Hz).

^{13}C NMR (101 MHz, $CDCl_3$): δ_C 14.21, 22.76, 25.98, 28.63, 29.31, 29.36, 31.65, 31.93, 39.55, 65.39, 173.96, 179.19 (d, $J_{C,P}$ = 3.5 Hz).

^{31}P NMR (162 MHz, $CDCl_3$): δ_P 32.70,

ESI MS: 337 ($[M - H]^-$).

HR ESI MS: calcd for $C_{14}H_{26}O_7P$: 337.14216; found: 337.14156.

2. Mass Transitions for LC-MS/MS analysis of Prodrugs 1-5

Table S1: Mass transitions and conditions for intact prodrugs used for LC/MS/MS analysis

Ionization Mode	ESI, MRM(+)			
Compound	Q1	Q3	CE	S-Lens
1	375.121	107.005, 191.031	35, 23	58
2	343.100	148.970, 191.011	31, 19	49
3	283.160	148.982, 191.031	24, 15	55
4	316.855	91.007	32	74
5	339.003	148.957, 190.987	29, 18	87
Losartan (IS)	423.200	180.088, 207.107	35, 22	99