Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Discovery of GS-8374, a Potent Human Immunodeficiency Virus Type 1 Protease Inhibitor with Superior Resistance Profile

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Biological Assays

HIV-1 protease enzymatic assay

The assay was carried out in 96-well format using a fluorogenic synthetic hexapeptide substrate (2-aminobenzoyl)Thr-Ile-Nle-(p-nitro)Phe-Gln-Arg (Bachem, Torrance, CA). Exact concentration of active protease was defined by active site titration. For Ki determination, serial dilutions of the tested inhibitors were prepared in a reaction buffer (100 mM ammonium acetate pH 5.3, 1 M NaCl, 1 mM EDTA, 1 mM DTT, 10% DMSO). Mixtures of the inhibitor and ~1 nM enzyme were pre-incubated for 15 min at 37 °C. The reaction was initiated by adding substrate to a final concentration of 40 μM. Real-time reaction kinetics was measured at 37 °C using Gemini 96-well plate fluorimeter (Molecular Devices, Sunnyvale, CA) at λ(Ex)=330 nm and λ(Em)=420 nm. Apparent inhibition constant (Ki_{app}) values were calculated using Prism4 software according to an algorithm for tight binding inhibition (EnzFitter software, Biosoft, Cambridge, U.K.). Inhibition constants (Ki) were calculated using equation Ki_{app}=Ki (1+[S]/Km) with the substrate Km value of 8 μM.

Viral strains

HIV-1 strain IIIB (Advanced Biotechnologies, Columbia, MD) was used for the acute infection of MT-2 cells. To generate the other protease mutant viruses, protease-encoding sequences were amplified from plasma samples of selected PI-experienced patients. Viral RNA was purified using QiaAmp Viral RNA kit (Qiagen, Valencia, CA) and used for the first-strand DNA synthesis with a primer 5′-CTGTATTTCTGCTATTAAGTCTTTTGATGGG-3′ and Ready-To-Go First-Strand beads (Amersham Biosciences, Piscataway, NJ). Protease-encoding sequence together with flanking sequences were amplified by nested PCR using Expand High Fidelity PCR System (Roche Applied Science) and two sets of primers (5′-TACTAATGCTTTTATTTTTTC-3′ and 5′-GCTAATTTTTTAGGGAAGATCTG-3′ for the first round amplification; 5′-CTAATGCTTTTATTTTTTCTT-3′ and 5′-TTTTTAGGGAAGATCTGGCCTTC-3′ for the second round amplification). Amplified DNA fragments were sequenced and transfected into exponentially growing
Sup-T1 cells together with a modified HIV-1 HXB2 DNA backbone that had the protease coding sequence deleted. Supernatants from the transfected cells were

harvested after a widespread cytopathic effect was observed. Virus strain containing M46I/I50V protease mutations was selected in vitro from the wild-type HIV-1 IIIB strain in the presence of increasing concentrations of amprenavir (up to 5 μM) over the period of six months. All viral stocks were prepared by harvesting supernatants from infected MT-2 or Sup-T1 cells. Following the RNA isolation and DNA amplification described above, protease sequence of each virus was verified using automated sequencing. Prepared stocks of recombinant viruses were tittered in MT-2 cells and used for antiviral susceptibility studies.

Antiviral activity assays

MT-2 cells (Stanford University, Palo Alto, CA) were maintained in RPMI-1640 medium supplemented with antibiotics and 10% fetal bovine serum (FBS). Cells were passaged twice a week and kept at the density of $< 0.6x10^6$/mL. Cells were infected in bulk with HIV-1 III_B (Advanced Biotechnologies Inc., Columbia, MD) at a multiplicity of infection (MOI) of 0.01 for 2 hours at 37 °C and mixed in 96-well plates with 5-fold serial dilutions of tested compounds at a density of 20,000 cells/well in a final assay volume of 200 μL. Following 5-day incubation at 37°C, the virus-induced cytopathic effect was determined using a cell viability assay. One hundred μL media was removed from each well and replaced with 100 μL of phosphate-buffered saline containing 1.7 mg/mL XTT [2,3,-bis(methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; Sigma, St. Louis, MO) and 5 μg/mL PMS (phenazine methosulfate; Sigma). Following 1-hour incubation at 37°C, 20 μL of 2% Triton X-100 was added to each well and absorbance was read at 450 nm with a background subtraction at 650 nm. Results were processed by regression analysis to determine EC_{50} values for the tested compounds. Similar assays were performed in the presence of 50% pooled human serum (Hyclone, Logan, UT).

HIV antiviral assay with human serum proteins

The effect of human serum (HS) and serum proteins on the antiviral activity was evaluated by the addition of HS or specific serum proteins to the infected cells after the 3-hour infection step described above. The antiviral assay was carried out in parallel in the absence and presence of HS. HS was used at 40% (v/v) concentration.

The assay incubation time, read-out, and data analysis were same as described in the above section.

Cytotoxicity assays

MT-2 cells. Cells were mixed in 96-well plates with 5-fold serial dilutions of tested compounds at a density of 20,000 cells/well in a final assay volume of 200 μL. After a 5-day incubation at 37 °C, cells were stained with XTT as described above. The cell viability was expressed as a percentage of the signal from untreated samples (0% cytotoxicity) after the subtraction of signal from samples treated with 1 μM of podophyllotoxin (Sigma) (100% cytotoxicity). The concentration of each drug that reduced the cell viability by 50% (CC_{50}) was determined by non-linear regression analysis using the Prism software.

HepG2 cells. HepG2 human hepatoma cells (ATCC, Manassas, VA) were maintained in DMEM supplemented with 2 mM L-glutamine, 10% FBS, 1 mM sodium pyruvate, and antibiotics. Cells were plated in 96-well plates at a density of 8,000 cells/well in a media volume of 200 μL. The next day, 100 μL of media was removed from each sample well and replaced with fresh media containing 5-fold serial dilutions of tested compounds. After incubation at 37°C for 72 hours, cells were stained with XTT and the data were processed as described above.

DMPK Evaluations

Hepatic Microsomal Stability

Pooled hepatic micriosomal fractions and the components for the NADPH regenerating system were obtained from BD Biosciences (Bedford MA). HIV-PIs, apart from compound **1** (GS-8374), were purified from clinical dose forms. Cobicistat was prepared by Gilead Sciences Medicinal Chemistry Department. Incubations were performed at 37°C in duplicate. Final reaction conditions were: 3 μM test compound, 1 mg microsomal protein/mL, 1.25 mM NADP, 3.3 mM glucose 6 phosphate, 0.4 U/mL glucose 6 phosphate dehydrogenase and 3.3 mM $MgCl_2$ in 50 mM potassium phosphate buffer, pH 7.4. Aliquots were removed over a period of 60 minutes and quenched with a mixture of acetonitrile, methanol and water (1:2:2 v/v/v) containing th internal standard for mass spectrometry (MS). Analytes were quantified

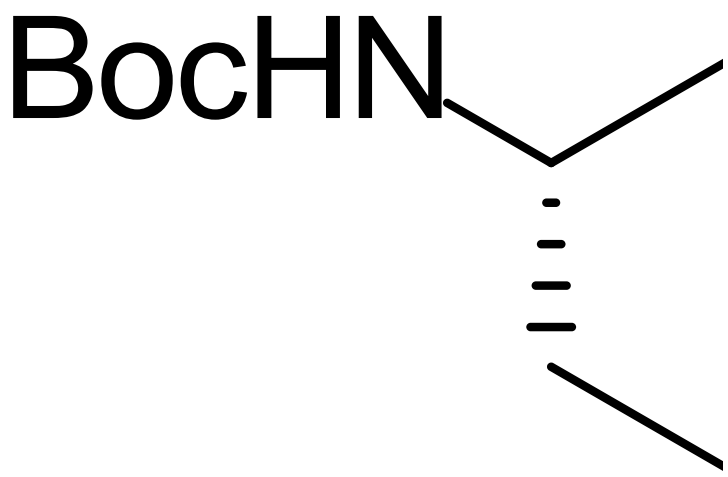
by analyte/internal standard peak area ratios (PAR) measured on a Micromass Quattro Premier XL tandem triple quadrupole mass spectrometer coupled to an Agilent 1200 Series HPLC system with a Leap Technologies HTC PAL autosampler. The connection was through an electrospray interface operating in positive ionisation mode. The column used was a Phenomenex MercuryMS, Synergi 2 Max-RP (100 Å pore size, 2 µm particle size, 20 x 2.0 mm). The initial mobile phase was a mixture of acetonitrile, formic acid and water (1 : 0.2 : 98.8 v/v/v) and elution was achieved by a series of linear gradients with increasing proportions of acetonitrile. The sample injection volume was 10 µL. The rate of loss of parent was calculated using the in vitro half-life method (Obach, 1999). In some cases the rate of metabolism was determined in the presence of 1 µM ritonavir or 1 µM cobicistat.

Pharmacokinetic studies

Single dose pharmacokinetic studies were performed in beagle dogs (3 animals per group). GS-8374 was formulated as a solution and was administered by oral gavage or by intravenous infusion over 30 minutes. In some cases the oral dose was coformulated with cobicistat. Serial blood samples were collected and plasma prepared. Quantification was by LC-MS/MS using methods similar to those used for hepatic microsomal stability, except quantification was by reference to an extracted standard curve. The mass spectrometer was a Thermo Finnigan TSQ Ultra and the column was a ThermoHypersil HyPurity C18 (30 x 2.1 mm, 5 µm particle size). Pharmacokinetic parameters were calculated using non-compartmental methods.

Experimental Procedures

Preparation of GS-8374 (compound 1), compounds 4 and 5



[a] Reagents and conditions: (i) isobutylamine, 2-propanol (iPrOH), reflux. (ii) 4-methoxybenzenesulfonyl chloride, diisopropylethylamine, CH_2Cl_2, 0 ºC. (iii) TFA. (iv) bis-(4-nitrophenyl)carbonate, Et_3N, CH_2Cl_2, rt. (v) N,N-dimethylaminopyridine, CH_3CN. (vi) H_2 (1atm), 10% Pd/C, EtOH, EtOAc. (vii) (diethoxyphosphoryl)methyl trifluoromethanesulfonate, Cs_2CO_3, THF. (viii) CH_3I, Cs_2CO_3, CH_3CN.

Compound 13: A solution of epoxide **12** (30 g, 81.2 mmol), obtained through commercial sources, and isobutylamine (80 mL, 812 mmol) in propan-2-ol (300 mL) was stirred at 80^{o}C for 1 hour. After removing the solvent under reduced pressure, the crude product **13** obtained as an off-white solid (36 g, 100% yield). ^{1}H NMR

($CDCl_3$) δ 7.45-6.91 (m, 9H), 5.02 (s, 2H), 4.62 (m, 1H), 3.75 (m, 1H), 3.42 (m, 1H),2.91-2.34 (m, 7H), 1.73 (m, 1H) 1.37 (s, 9H) 0.92 (m, 6H); MS (ESI) m/z 443 $(M+H)^+$.

Compound 14: Compound **13** (36 g, 81.3 mmol) in a solution of CH_2Cl_2 (400 mL) was cooled to 0 ^oC, 4-methoxybenzene-1-sulfonyl chloride (16.8 g, 81.3 mmol) and TEA (23 mL, 163 mmol) were added and the resulting mixture was stirred for 30 min at 0^oC and then for 1 hour at room temperature. The solvent was removed under reduced pressure, the crude product was recrystallized from EtOAc to give compound **14** as a white solid (47 g, 96% yield). ^1H NMR ($CDCl_3$) δ 7.75-6.91 (m, 13H), 5.02 (s, 2H), 4.62 (m, 1H), 3.94-3.68 (m, 5H), 3.17-2.78 (m, 7H), 1.91 (m, 1H) 1.18 (s, 9H) 0.92 (m, 6H); MS (ESI) m/z 613 $(M+H)^+$.

Compound 15: To a solution of compound **14** (5.5 g, 9 mmol) in CH_2Cl_2 (18 mL) was added trifloroacetic acid (9 mL), the mixture was stirred at room temperature for 3 hours. The resulting mixture was evaporated to a small volume, and the residue was taken into partition between EtOAc and saturated aqueous NaCl. The organic layers were collected, dried over Na_2SO_4, filtered and evaporated to dryness to provide crude product compound **15** as an off-white solid (4.4 g, 95% yield), which was used without further purification. ^1H NMR ($CDCl_3$) δ 7.75-6.91 (m, 13H), 5.01 (s, 2H), 4.18 (m, 2H), 3.91 (s, 3H), 3.82 (m, 2H), 3.17-2.78 (m, 7H), 1.85 (m, 1H), 0.92 (m, 6H); MS (ESI) m/z 513 $(M+H)^+$.

Compound 17 To a solution of **16** (1.6 g, 13 mmol) and bis-(4-nitrophenyl)carbonate (6 g, 19.7 mmol) in CH_2Cl_2 (40 mL) was added TEA (2.8 mL, 20 mmol) and the resulting mixture was stirred at room temperature for 48 hours. After the solvent was removed, the residue was taken into partition between EtOAc and saturated aqueous NaCl. The organic layers were collected, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Purification with flash column chromatograph (silica gel; 10%-50% EtOAc /hexane) afforded the compound **17** as a white solid (2.4 g, 67% yield). ^1H NMR ($CDCl_3$) δ 8.30 (d, J = 9.4 Hz, 2H), 7.39 (d, J = 9.4 Hz, 2H), 5.78 (d, J = 5.1 Hz, 1H), 5.25 (m, 1H), 4.19-3.94 (m, 4H), 3.18 (m, 1H), 2.18-2.02 (m, 2H); MS (ESI) m/z 296 $(M+H)^+$.

Compound 18: To a solution of compounds **15** (4 g, 7.8 mmol) and **17** (2.3 g, 7.8 mmol) in acetonitrilee (46 mL) at 0^{o}C was added DMAP (1.9 g , 15.6 mmol) and the mixture was stirred at 0 ^{o}C for 1 hour. After the solvent was removed, the residue was taken into partition between EtOAc and saturated aqueous NaCl. The organic phase was collected, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification with flash column chromatograph (silica gel; 30%-50% EtOAc/hexanes) provided the compound **18** as a white solid (4.1 g, 80% yield). ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 8.7 Hz, 2H),7.44-7.33 (m, 5H), 7.13 (d, J = 8.7z, 2H), 6.99 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 5.66 (d, J = 5.1 Hz, 1H), 5.08-4.90 (m, 3H), 3.98-3.70 (m, 6H), 3.18-2.76 (m, 8H), 1.89-1.57(m, 7H), 0.94-0.88 (m, 6H). MS (ESI) m/z 669 $(M+H)^{+}$.

Compound 4: A solution of compound **18** (4 g) in EtOH (200 mL) and EtOAc (100 mL) was added 10% Pd/C (400 mg) and allowed to stir at room temperature under H_2 atmosphere (balloon) for 6 hours. The catalyst was removed by filtration through celite. The residue was washed and the filtrates were combined and evaporated under reduced pressure. Purification with flash column chromatograph (silica gel; 10%-50% EtOAc/hexanes) afforded compound **4** as a white solid (3.28 g, 95 %). HPLC purity: 98.9%. ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.7 Hz, 2H), 7.08 (d,J = 8.7z, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.75 (d, J = 8.7 Hz, 2H), 5.66 (d, J = 5.1 Hz, 1H), 5.08-4.90 (m, 2H), 3.98-3.70 (m, 6H), 3.18-2.76 (m, 8H), 1.89-1.57(m, 7H), 0.94-0.88 (m, 6H). MS (ESI) m/z 579 $(M+H)^{+}$.

GS-8374 (compound 1) To a solution of phenol **4** (2 g, 3.5 mmol) and (diethoxyphosphoryl)methyl trifluoromethanesulfonate (1.4 g, 4.6 mmol) in acetonitrile (50 mL) at 0 ^{o}C was added Cs_2CO_3 (1.1 g, 5.3 mmol) in four portions over 20 minutes, and the mixture was stirred at 0 ^{o}C for 40 minutes and then room temperature for 1 hour. After the solvent was removed, the residue was taken into partition between EtOAc and saturated aqueous NaCl. The organic phase was separated, dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. Purification with flash column chromatograph (silica gel; 1 %-4 % MeOH /CH_2Cl_2) afforded compound **1**(GS-8374) as a white foam (2.2 g, 90% yield). HPLC

purity: 99.1%. ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.7 Hz, 2H), 7.16 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.66 (d, J = 5.1 Hz, 1H), 5.08-4.90 (m, 2H), 4.29-4.19 (m, 6H), 3.98-3.68 (m, 10H), 318-2.76 (m, 7H), 1.89-1.51 (m, 3H), 1.38 (t, J = 7.2 Hz, 6H), 0.94-0.88 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 19.4; MS (ESI) 752 m/z $(M+Na)^+$.

Compound 5: To a solution of phenol (150 mg, 0.26 mmol) and CH_3I (0.017mL 0.28 mmol) in acetonitrile (3 mL) at 0^{o}C was added Cs_2CO_3 (97 mg, 0.3 mmol) and the mixture was stirred at room temperature for overnight. After the solvent was removed, the residue was partitioned between EtOAc and saturated aqueous NaCl. The organic phase was separated, dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. Purification with flash column chromatograph (silica gel; 1%-4% MeOH /CH_2Cl_2) yielded compound **5** as a white foam (100 mg, 65% yield). HPLC purity: 96.3%. ^{1}H NMR ($CDCl_3$) δ 7.70 (d, 2H), 7.13 (d, 2H), 6.99 (d, 2H), 6.82 (d,2H), 5.66 (d, 1H), 5.05-4.95 (m, 2H), 3.98-3.67 (m,13H), 3.19-2.76 (m, 7H), 1.89-1.57(m, 3H), 0.94-0.88 (m, 6H), (ESI) m/z 593 $(M+H)^+$.

Preparation of compounds 6 and 7

[a] Reagents and conditions: (i) $PhNTf_2$, Cs_2CO_3, CH_2Cl_2. (ii) $Pd(OAc)_2$/dppp, DMF/MeOH/Et_3N, 70 °C. (iii) $HPO(OEt)_2$, $Pd(PPh_3)_4$, 4-methylmorpholine, CH_3CN, 75 °C

Compound 19: To a solution of phenol **4** (240 mg, 0.41mmol) in anhydrous CH_2Cl_2 (5 mL) was added N-phenyltrifluoromethanesulfonimide (161 mg, 0.45 mmol) and Cs_2CO_3 (147 mg, 0.45 mmol). The mixture was stirred at room temperature for overnight (~16 hours). The solvents were removed under reduced pressure and the residue was purified by flash column chromatograph (silica gel; 5-10% MeOH/CH_2Cl_2) to provide compound **19** as a white solid (280 mg, 95%). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 8.6 Hz, 2 H), 7.32 (d, J = 8.6 Hz, 2 H), 7.19 (d, J = 8.5 Hz, 2 H), 7.00 (d, J = 8.5 Hz, 2 H), 5.65 (d, J = 5.1 Hz, 1 H), 5.0 (m, 2 H), 4.0-3.8 (m, 3 H), 3.88 (s, 3 H), 3.75 (m, 3 H), 3.2-2.75 (m, 7 H), 1.8 (m, 1 H), 1.7 (m, 1 H), 1.55 (m, 1 H), 0.93 (d, J = 6.7 Hz, 3 H), 0.89 (d, J = 6.7 Hz, 3 H). MS (ESI) m/z 711 $(M+ H)^+$.

Compound 6: To a solution of **23** (71 mg, 0.1 mmol) in anhydrous DMF (0.5 mL) and methanol (0.5 ml) was added 1,3-bis(diphenylphosphino)propane (dppp) (3 mg), followed by $Pd(OAc)_2$ (2 mg) and triethylamine (60 μl). The mixture was heated at 70 °C under carbon monoxide (balloon) for 20 hour. The solvents were removed under reduced pressure and the crude product was purified by flash column chromatograph (silica gel; 10%-60% EtOAc/hexanes) to afford methyl ester as a white powder (37 mg, 60%). HPLC purity: 96.5%. ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 8 Hz, 2 H), 7.70 (d, J = 9.2 Hz, 2 H), 7.30 (d, J = 8 Hz, 2 H), 6.99 (d, J = 9.2 Hz, 2 H), 5.64 (d, J = 4.8 Hz, 1 H)), 4.99 (m, 2 H), 4.0-3.8 (m, 10 H), 3.72-3.68 (m, 4 H), 3.2-2.9 (m, 7 H), 1.80 (m, 1 H), 1.50 (m, 1 H), 0.94-0.88 (m, 6 H); MS (ESI) 620.9 $(M+ H)^+$.

Compound 7: To a solution of compound 23 (71 mg, 0.1 mmol) in acetonitrile (1 ml) was added 4-methylmorpholine (15 μl, 0.14 mmol), followed by diethyl phosphite (15 μl, 0.11 mmol) and tetrakis(triphenylphosphine)palladium(0) (10 mg). The mixture was purged with nitrogen and heated at 75 °C for 16 hours. The solvents were removed under reduced pressure and the residue was purified by flash column chromatograph (silica gel; EtOAc) to provide compound **28** as a white powder (50 mg, 72%). HPLC purity: 96.0%. ^{1}H NMR ($CDCl_3$) δ 7.75-7.70 (m, 4 H), 7.35-7.32 (m, 2 H), 6.99 (d, J = 9 Hz, 2 H), 5.64 (d, J = 5.1 Hz, 1 H), 5.0 (m, 2 H), 4.16 -4.07 (m, 4 H), 3.96-3.60 (m, 11 H), 3.15-2.81 (m, 7 H), 1.79 (m, 1 H), 1.7-1.45 (m, 1 H),

1.32 (t, J = 7.1 Hz, 6 H), 0.93-0.87 (m, 6 H); ^{31}P NMR ($CDCl_3$) δ 18.6; MS (ESI) 699.0 $(M+ H)^+$.

Preparation of compound 8

[a] Reagents and conditions: (i) 1,3-bis(diphenylphosphino)propane (dppp), CO(1atm), $Pd(OAc)_2$, TEA, triethylsilane, DMF, 70 ºC, 20 h. (ii) $NaBH_4$, THF/H_2O, -10 ºC. (iii) MsCl, Et_3N, THF/CH_2Cl_2, 0 ºC-rt; LiBr, rt. (iv) $P(OEt)_3$, toluene, 120 ºC, ~16 hours.

Compound 20: To a solution of **19** (500 mg, 0.7 mmol) in anhydrous DMF (3.5 mL) was added 1,3-bis(diphenylphosphino)propane (dppp) (17.4 mg, 0.042 mmol) and $Pd(OAc)_2$ (9.5 mg, 0.042 mmol) under CO balloon. Then TEA (0.4 mL, 2.8 mmol) and triethylsilane (0.23 mL, 1.4 mmol) were added slowly. The mixture stirred at 70 ºC for 20 hours. The solvents were removed under reduced pressure and the crude product was purified by column chromatograph (silica gel; 10-40% EtOAc/CH_2Cl_2) to afford compound **20** as a white solid (336 mg, 81%). ^{1}H NMR ($CDCl_3$) δ 9.99 (s, 1H), 7.81 (d, J = 8.7 Hz, 2H), 7.71 (d,J = 8.7z, 2H), 7.42 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.1 Hz, 1H), 5.05-4.99 (m, 2H), 3.98-3.70 (m, 6H), 3.18-2.76 (m, 10H), 1.89-1.57(m, 4H), 0.95-0.88 (m, 6H). MS (ESI) m/z 591 $(M+ H)^+$.

Compound 21: To a stirred solution of aldehyde **20** (0.80 g 1.35 mmol) in THF (9 mL) and H_2O (1 mL) at -10°C was added $NaBH_4$ (0.13 g, 3.39 mmol). The reaction mixture was stirred for 1 hour at -10°C and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with $NaHSO_4$, H_2O, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Purification with flash column chromatograph (silica gel; 6% 2-propanol/CH_2Cl_2) afforded compound **21** as

a white solid (0.56 mg, 70% yield). ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 9.0 Hz, 2 H), 7.29 (d, J = 7.9 Hz, 2 H), 7.22 (d, J = 8.2 Hz, 2 H), 6.99 (d, J = 8.6 Hz, 2 H), 5.65 (d, J = 5.5 Hz, 1 H), 5.05-4.90 (m, 2 H), 4.66 (s, 2 H), 4.0-3.8 (m, 3 H), 3.88 (s, 3 H), 3.8-3.6 (m, 3 H), 3.2-2.8 (m, 7 H), 1.9-1.6 (m, 3 H), 0.97-0.85 (m, 6 H); MS (ESI) 592.9 (M+ H)$^{+}$.

Compound 22: To a stirred solution of alcohol **21** (77 mg 0.13 mmol) in THF (1 mL) and CH_2Cl_2 (1 mL) at 0 °C was added triethylamine (0.027 mL, 0.20 mmol) and methanesulfonyl chloride (0.011 mL, 0.14 mmol). The reaction mixture was stirred at 0 °C for 30 min and warmed to room temperature for 3 hours. Lithium bromide (60 mg, 0.69 mmol) was added and the resulting mixture was stirred for 45 min. The mixture was then concentrated and the residue was partitioned between CH_2Cl_2 and H_2O. The organic phase was collected, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatograph (silica gel; 2% MeOH/CH_2Cl_2) to give compound **22** (60 mg, 70% yield). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 9 Hz, 2 H), 7.31 (d, J = 8.2 Hz, 2 H), 7.21 (d, J = 7.5 Hz, 2 H), 7.00 (d, J = 9 Hz, 2 H), 5.64 (d, J = 5.1 Hz, 1 H), 5.0-4.95 (m, 2 H), 4.46 (s, 2 H), 4.0-3.8 (m, 3 H), 3.88 (s, 3 H), 3.73-3.67 (m, 3 H), 3.2-2.8 (m, 7 H), 1.85 (m, 1 H), 1.64 (m, 1 H), 1.56 (m, 1H), 0.97-0.87 (m, 6 H); MS (ESI) 654.9 and 656.9 (M+ H)$^{+}$.

Compound 8: A mixture of bromide **22** (49 mg 0.075 mmol) and triethylphosphite (0.13 mL, 0.75 mmol) in toluene (1.5 mL) was heated at 120 °C for over night (~16 hours). The resulting mixture was cooled to room temperature and then concentrated. The crude product was purified by flash column chromatograph (silica gel; 6% MeOH/CH_2Cl_2) to give compound **8** as a white solid (35 mg, 66% yield). Purity: 95%. ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 8.7 Hz, 2H), 7.27-7.16 (m, 4H), 7.00 (d, J = 8.7 Hz, 2H), 5.66 (d, J = 5.1 Hz, 1H), 5.00 (m, 2H), 4.04-3.73 (m, 13H), 3.13-2.80 (m, 9H), 1.82-1.64 (m, 3H), 1.25 (t, J = 6.9 Hz, 6H), 0.92 (t, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); ^{31}P NMR ($CDCl_3$) δ 26.4; MS (ESI) m/z 735 (M+Na)$^{+}$.

Preparation of compound 9

[a] Reagents and conditions: (i) Ph_3P, Et_3N, CH_2Cl_2, 0 ºC-rt. (ii) **23**, diisopropylethylamine, toluene. (iii) H_2 (1atm), 10% Pd/C, EtOH.

Compound 25: To a stirred solution of triphenylphosphine 445 mg (1.7 mmol) in anhydrous CH_2Cl_2 (2.5 mL) was added a solution of **23** (450 mg, 1.5 mmol) in CH_2Cl_2 (0.5mL) dropwise at 0 °C under N_2. The mixture was allowed to warm to room temperature and then stirred overnight (~16 hours). The solvent was removed under reduced pressure to about one-third of the volume and the remaining oil triturated with ether. A white solid was formed and collected by filtration. The solid was washed with ether (10 mL x 2) to give compound **24** as a white solid (716 mg, 85%).

To a stirred solution of aldehyde **20** (44 mg, 0.074 mmol), in anhydrous toluene (1 mL) and DMF (0.15 mL) was added **24** at room temperature under N_2, followed by addition of diisopropylethylamine (77 μL, 0.44 mmol). The resulting mixture was slowly heated to 100 °C for 20 hours. The solvents were removed under reduced pressure and the residue was purified by flash column chromatograph (silica gel column; 1-5% MeOH/CH_2Cl_2) to afford compound **25** as a white solid (35 mg, 65%). ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 2H), 7.42 (d, 2H), 7.25 (d, 2H), 6.99 (d, 2H), 6.22 (m ,2H), 5.65 (d, 1H), 5.03-4.95 (m, 2H), 4.11 (m, 3H), 3.98-3.70 (m,10H), 3.18-2.78 (m, 7H),1.89-1.67(m, 4H), 1.33 (m, 6H), 0.94-0.88 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 19.5; MS (ESI) m/z 725 $(M+ H)^+$.

Compound 9: Compound **25** (20 mg) in a solution of EtOH (4 mL) was added 10% Pd/C (4mg) and the resulting mixture was allowed to stir at room temperature under H_2 atmosphere (balloon) for 3 hours. The catalyst was removed by filtration through celite. The residue was washed and the filtrates were combined and evaporated under reduced pressure. The resulting residue was purified by reverse phase HPLC (25-100% acetonitrile/H_2O) to yield compound **9** as a white solid (19 mg, 96 %). HPLC purity: 97.4%. ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 2H), 7.14 (m, 4H), 6.99 (d, 2H), 6.88 (d,2H), 5.65 (d, 1H), 5.03-4.95 (m, 3H), 4.11 (m, 3H), 3.98-3.70 (m,10H), 3.18-2.78 (m, 7H), 2.01 (m, 4H), 1.89-1.67(m, 3H), 1.33 (m, 6 H), 0.94-0.88 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 30.6. MS (ESI) m/z 727 $(M+H)^+$.

Preparation of compounds 10 and 11

H

[a] Reagents and conditions: (i) (bis(benzyloxy)phosphoryl)methyl trifluoromethanesulfonate, Cs_2CO_3, CH_3CN. (ii) H_2 (1atm), 10% Pd/C, EtOH, (iii) For compound **10**: DCC, MeOH, pyridine or for compound **11**: DCC, iPrOH, pyridine.

Compound 26: To a solution of phenol **4** (2.89 g, 5 mmol) and (bis(benzyloxy)phosphoryl)methyl trifluoromethanesulfonate (4.24 g, 10 mmol) in acetonitrile (60 mL) at 0 °C was added Cs_2CO_3 (4 g, 12.5 mmol) in four portions over 20 minutes, and the mixture was stirred at 0 °C for 1 hour and then room temperature for 3 hours. After the solvent was removed, the residue was partitioned between EtOAc and saturated aqueous NaCl. The organic phase was collected, dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. Purification with flash column chromatograph (silica gel; 1 % to 4 % MeOH /CH_2Cl_2) afforded the

compound **26** (3.1 g, 74% yield). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 8.7 Hz, 2H), 7.38 (m, 10H), 7.16 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.1 Hz, 1H), 5.18 (m, 4H), 5.08-4.90 (m, 2H), 4.29-4.19 (m, 6H), 3.98-3.68 (m, 6H), 318-2.76 (m, 7H), 1.89-1.51 (m, 3H), 0.94-0.88 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 20.4; MS (ESI) m/z 853 $(M+ H)^+$.

Compound 27: Compound **19** (8 g) in a solution of EtOH (120 mL) and EtOAc (40mL) was added 10% Pd/C (800 mg) allowed to stir at room temperature under H_2 atmosphere (balloon) for 3 hours. The catalyst was removed by filtration through celite. The residue was washed and the filtrates were combined and evaporated under reduced pressure to yield compound **20** a white solid (5.8 g, 92 %). ^{1}H NMR (CD_3OD) δ 7.72 (d, J = 8.7 Hz, 2H), 7.16 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.1 Hz, 1H), 5.08-4.90 (m, 2H), 4.29-4.19 (m, 6H), 3.98-3.68 (m, 6H), 318-2.76 (m, 7H), 1.89-1.51 (m, 3H), 0.94-0.88 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 17.6; MS (ESI) m/z 673 $(M+ H)^+$.

Compound 10: To a solution of **20** (67 mg, 0.1 mmol) and CH_3OH (0.1 mL 2.5 mmol) in pyridine (1 mL) was added DCC (83 mg, 0.4 mmol) and the mixture was heated at 70 °C for 2 hours, then at room temperature for overnight. After the solvent was removed, the residue was taken into partition between EtOAc and saturated aqueous NaCl. The organic phase was separated, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Purification with flash column chromatograph (silica gel; 1%-4% MeOH/CH_2Cl_2) afforded compound **10** as a white solid (39 mg, 56% yield). HPLC purity: 96.7%. ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 2H), 7.16 (d, 2H), 7.00 (d, 2H), 6.88 (d,2H), 5.66 (d, 1H), 4.99 (m, 2H), 4.26 (d, 2H), 3.98-3.70 (m,15H), 3.18-2.77 (m, 8H), 1.89-1.47(m, 3H), 0.94-0.88 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 21.8. MS (ESI) m/z 701 $(M+H)^+$.

Compound 11: Compound **11** was prepared using the similar procedure as the preparation of compound **10**, except replacing CH_3OH with iPrOH. Purification with flash column chromatograph (silica gel; 1%-4% MeOH/CH_2Cl_2) provided compound **11** as a white solid (45 mg, 60% yield). HPLC purity: 99.7%. ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 2H), 7.15 (d, 2H), 6.99 (d, 2H), 6.88 (d,2H), 5.66 (d, 1H), 5.02-4.80 (m,

4H), 4.16 (d, 2H), 3.98-3.70 (m,10H), 3.18-2.78 (m, 7H), 1.89-1.67(m, 3H), 1.37 (m, 12 H), 0.94-0.88 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 17.3. MS (ESI) m/z 757 $(M+H)^+$.