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Protocol Article

A practical synthesis of a novel DPAGT1 inhibitor, aminouridyl phenoxy piperidinbenzyl butanamide (APPB) for in vivo studies

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A B S T R A C T

Immunotherapy that targets *N*-linked glycans has not yet been developed due in large part to the lack of specificity of *N*-linked glycans between normal and malignant cells. *N*-Glycan chains are synthesized by the sequential action of glycosyl transferases in the Golgi apparatus. It is an overwhelming task to discover drug-like inhibitors of glycosyl transferases that block the synthesis of specific branching processes in cancer cells, killing tumor cells selectively. It has long been known that *N*-glycan biosynthesis can be inhibited by disruption of the first committed enzyme, dolichyl-phosphate *N*-acetylglucosaminophosphotransferase 1 (DPAGT1). Selective DPAGT1 inhibitors have the promising therapeutic potential for certain solid cancers that require increased branching of *N*-linked glycans in their growth progressions. Recently, we discovered that an anti-*Clostridium difficile* molecule, aminouridyl phenoxy piperidinbenzyl butanamide (APPB) showed DPAGT1 inhibitory activity with the IC₅₀ value of 0.25 μM. It was confirmed that APPB inhibits *N*-glycosylation of β-catenin at 2.5 nM concentration. A sharp difference between APPB and tunicamycin was that the hemolytic activity of APPB is significantly attenuated (IC₅₀ > 200 μM RBC). Water solubility of APPB is >350-times greater than that of tunicamycin (78.8 mg/mL for APPB, <0.2 mg/mL for tunicamycin). A novel DPAGT1 inhibitor, APPB selectively inhibits growth of the solid tumors (e.g. KB, LoVo, SK-OV-3, MDA-MB-432S, HCT116, Panc-1, and AsPC-1) at low μM concentrations, but does not inhibit growth of a leukemia cell (L1210) and the healthy cells (Vero and HPNE) at these concentrations. *In vitro* metabolic stability using rat liver microsomes indicated that a half-life (*t*_{1/2}) of APPB is sufficiently long (>60 min) for *in vivo* studies (PK/PD, safety profiles, and *in vivo* efficacy) using animal models. We have refined all steps in the previously reported synthesis for APPB for larger-scale. This article summarizes protocols of gram-scale synthesis of APPB and its physicochemical data, and a convenient DPAGT1 assay.

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Specifications Table

Subject Area:	Chemistry
More specific subject area:	Medicinal Chemistry
Protocol name:	A practical synthesis of a novel DPAGT1 Inhibitor, aminouridyl phenoxypiperidinbenzyl butanamide (APPB) for <i>in vivo</i> studies
Reagents/tools:	All were operated with standard tools available in general synthetic and biochemistry lab.
Experimental design:	All synthetic steps were demonstrated in gram-quantity. Selectivity of all asymmetric reactions is greater than 15:1 ratio.
Trial registration:	N/A
Ethics:	N/A

Value of the Protocol

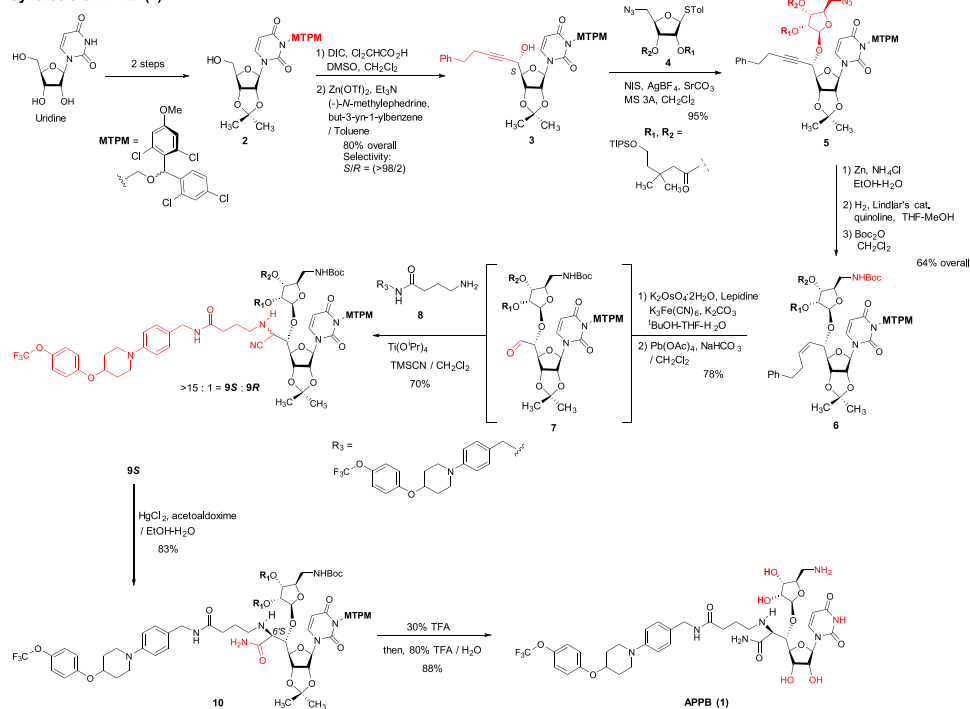
- All reactions were performed in over one gram-scale; the desired product was synthesized >1.0 g quantity.
- Synthesis of a novel DPAGT1 inhibitor
- Physicochemical property of a therapeutically interesting DPAGT1 inhibitor is summarized.

Description of protocol

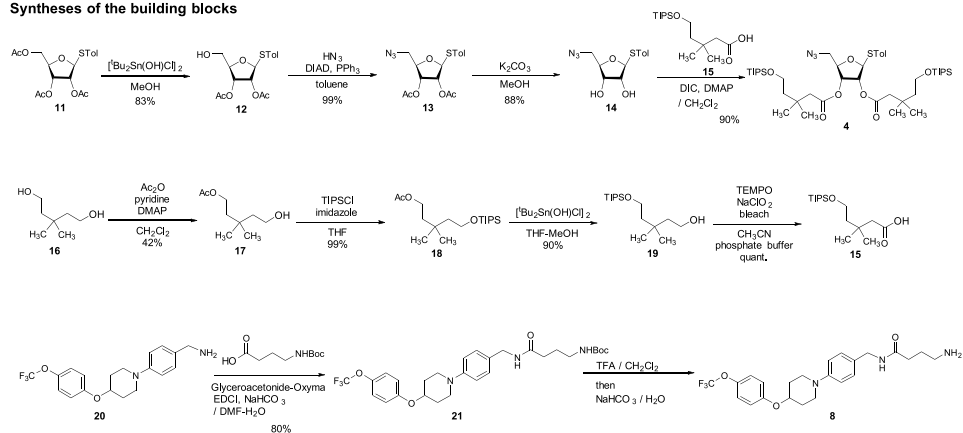
Synthesis of a novel DPAGT1 inhibitor, aminouridyl phenoxypiperidinbenzyl butanamide (APPB, 1)

The monomethoxytetrachlorodiphenylmethoxymethyl (MTPM)-protected uridine **2** was prepared according to the previously reported procedure [1]. The *primary* alcohol of **2** was oxidized by a modified Swern condition to provide the corresponding aldehyde in quantitative yield, which was then subjected to Carreira's asymmetric alkylation reaction using (–)-*N*-methylephedrine [2], yielding the (*S*)-propargyl alcohol **3** in 80% yield with selectivity of >98:2. NIS-AgBF₄ promoted ribosylation of (*S*)-propargyl alcohol **3** with **4** furnished the β-riboside **5** exclusively in 95% yield. The azido group of **5** was reduced with Zn metal in the presence of aq. NH₄Cl, and the triple bond was partially reduced with Lindlar's catalyst. The generated free-amine was protected with (Boc)₂O to furnish **6** in 64% overall yield. The alkene moiety of **6** was subjected to a two-step procedure (osmylation and oxidative cleavage with Pb(OAc)₄), providing the crude aldehyde **7**. Ti(OⁱPr)₄-mediated Strecker reaction of **7** with the 4-aminobutanamide derivatives **8** provided the *S*-diastereomer **9S** in 70% yield with greater than 15:1 *S/R* ratio. The desired diastereomer, **9S** was subjected to hydration reaction with HgCl₂-acetoaldehyde, furnishing the amide **10** in 83% overall yield. Global deprotection of **10** was performed in one-pot two step reaction using 30% TFA in CH₂Cl₂

Synthesis of APPB (1)



Syntheses of the building blocks



Scheme 1. Synthesis of APPB (1).

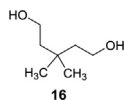
followed by 80% TFA in H₂O; the crude product was purified by DOWEX 50W x 4 ion exchange resin followed by preparative HPLC to furnish **1** in 88% overall yield (Scheme 1).

General

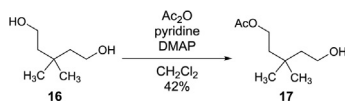
All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. THF, CH₂Cl₂, and DMF were purified via Innovative Technology's Pure-Solve System. All reactions were performed under nitrogen atmosphere. Reactions were monitored by TLC

using 0.25 mm coated commercial silica gel plates (EMD, Silica Gel 60F₂₅₄). TLC spots were visualized by UV light at 254 nm, or developed with ceric ammonium molybdate or anisaldehyde or copper sulfate or ninhydrin solutions by heating on a hot plate. Reactions were also monitored by using SHIMADZU LCMS-2020 with solvents: A: 0.1% formic acid in water, B: acetonitrile. Flash chromatography was performed with SiliCycle silica gel (Purasil 60 Å, 230–400 Mesh). ¹H NMR spectral data were recorded on 400, and 500 MHz instruments. ¹³C NMR spectral data were recorded on 100 and 125 MHz instruments. For all NMR spectra, chemical shifts (δH, δC) were quoted in parts per million (ppm), and *J* values were quoted in Hz. ¹H and ¹³C NMR spectra were calibrated with residual undeuterated solvent (CDCl₃: δH = 7.26 ppm, δC = 77.16 ppm; CD₃CN: δH = 1.94 ppm, δC = 1.32 ppm; CD₃OD: δH = 3.31 ppm, δC = 49.00 ppm; DMSO-*d*₆: δH = 2.50 ppm, δC = 39.52 ppm; D₂O: δH = 4.79 ppm) as an internal reference. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, dd = double doublets, t = triplet, q = quartet, quin = quintet, hept = heptet, m = multiplet, br = broad. Infrared (IR) spectra were recorded on a Perkin-Elmer FT1600 spectrometer. HPLC analyses were performed with a Shimadzu LC-20AD HPLC system. HR-MS data were obtained from a Waters Synapt G2-Si (ion mobility mass spectrometer with nano-electrospray ionization).

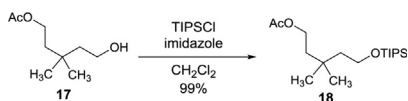
Synthetic procedure for **1**



3,3-Dimethylpentane-1,5-diol (16): The title compound was synthesized according to the reported procedure [1,3]: TLC (hexanes/EtOAc 20:80) *R_f* = 0.20; IR (thin film) ν_{\max} = 3317 (br), 2955, 2934, 1676, 1469, 1366, 1030, 1006, 990 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.73 (t, *J* = 7.0 Hz, 4H), 2.04 (brs, 2H), 1.57 (t, *J* = 7.0 Hz, 4H), 0.94 (s, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 59.60 (2C), 44.06 (2C), 31.67, 28.08 (2C); HRMS (ESI+) *m/z* calcd for C₇H₁₆O₂ 132.1150, found 132.1144.

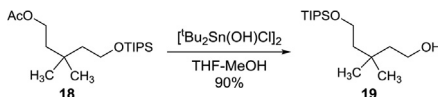


5-Hydroxy-3,3-dimethylpentyl acetate (17): To a stirred solution of **16** (47.5 g, 359.3 mmol) in CH₂Cl₂ (500 mL) were added pyridine (31.8 mL, 395.2 mmol), Ac₂O (33.9 mL, 359.3 mmol) and DMAP (0.44 g, 3.59 mmol) at 0 °C. The reaction mixture was stirred for 12 h at rt, and all volatiles were evaporated *in vacuo*. Purification by silica gel column chromatography (hexanes/EtOAc 90:10 to 50:50) to gave **17** (26.3 g, 150.9 mmol, 42%): TLC (hexanes/EtOAc 67:33) *R_f* = 0.20; ¹H NMR (400 MHz, Chloroform-*d*) δ 4.13 (t, *J* = 7.5 Hz, 2H), 3.72 (t, *J* = 7.5 Hz, 2H), 2.04 (s, 3H), 1.57 (dt, *J* = 14.8, 7.5 Hz, 4H), 0.95 (s, 6H); HRMS (ESI+) *m/z* calcd for C₉H₁₈O₃ 174.1256, found 174.1249.

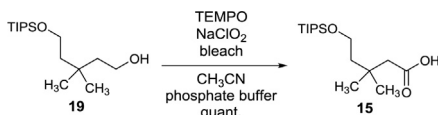


3,3-Dimethyl-5-((triisopropylsilyloxy)pentyl) acetate (18): To a stirred solution of **17** (26.3 g, 150.9 mmol) and imidazole (20.6 g, 301.8 mmol) in dry CH₂Cl₂ (500 mL) were added TIPSCl (48.4 mL, 226.4 mmol) and DMAP (0.18 g, 1.51 mmol) at 0 °C. The reaction mixture was warmed to rt and stirred for 16 h. The reaction was quenched with saturated NaHCO₃ (aq.) and extracted with CH₂Cl₂. The combined organic extracts were dried over Na₂SO₄ and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 97:3) to obtain **18** (49.4 g, 149.4 mmol, 99%): TLC (hexanes/EtOAc 90:10) *R_f* = 0.70; ¹H NMR (400 MHz, Chloroform-*d*) δ 4.12 (t, *J* = 7.6 Hz, 2H),

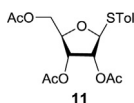
3.74 (t, $J = 7.2$ Hz, 2H), 2.03 (s, 3H), 1.59 (t, $J = 7.6$ Hz, 2H), 1.53 (t, $J = 7.2$ Hz, 2H), 1.11–1.02 (m, 21H), 0.94 (s, 6H); HRMS (ESI+) m/z calcd for $C_{18}H_{39}O_3Si$ [M+H] 331.2668, found 331.2685.



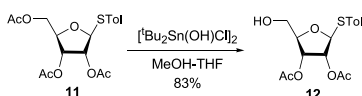
3,3-Dimethyl-5-((triisopropylsilyloxy)pentan-1-ol (19): To a stirred solution of **18** (49.4 g, 149.4 mmol) in MeOH/THF (4:1, 300 mL) was added $[tBu_2Sn(OH)Cl]_2$ (0.86 g, 1.50 mmol). After 20 h at rt, all volatiles were evaporated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 95:5 to 90:10) to provide **19** (38.8 g, 134.5 mmol, 90%); TLC (hexanes/EtOAc 80:20) $R_f = 0.40$; IR (thin film) $\nu_{max} = 3343$ (br), 2941, 2891, 2866, 1463, 1384, 1366, 1096, 1065, 1012, 995, 881, 745, 678, 656 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 3.76 (t, $J = 6.9$ Hz, 2H), 3.72 (t, $J = 7.2$ Hz, 2H), 1.57 (td, $J = 7.1, 2.8$ Hz, 4H), 1.12–1.03 (m, 21H), 0.94 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 60.30, 59.85, 44.31, 31.67, 28.14 (2C), 18.05 (6C), 11.95 (3C); HRMS (ESI+) m/z calcd for $C_{16}H_{36}O_2Si$ 288.2485, found 288.2473.



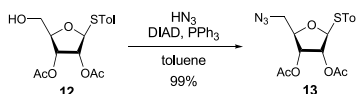
3,3-Dimethyl-5-((triisopropylsilyloxy)pentanoic acid (15): To a stirred solution of **19** (38.8 g, 134.5 mmol) and TEMPO (1.05 g, 6.73 mmol) in MeCN (135 mL) and phosphate buffer (pH = 6.8, 135 mL) were added $NaClO_2$ (14.6 g, 141.4 mmol) and bleach (8.25%, 65 mL) at 35 °C. After being stirred for 4 h, the reaction mixture was extracted with EtOAc and combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 90:10) to give **15** (40.7 g, 134.5 mmol, 100%) as an orange oil; TLC (hexanes/EtOAc 50:50) $R_f = 0.50$; IR (thin film) $\nu_{max} = 2942, 2866, 1705, 1463, 1246, 1097, 996, 881, 738, 678$ cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 3.88 (t, $J = 5.8$ Hz, 2H), 2.38 (s, 2H), 1.71 (t, $J = 5.8$ Hz, 2H), 1.20–1.11 (m, 3H), 1.09 (s, 12H), 1.08 (s, 6H), 1.07 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 60.7, 46.8, 42.6, 32.4, 28.5 (2C), 17.9 (6C), 11.8 (3C); HRMS (ESI+) m/z calcd for $C_{16}H_{34}O_3NaSi$ [M+Na] 325.2175, found 325.2171.



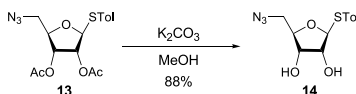
(2R,3S,4S,5S)-2-(Acetoxymethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diyl diacetate (10): The title compound was synthesized according to the reported procedure [1]; TLC (hexanes/EtOAc 50:50) $R_f = 0.60$; $[\alpha]_D^{20} -0.411$ ($c = 0.51, CHCl_3$); IR (thin film) $\nu_{max} = 1742, 1371, 1214, 1091, 1045, 1017, 899, 810$ cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, $J = 8.1$ Hz, 2H), 7.14 (d, $J = 7.7$ Hz, 2H), 5.25–5.22 (m, 1H), 5.21–5.17 (m, 2H), 4.26–4.20 (m, 2H), 4.07 (dd, $J = 12.9, 5.5$ Hz, 1H), 2.34 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.50, 169.63, 169.42, 138.80, 134.18 (2C), 129.81 (2C), 127.45, 87.95, 79.97, 73.67, 71.41, 63.46, 21.15, 20.75, 20.53 (2C); HRMS (ESI+) m/z calcd for $C_{18}H_{22}O_7NaS$ [M+Na] 405.0984, found: 405.0970.



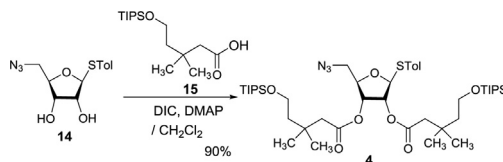
(2R,3S,4S,5S)-2-(Hydroxymethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diyl diacetate (12): To a stirred solution of **10** (24.3 g, 62.8 mmol) in MeOH/THF (4:1, 300 mL) was added [^tBu₂Sn(OH)Cl]₂ (0.72 g, 1.26 mmol). After 20 h at rt, all volatiles were evaporated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 67:33) to provide **11** (17.9 g, 52.7 mmol, 83%): TLC (hexanes/EtOAc 60:40) *R*_f=0.40; [α]²¹_D −0.298 (*c* = 1.37, CHCl₃); IR (thin film) ν_{\max} = 3484 (br), 3021, 2924, 2877, 1746, 1493, 1432, 1373, 1239, 1222, 1102, 1093, 1046, 1017, 810 cm^{−1}; ¹H NMR (400 MHz, CDCl₃) δ 7.43 (d, *J* = 7.8 Hz, 2H), 7.16 (d, *J* = 7.8 Hz, 2H), 5.27 (d, *J* = 5.6 Hz, 1H), 5.24 (t, *J* = 4.6 Hz, 1H), 5.20 (d, *J* = 5.8 Hz, 1H), 4.13 (q, *J* = 3.7 Hz, 1H), 3.74 (dd, *J* = 12.3, 2.8 Hz, 1H), 3.58 (dd, *J* = 12.2, 3.2 Hz, 1H), 2.34 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 169.89, 169.39, 138.92, 133.88 (2C), 129.93 (2C), 127.45, 87.76, 83.46, 73.89, 71.40, 62.08, 21.17, 20.62, 20.57; HRMS (ESI +) *m/z* calcd for C₁₆H₂₁O₆S [M+H] 341.1059, found 341.1075.



(2R,3S,4S,5S)-2-(Azidomethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diyl diacetate (13): To a stirred solution of **12** (17.9 g, 52.7 mmol) and PPh₃ (27.6 g, 105.1 mmol) in dry toluene (100 mL) were added HN₃ (1.0 M in toluene, 262.9 mL, 262.9 mmol) and DIAD (20.7 mL, 105.1 mmol). The reaction mixture was stirred for 8 h at rt, and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 80:20 to 70:30) to afford **13** (19.0 g, 52.0 mmol, 99%): TLC (hexanes/EtOAc 75:25) *R*_f=0.40; [α]²¹_D −0.899 (*c* = 3.93, CHCl₃); IR (thin film) ν_{\max} = 3023, 2924, 2101, 1746, 1493, 1436, 1372, 1233, 1217, 1094, 1064, 1044, 1016, 965, 899, 810 cm^{−1}; ¹H NMR (400 MHz, CDCl₃) δ 7.43 (d, *J* = 8.1 Hz, 2H), 7.15 (d, *J* = 7.9 Hz, 2H), 5.27 (d, *J* = 5.2 Hz, 1H), 5.19 (t, *J* = 5.3 Hz, 1H), 5.11 (t, *J* = 5.2 Hz, 1H), 4.15 (q, *J* = 5.0 Hz, 1H), 3.42 (d, *J* = 1.2 Hz, 1H), 3.41 (d, *J* = 2.2 Hz, 1H), 2.34 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 169.63, 169.35, 138.74, 133.86 (2C), 129.81 (2C), 127.60, 88.27, 80.97, 73.74, 71.73, 52.46, 21.14, 20.50, 20.49; HRMS (ESI+) *m/z* calcd for C₁₆H₂₀N₃O₅S [M+H] 366.1124, found: 366.1133.

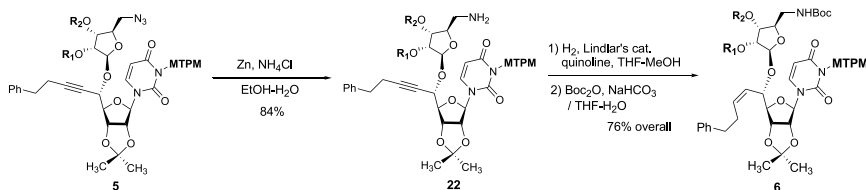


(2R,3R,4S,5S)-2-(Azidomethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diol (13): To a stirred solution of **13** (19.0 g, 52.0 mmol) in MeOH (200 mL) was added K₂CO₃ (10.0 g, 72.5 mmol). After being stirred for 30 min, the reaction mixture was filtered and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 70:30 to 50:50) to afford **14** (12.9 g, 45.9 mmol, 88%): TLC (hexanes/EtOAc 33:67) *R*_f=0.60; [α]²¹_D −0.152 (*c* = 0.34, CHCl₃); IR (thin film) ν_{\max} = 3385 (br), 2923, 2103, 1493, 1437, 1399, 1286, 1117, 1065, 1042, 1017, 809 cm^{−1}; ¹H NMR (400 MHz, CDCl₃) δ 7.42 (d, *J* = 7.7 Hz, 2H), 7.14 (d, *J* = 7.8 Hz, 2H), 5.19 (d, *J* = 4.7 Hz, 1H), 4.11 (t, *J* = 4.4 Hz, 1H), 4.04 (d, *J* = 3.7 Hz, 2H), 3.49 (dd, *J* = 13.0, 2.9 Hz, 1H), 3.42 (dd, *J* = 13.0, 4.2 Hz, 1H), 2.57 (brs, 2H), 2.34 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 138.34, 133.17 (2C), 129.82 (2C), 128.68, 90.76, 82.62, 74.88, 72.24, 52.68, 21.15; HRMS (ESI+) *m/z* calcd for C₁₂H₁₆N₃O₃S [M+H] 282.0912, found: 282.0928.



(2R,5S)-2-(Azidomethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-((triisopropylsilyl)oxy)pentanoate) (4): To a stirred solution of **14** (12.9 g, 45.9 mmol) and **15** (34.7 g, 114.8 mmol) in CH₂Cl₂ (231 mL) were added DMAP (1.12 g, 9.17 mmol) and DIC (18.0 mL, 114.8 mmol) at 0 °C. The reaction mixture was stirred for 16 h at rt and concentrated *in vacuo*. The crude mixture

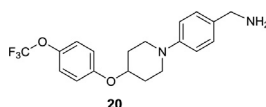
(dd, $J = 23.1, 8.5$ Hz, 1H), 7.32–7.27 (m, 4H), 7.24–7.16 (m, 4H), 6.84 (d, $J = 7.3$ Hz, 2H), 6.51 (d, $J = 3.7$ Hz, 1H), 5.71–5.64 (m, 2H), 5.60–5.49 (m, 2H), 5.20–5.16 (m, 3H), 4.79 (ddd, $J = 7.5, 6.5, 3.1$ Hz, 1H), 4.64 (td, $J = 5.9, 2.6$ Hz, 1H), 4.57 (ddt, $J = 11.4, 6.3, 1.9$ Hz, 1H), 4.28 (dt, $J = 6.2, 2.8$ Hz, 1H), 4.19 (tt, $J = 6.1, 3.0$ Hz, 1H), 3.79–3.72 (m, 7H), 3.50 (ddd, $J = 13.0, 7.6, 3.3$ Hz, 1H), 3.35 (dd, $J = 13.0, 3.4$ Hz, 1H), 2.83 (t, $J = 7.4$ Hz, 2H), 2.55 (td, $J = 7.4, 1.8$ Hz, 2H), 2.29 (t, $J = 1.6$ Hz, 2H), 2.24 (dd, $J = 5.1, 2.1$ Hz, 2H), 1.62–1.55 (m, 7H), 1.36 (d, $J = 2.0$ Hz, 3H), 1.08–1.00 (m, 54H); ^{13}C NMR (101 MHz, CDCl_3) δ 175.6, 171.0, 170.9, 170.71, 170.70, 170.6, 162.2, 162.1, 159.5, 150.8, 150.7, 140.4, 140.19, 140.15, 140.13, 136.92, 136.91, 135.4, 135.3, 133.9, 133.8, 133.7, 131.2, 129.4, 129.3, 128.5 (2C), 128.4 (2C), 126.5, 126.4, 126.2, 126.1, 125.6, 125.5, 115.29, 115.25, 114.23, 114.22, 104.61, 104.55, 101.83, 101.82, 88.8, 88.2, 84.44, 84.35, 83.9, 81.4, 81.3, 80.6, 79.9, 76.5, 75.9, 75.8, 74.1, 71.8, 71.7, 71.4, 70.7, 69.6, 69.5, 68.9, 68.8, 59.97, 59.96, 55.7, 46.2, 46.0, 44.7, 44.6, 34.7, 34.51, 34.49, 32.7, 32.61, 32.57, 28.0, 27.38, 27.35, 27.3, 27.1, 25.34, 25.27, 20.9, 18.1 (12C), 11.9 (6C); HRMS (ESI+) m/z calcd for $\text{C}_{74}\text{H}_{106}\text{Cl}_4\text{N}_5\text{O}_{15}\text{Si}_2$ [$\text{M}+\text{H}$] 1500.5978, found: 1500.5992.



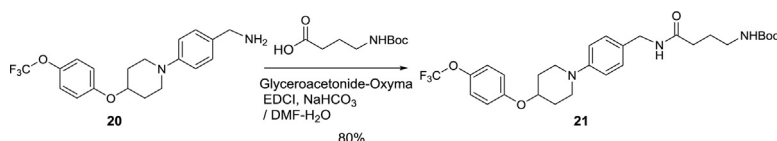
(2R,3R,4R,5R)-2-(Aminomethyl)-5-(((1S)-1-((3aR,4R,6R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-phenylpent-2-yn-1-yl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyloxy)pentanoate) (22): A suspended solution of **5** (7.03 g, 4.68 mmol), NH_4Cl (7.50 g, 140.3 mmol) and Zn (9.17 g, 140.3 mmol) in $\text{EtOH}/\text{H}_2\text{O}$ (9:1, 50 mL) was stirred at 80°C for 12 h and cooled to rt. The precipitates were filtered and the combined organic solution was concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/ EtOAc 50:50 to $\text{CHCl}_3/\text{MeOH}$ 96:4) to afford the primary amine **22** (5.80 g, 3.93 mmol, 84%): TLC ($\text{CHCl}_3/\text{MeOH}$ 90:10) $R_f = 0.60$; $[\alpha]_D^{21} -0.013$ ($c = 1.35, \text{CHCl}_3$); IR (thin film) $\nu_{\text{max}} = 2941, 2866, 1742, 1721, 1675, 1600, 1556, 1461, 1382, 1278, 1215, 1099, 1070, 1050, 999, 882 \text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 7.64 (s, 1H), 7.50 (dd, $J = 31.4, 8.4$ Hz, 1H), 7.33–7.30 (m, 2H), 7.28 (d, $J = 7.5$ Hz, 3H), 7.24–7.15 (m, 5H), 6.85 (d, $J = 9.5$ Hz, 2H), 6.49 (d, $J = 6.1$ Hz, 1H), 5.75 (dd, $J = 8.5, 1.9$ Hz, 1H), 5.72–5.66 (m, 1H), 5.59–5.46 (m, 2H), 5.30 (d, $J = 5.3$ Hz, 1H), 5.22–5.13 (m, 2H), 4.82 (dt, $J = 6.3, 3.1$ Hz, 1H), 4.78 (d, $J = 7.0$ Hz, 1H), 4.65 (dd, $J = 14.5, 7.6$ Hz, 1H), 4.28 (dt, $J = 7.4, 3.5$ Hz, 1H), 4.17 (quin, $J = 3.9$ Hz, 1H), 3.87 (t, $J = 5.8$ Hz, 1H), 3.75 (dt, $J = 15.3, 6.3$ Hz, 6H), 3.14 (d, $J = 13.6$ Hz, 1H), 2.94–2.86 (m, 1H), 2.83 (t, $J = 7.4$ Hz, 2H), 2.55 (td, $J = 7.2, 2.0$ Hz, 2H), 2.35 (s, 1H), 2.30 (s, 2H), 2.25 (s, 2H), 2.23–2.17 (m, 1H), 1.70 (t, $J = 5.9$ Hz, 1H), 1.63–1.51 (m, 4H), 1.35 (d, $J = 5.1$ Hz, 2H), 1.25 (s, 1H), 1.12–0.95 (m, 51H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.46, 171.73, 171.39, 170.66, 162.24, 159.45, 150.84, 140.16, 136.82, 135.21, 135.04, 134.04, 133.95, 133.75, 131.18, 131.16, 131.14, 129.40, 129.35, 128.52, 128.43 (2C), 128.40 (2C), 128.37, 126.42, 126.29, 126.16, 125.40, 125.26, 115.30, 115.24, 114.01, 101.82, 89.55, 84.49, 74.87, 70.13, 60.70, 59.93, 55.69, 47.00, 46.16, 45.95, 44.74, 44.64, 42.72, 34.53, 34.51, 32.62, 32.58, 32.33, 29.69, 28.47, 27.38, 27.34, 27.29, 27.03, 25.21, 25.19, 20.92, 18.04 (12C), 17.92, 11.87 (6C), 11.78; HRMS (ESI+) m/z calcd for $\text{C}_{74}\text{H}_{108}\text{Cl}_4\text{N}_5\text{O}_{15}\text{Si}_2$ [$\text{M}+\text{H}$] 1474.6073, found: 1475.6091.

(2R,3R,4R,5R)-2-(((tert-Butoxycarbonyl)amino)methyl)-5-(((1S,Z)-1-((3aR,4R,6R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-phenylpent-2-en-1-yl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyloxy)pentanoate) (6): To a stirred solution of **22** (5.80 g, 3.93 mmol) and quinoline (10 mL) in $\text{THF}-\text{MeOH}$ (1:1, 200 mL) was added Lindlar catalyst (2.90 g). H_2 gas was introduced and the reaction mixture was stirred under H_2 atmosphere (1000 psi). After being stirred for 20 h, the reaction mixture was added Lindlar catalyst (2.90 g). The reaction mixture was stirred for 20 h under H_2 atmosphere (1000 psi) at rt. The solution was filtered through Celite, concentrated *in vacuo*. The crude mixture was used for the next reaction without purification. To a stirred solution of the crude mixture in CH_2Cl_2 (40 mL) was Boc_2O (1.29 g,

5.89 mmol). After being stirred for 12 h at rt, the reaction mixture was quenched with 1N HCl and extracted with EtOAc. The combined organic solution was washed with saturated aq. NaHCO₃, dried over Na₂SO₄ and concentrated *in vacuo*. The crude mixture was passed through a silica gel pad (hexanes/EtOAc 80:20 to 70:30), to afford **6** (4.71 g, 2.99 mmol, 76%): TLC (hexanes/EtOAc 75:25) *R_f* = 0.40; [α]²¹_D −0.015 (*c* = 0.86, CHCl₃); IR (thin film) *ν*_{max} = 3403 (br), 2957, 2941, 2866, 1720, 1675, 1600, 1556, 1507, 1456, 1382, 1367, 1278, 1247, 1218, 1161, 1100, 1071, 1049, 1013, 999, 882 cm^{−1}; ¹H NMR (400 MHz, CDCl₃) δ 7.54 (dd, *J* = 8.4, 6.7 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.30 (t, *J* = 2.1 Hz, 2H), 7.25–7.21 (m, 2H), 7.21–7.12 (m, 5H), 6.82 (d, *J* = 10.7 Hz, 2H), 6.51 (d, *J* = 13.6 Hz, 1H), 5.84–5.74 (m, 2H), 5.72 (d, *J* = 8.1 Hz, 1H), 5.62–5.50 (m, 2H), 5.47 (t, *J* = 8.0 Hz, 1H), 5.14 (t, *J* = 4.2 Hz, 1H), 5.07–4.97 (m, 2H), 4.90 (s, 1H), 4.75 (ddd, *J* = 24.4, 6.4, 2.0 Hz, 1H), 4.58–4.45 (m, 2H), 4.19 (dt, *J* = 8.4, 4.2 Hz, 1H), 4.01 (dt, *J* = 6.6, 4.3 Hz, 1H), 3.76 (d, *J* = 5.1 Hz, 4H), 3.75–3.70 (m, 4H), 3.32 (d, *J* = 5.0 Hz, 2H), 2.79–2.58 (m, 2H), 2.57–2.41 (m, 2H), 2.36–2.28 (m, 1H), 2.26–2.19 (m, 5H), 1.66–1.52 (m, 4H), 1.41 (s, 6H), 1.33 (d, *J* = 4.9 Hz, 2H), 1.05 (q, *J* = 2.7 Hz, 5H), 0.99 (dd, *J* = 9.6, 4.0 Hz, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 170.87, 159.38, 155.94, 150.85, 141.06, 136.87, 136.80, 135.57, 135.31, 135.27, 133.86, 133.66, 133.56, 131.23, 131.20, 129.29, 129.27, 128.52, 128.51, 128.37 (2C), 126.16, 126.15, 126.06, 126.03, 125.99, 125.64, 125.52, 125.46, 125.43, 115.24, 115.23, 114.17, 114.11, 84.61, 81.15, 81.03, 79.30, 79.25, 74.72, 74.29, 70.50, 69.81, 59.95, 59.91, 55.66, 55.65, 46.18, 46.17, 45.92, 44.80, 44.79, 41.64, 35.37, 35.34, 32.56, 32.55, 32.52, 32.50, 29.70, 28.34, 27.27, 27.24, 27.22, 27.10, 27.08, 25.25, 18.05 (12C), 17.88, 11.88 (6C), 11.74; HRMS (ESI+) *m/z* calcd for C₇₉H₁₁₈Cl₄N₃O₁₇Si₂ [M+H] 1576.6754, found: 1576.6771.

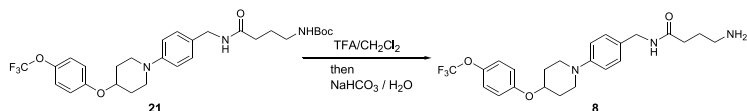


(4-(4-(4-(Trifluoromethoxy)phenoxy)piperidin-1-yl)phenyl)methanamine (20): The title compound was synthesized according to the reported procedure [5]: ¹H NMR (400 MHz, CDCl₃) δ 7.21 (d, *J* = 8.2 Hz, 2H), 7.14 (d, *J* = 8.6 Hz, 2H), 6.97–6.87 (m, 4H), 4.43 (tt, *J* = 7.7, 3.8 Hz, 1H), 3.79 (s, 2H), 3.49 (ddd, *J* = 11.7, 7.2, 3.7 Hz, 2H), 3.09 (ddd, *J* = 12.2, 8.2, 3.6 Hz, 2H), 2.15–2.06 (m, 2H), 1.98–1.88 (m, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 155.8, 150.2, 142.8, 134.6, 128.0 (2C), 122.5 (2C), 116.83 (2C), 116.76 (2C), 72.9, 46.9 (2C), 45.9, 30.4 (2C); HRMS (ESI+) *m/z* calcd for C₁₉H₂₂F₃N₂O₂ [M+H] 367.1633, found 367.1628.

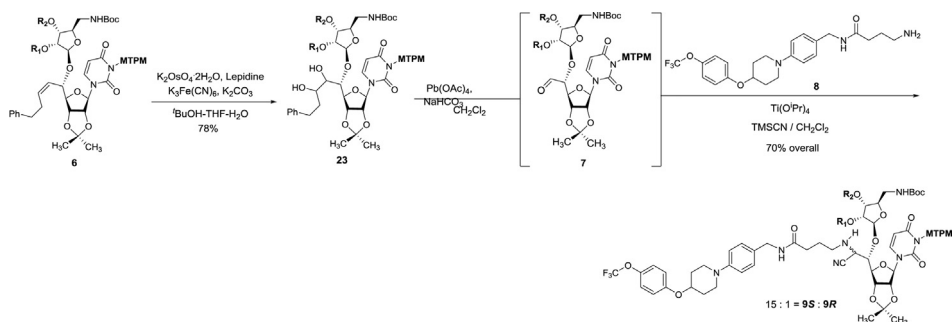


tert-Butyl (4-oxo-4-((4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)amino)butyl)carbamate (21): To a stirred solution of 4-aminobutyric acid (2.50 g, 24.0 mmol) and NaHCO₃ (6.00 g, 72.0 mmol) in THF-H₂O (1:1, 24 mL) was added Boc₂O (5.76 g, 26.4 mmol). After being stirred for 8 h at rt, the reaction mixture was quenched with 1N HCl and extracted with CHCl₃. The combined organic solution was dried over Na₂SO₄ and concentrated *in vacuo*. To a stirred solution of the crude mixture, **20** (4.45 g, 12.14 mmol), NaHCO₃ (5.10 g, 60.7 mmol) and Glyceroacetone-Oxyma (5.54 g, 24.3 mmol) in DMF-H₂O (9:1, 60 mL), was added EDCI (4.65 g, 24.3 mmol). After being stirred for 13 h at rt, the reaction mixture was quenched with H₂O and extracted with EtOAc. The combined organic solution was washed with 1N HCl (aq.), saturated NaHCO₃ (aq.), dried over Na₂SO₄ and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 33:67 to 20:80) to afford **21** (5.35 g, 9.71 mmol, 80%) [4]: TLC (hexanes/EtOAc 20:80) *R_f* = 0.30; IR (thin film) *ν*_{max} = 3303 (br), 2931, 1692, 1637, 1613, 1542, 1504, 1465, 1366, 1264, 1238, 1219, 1193, 1159, 1120, 1111, 1036, 918, 841, 827, 772 cm^{−1}; ¹H NMR (400 MHz, Chloroform-*d*) δ 7.19 (dd, *J* = 8.4, 4.7 Hz, 2H), 7.14 (d, *J* = 8.8 Hz, 2H), 6.91 (dd, *J* = 9.1, 1.1 Hz, 4H), 6.22 (brs, 1H), 4.77 (brs, 1H), 4.72 (brs, 1H), 4.44 (tt, *J* = 7.2,

3.5 Hz, 1H), 4.35 (d, $J = 5.6$ Hz, 1H), 4.29 (d, $J = 5.5$ Hz, 1H), 3.48 (ddt, $J = 11.6, 7.6, 3.8$ Hz, 2H), 3.20–3.05 (m, 4H), 2.22 (t, $J = 7.1$ Hz, 2H), 2.18–2.07 (m, 2H), 1.98–1.89 (m, 2H), 1.81 (quin, $J = 6.9$ Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.31, 157.99, 156.40, 155.72, 155.70, 142.76, 128.90 (2C), 128.56 (2C), 122.52 (3C), 116.76 (3C), 79.32, 72.55, 46.87, 44.10, 43.12, 39.76, 33.69, 30.15, 28.38 (3C), 26.35; HRMS (ESI+) m/z calcd for $\text{C}_{28}\text{H}_{37}\text{F}_3\text{N}_3\text{O}_5$ $[\text{M}+\text{H}]^+$ 552.2685, found: 552.2701.



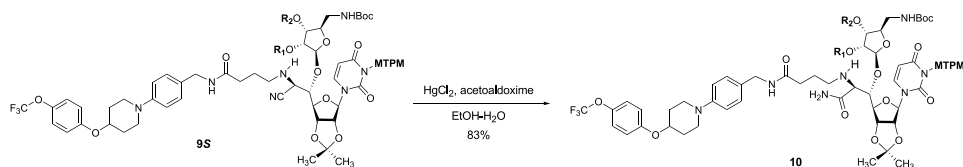
4-Amino-N-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)butanamide (8): To a stirred solution of **22** (3.81 g, 6.99 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL). The reaction mixture was stirred for 3 h at rt, and all volatile were evaporated *in vacuo*. The residue was neutralized with aq. NaHCO_3 extracted with CHCl_3 . The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture of **8** was used for next reaction without purification.



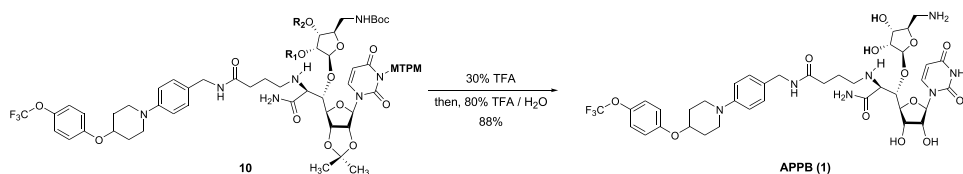
(2R,3R,4R,5S)-2-(((tert-Butoxycarbonyl)amino)methyl)-5-(((1S)-1-((3aR,4R,6R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-2,3-dihydroxy-5-phenylpentyl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((trisopropylsilyl)oxy)pentanoate) (23): To a stirred solution of **6** (4.71 g, 2.99 mmol) and lepidine (2.37 mL, 17.9 mmol) in $t\text{-BuOH}/\text{THF}/\text{H}_2\text{O}$ (1:1:1, 180 mL) were added K_2CO_3 (2.06 g, 14.9 mmol), $\text{K}_3\text{Fe}(\text{CN})_6$ (4.91 g, 14.9 mmol) and $\text{K}_2\text{OsO}_4 \cdot 2\text{H}_2\text{O}$ (1.10 g, 2.99 mmol) at rt. After being stirred for 12 h, the reaction mixture were added K_2CO_3 (2.06 g, 14.9 mmol), $\text{K}_3\text{Fe}(\text{CN})_6$ (4.91 g, 14.9 mmol) and $\text{K}_2\text{OsO}_4 \cdot 2\text{H}_2\text{O}$ (1.10 g, 2.99 mmol). After 20 h, the reaction mixture was diluted with EtOAc and quenched with saturated aq. Na_2SO_3 . The heterogeneous mixture was stirred for 30 min, and extracted with EtOAc. The combined organic solution was washed with 1N HCl, saturated aq. NaHCO_3 , dried over Na_2SO_4 , and concentrated *in vacuo*. The crude mixture was passed through a silica gel pad (hexanes/EtOAc 75:25 to 50:50) to afford **23** (3.76 g, 2.33 mmol, 78%) as diastereomeric mixture. This mixture was used for next reaction without further purification. Data for less-polar diastereomer: TLC (hexanes/EtOAc 67:33) $R_f = 0.30$; $[\alpha]_D^{22}$ 0.210 ($c = 1.62$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3444$ (br), 2941, 2866, 1741, 1719, 1675, 1600, 1556, 1457, 1382, 1367, 1278, 1249, 1216, 1160, 1098, 1070, 1049, 1013, 998, 882, 867, 754, 681 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.52 (dd, $J = 8.4, 3.6$ Hz, 1H), 7.31 (d, $J = 2.0$ Hz, 2H), 7.30–7.27 (m, 2H), 7.25–7.14 (m, 6H), 6.85 (d, $J = 3.4$ Hz, 2H), 6.50 (d, $J = 5.9$ Hz, 1H), 5.75 (dd, $J = 17.6, 8.0$ Hz, 1H), 5.63 (d, $J = 22.1$ Hz, 1H), 5.58–5.52 (m, 2H), 5.48 (d, $J = 9.7$ Hz, 1H), 5.21 (q, $J = 7.3, 6.2$ Hz, 2H), 5.11 (d, $J = 6.8$ Hz, 1H), 5.01 (dd, $J = 8.4, 4.7$ Hz, 1H), 4.85–4.78 (m, 2H), 4.25 (d, $J = 5.6$ Hz, 1H), 4.16 (dt, $J = 8.6, 4.4$ Hz, 1H), 4.03 (dd, $J = 14.2, 5.1$ Hz, 1H), 3.90 (d, $J = 1.8$ Hz, 1H), 3.78 (d, $J = 1.8$ Hz, 4H), 3.77–3.71 (m, 4H), 3.69–3.62 (m, 2H), 3.39–3.22 (m, 2H), 2.97–2.86 (m, 2H), 2.77–2.66 (m, 2H), 2.34–2.18 (m, 5H), 2.12–2.00 (m, 1H), 1.91–1.67 (m, 2H), 1.64–1.51 (m, 4H), 1.42 (s, 6H), 1.35 (d, $J = 3.9$ Hz, 3H), 1.13–0.99 (m, 41H), 0.99–0.94

(m, 6H), 0.86 (dtd, $J = 9.1, 6.6, 2.2$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 170.85, 170.74, 170.70, 170.70, 162.10, 162.09, 159.44, 159.44, 156.05, 150.59, 150.53, 141.92, 141.89, 136.87, 136.84, 135.25, 135.09, 133.96, 133.77, 131.21, 131.17, 129.37, 129.32, 128.43 (2C), 128.38 (2C), 126.22, 126.14, 125.81, 125.36, 125.26, 115.27, 114.97, 80.36, 80.34, 79.85, 79.67, 79.58, 74.64, 74.62, 74.60, 73.82, 73.77, 73.72, 70.31, 70.31, 59.94, 59.90, 55.69, 46.13, 45.92, 44.72, 34.63, 34.50, 32.58, 32.57, 32.55, 32.54, 31.76, 29.69, 29.03, 28.35, 27.28, 27.22, 26.88, 25.32, 25.25, 20.68, 18.04 (1C), 11.88 (3C), 11.86 (3C), 11.43; HRMS (ESI+) m/z calcd for $\text{C}_{79}\text{H}_{120}\text{Cl}_4\text{N}_3\text{O}_{19}\text{Si}_2$ [M+H] 1610.6809, found: 1610.6827. Data for polar diastereomer: TLC (hexanes/EtOAc 67:33) $R_f = 0.20$; $[\alpha]_D^{22} 0.071$ ($c = 1.08, \text{CHCl}_3$); IR (thin film) $\nu_{\text{max}} = 3413$ (br), 2941, 2866, 1719, 1675, 1457, 1367, 1278, 1248, 1219, 1160, 1099, 1070, 1049, 882, 772 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.58 (d, $J = 8.5$ Hz, 1H), 7.31–7.29 (m, 2H), 7.28 (s, 2H), 7.24–7.11 (m, 6H), 6.84 (d, $J = 1.4$ Hz, 2H), 6.51 (d, $J = 6.6$ Hz, 1H), 5.90 (dd, $J = 6.3, 2.7$ Hz, 1H), 5.84 (t, $J = 8.2$ Hz, 1H), 5.61–5.41 (m, 2H), 5.23–5.10 (m, 2H), 5.04 (t, $J = 5.8$ Hz, 2H), 4.86–4.77 (m, 1H), 4.68 (ddd, $J = 21.0, 6.3, 2.8$ Hz, 1H), 4.57 (dt, $J = 10.8, 3.8$ Hz, 1H), 4.25–4.14 (m, 1H), 4.06–3.98 (m, 1H), 3.92–3.84 (m, 1H), 3.80–3.71 (m, 6H), 3.47–3.23 (m, 2H), 2.92–2.83 (m, 2H), 2.77–2.66 (m, 2H), 2.31–2.20 (m, 4H), 2.19–2.06 (m, 2H), 1.92–1.66 (m, 3H), 1.63–1.53 (m, 6H), 1.42 (d, $J = 3.7$ Hz, 2H), 1.36 (s, 6H), 1.10–0.94 (m, 50H), 0.91–0.81 (m, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.04, 171.01, 170.93, 170.92, 162.00, 159.38, 150.79, 136.92, 136.91, 135.45, 131.30, 131.28, 129.29, 129.28, 128.46 (2C), 128.42 (2C), 126.09, 125.95, 125.93, 115.24, 81.03, 81.01, 79.95, 79.67, 75.03, 75.00, 74.98, 72.17, 70.38, 70.31, 69.52, 69.49, 59.95, 59.91, 55.69, 55.67, 46.13, 45.93, 44.86, 44.66, 35.27, 35.25, 34.64, 32.63, 32.59, 32.58, 31.95, 28.32, 27.38, 27.37, 27.36, 27.28, 27.27, 27.20, 26.89, 25.26, 18.05 (12C), 11.88 (3C), 11.87 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{79}\text{H}_{120}\text{Cl}_4\text{N}_3\text{O}_{19}\text{Si}_2$ [M+H] 1610.6809, found: 1610.6831.

(2R,3R,4R,5S)-2-(((tert-Butoxycarbonyl)amino)methyl)-5-((1S,2R)-2-cyano-1-((3aR,4R,6-R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl))-2-((4-oxo-4-((4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)amino)butyl)amino)ethoxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (9): To a stirred suspension of **23** (3.76 g, 2.33 mmol) and NaHCO_3 (0.98 g, 11.6 mmol) in CH_2Cl_2 (46.6 mL) was added $\text{Pb}(\text{OAc})_4$ (2.06 g, 4.66 mmol) at 0°C . The reaction mixture was stirred for 2 h at 0°C and quenched with saturated aq. NaHCO_3 , and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture of aldehyde **7** was used for the next reaction without purification. To a stirred solution of **7** (3.44 g, 2.33 mmol) and **8** (3.15 g, 6.99 mmol) in CH_2Cl_2 (30 mL) was added MS3A (7.5 g) followed by $\text{Ti}(\text{OiPr})_4$ (6.89 mL, 23.3 mmol). After 6 h, the reaction was added TMSCN (2.91 mL, 23.3 mmol) and stirred for 12 h at rt. After completion, the reaction mixture was quenched with saturated aq. NaHCO_3 , and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexanes/EtOAc 80:20 to 60:40) to afford **9S** (3.15 g, 1.63 mmol, 70% for 2 steps); TLC (hexanes/EtOAc 50:50) $R_f = 0.40$; $[\alpha]_D^{21} +0.102$ ($c = 0.75, \text{CHCl}_3$); IR (thin film) $\nu_{\text{max}} = 3342$ (br), 2941, 2866, 1718, 1675, 1505, 1464, 1243, 1164, 1101, 1071, 883, 772, 688 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.49 (dd, $J = 8.5, 4.3$ Hz, 1H), 7.32 (d, $J = 2.0$ Hz, 1H), 7.22–7.11 (m, 7H), 6.94–6.88 (m, 5H), 6.86 (d, $J = 6.5$ Hz, 2H), 6.50 (d, $J = 8.6$ Hz, 1H), 6.25–6.16 (m, 1H), 5.73 (dd, $J = 22.2, 8.0$ Hz, 1H), 5.60 (t, $J = 8.8$ Hz, 1H), 5.56–5.41 (m, 3H), 5.21 (d, $J = 4.4$ Hz, 1H), 5.05–4.98 (m, 2H), 4.94–4.77 (m, 2H), 4.53–4.37 (m, 3H), 4.25–4.16 (m, 2H), 4.05–3.98 (m, 1H), 3.80–3.69 (m, 6H), 3.68–3.63 (m, 1H), 3.56 (dd, $J = 17.3, 3.4$ Hz, 1H), 3.48 (ddt, $J = 11.6, 7.2, 4.0$ Hz, 2H), 3.44–3.29 (m, 1H), 3.08 (dq, $J = 9.5, 5.3, 4.8$ Hz, 2H), 2.95 (dt, $J = 11.4, 5.5$ Hz, 1H), 2.47 (td, $J = 12.0, 11.4, 5.7$ Hz, 1H), 2.36–2.14 (m, 5H), 2.13–2.05 (m, 2H), 1.97–1.85 (m, 3H), 1.84–1.75 (m, 1H), 1.58 (t, $J = 6.9$ Hz, 2H), 1.55–1.50 (m, 4H), 1.40 (s, 9H), 1.33 (d, $J = 4.8$ Hz, 3H), 1.28–1.23 (m, 3H), 1.08–1.02 (m, 42H), 1.01 (s, 6H), 0.94 (d, $J = 2.1$ Hz, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.4, 171.0, 170.9, 159.5, 155.8, 150.9, 150.7, 142.8, 136.9, 136.8, 135.3, 135.1, 134.13, 134.05, 133.86, 133.85, 133.78, 131.2, 131.1, 129.42, 129.37, 129.0, 126.4, 126.2, 125.5, 122.5 (2C), 121.8, 119.3, 118.4, 116.8 (2C), 116.6 (2C), 115.4, 115.3, 114.71, 114.66, 106.4, 102.3, 102.2, 84.8, 80.7, 80.6, 79.9, 79.8, 79.3, 76.2, 74.32, 74.30, 72.9, 60.38, 60.35, 60.0, 59.9, 55.72, 55.71, 52.0, 46.6, 46.2, 45.9, 44.84, 44.77, 42.99, 42.96, 42.4, 41.2, 33.53, 33.49, 32.6, 32.5, 30.3, 28.4, 27.3 (2C), 27.17, 27.16, 27.1, 25.4, 18.1 (12C), 14.2, 14.1, 11.91 (3C), 11.90 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{94}\text{H}_{135}\text{Cl}_4\text{F}_3\text{N}_7\text{O}_{20}\text{Si}_2$ [M+H] 1934.8007, found: 1934.8021.



(2S,3R,4R,5R)-2-((1S,2S)-3-Amino-1-((3aR,4R,6R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyl-tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-3-oxo-2-((4-oxo-4-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)amino)butyl)amino)propoxy)-5-(((tert-butoxycarbonyl)amino)methyl) tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (10): To a stirred solution of **9S** (3.15 g, 1.63 mmol) in EtOH/H₂O (9:1, 10 mL) were added HgCl₂ (0.89 g, 3.26 mmol) and acetaldoxime (0.99 mL, 16.3 mmol) at rt. After being stirred for 10 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was quenched with saturated aq. NaHCO₃, extracted with CHCl₃. The combined organic extracts were dried over Na₂SO₄ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (CHCl₃/MeOH 99.5:0.5–99.2:0.8–98.8:1.2) to afford **10** (2.64 g, 1.35 mmol, 83%); TLC (CHCl₃/MeOH 95:5) *R*_f = 0.30; [α]_D²¹ +0.144 (*c* = 0.53, CHCl₃); IR (thin film) ν_{\max} = 3335 (br), 2940, 2866, 1719, 1676, 1505, 1464, 1367, 1242, 1162, 1101, 1070, 882, 681 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.53 (dd, *J* = 8.6, 5.1 Hz, 1H), 7.30 (s, 1H), 7.28–7.22 (m, 2H), 7.21–7.12 (m, 6H), 6.91 (d, *J* = 8.5 Hz, 4H), 6.86 (d, *J* = 2.6 Hz, 2H), 6.51 (d, *J* = 8.7 Hz, 1H), 5.94 (brs, 1H), 5.79–5.67 (m, 3H), 5.56–5.47 (m, 2H), 5.17 (brs, 1H), 5.06 (s, 1H), 4.96 (brs, 1H), 4.82–4.73 (m, 2H), 4.43 (tt, *J* = 7.8, 3.8 Hz, 1H), 4.39–4.28 (m, 3H), 4.21 (brs, 1H), 4.13 (brs, 1H), 3.78 (s, 3H), 3.73 (q, *J* = 7.4 Hz, 5H), 3.67 (brs, 1H), 3.48 (ddd, *J* = 11.7, 7.2, 3.7 Hz, 2H), 3.41–3.28 (m, 1H), 3.17 (s, 1H), 3.09 (ddd, *J* = 12.2, 8.2, 3.3 Hz, 2H), 2.80–2.60 (m, 2H), 2.38–2.15 (m, 7H), 2.13–2.05 (m, 2H), 1.93 (ddd, *J* = 12.8, 8.0, 3.7 Hz, 2H), 1.85–1.79 (m, 2H), 1.54 (s, 3H), 1.42 (s, 9H), 1.34 (s, 3H), 1.04 (d, *J* = 2.8 Hz, 42H), 1.01 (s, 6H), 0.96 (s, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 162.1, 162.0, 159.6, 159.5, 156.2, 155.8, 150.9, 150.4, 142.80, 142.78, 136.88, 136.86, 135.23, 135.21, 133.9, 133.6, 131.33, 131.30, 131.29, 129.40, 129.37, 129.2, 129.1, 129.02, 128.98, 126.24, 126.22, 126.21, 125.40, 125.36, 124.5, 124.4, 123.20, 123.19, 122.5 (2C), 121.8, 120.1, 119.3, 116.8 (2C), 115.4, 80.4, 80.02, 79.99, 79.96, 79.95, 79.92, 79.87, 79.85, 79.83, 74.51, 74.50, 72.7, 70.4, 70.3, 69.5, 60.0, 59.9, 55.73, 55.72, 46.7, 46.19, 46.15, 46.13, 46.11, 46.10, 46.07, 46.0, 44.8, 34.7, 34.5, 32.61, 32.58, 30.2, 29.7, 29.64, 29.60, 28.50, 28.45, 28.42, 28.38, 28.34, 27.25 (2C), 27.19, 27.16, 25.31, 25.29, 25.27, 18.1 (12C), 14.1, 12.2, 11.9 (6C); HRMS (ESI+) *m/z* calcd for C₉₄H₁₃₇Cl₄F₃N₇O₂₁Si₂ [M+H] 1952.8112, found: 1952.8098.



4-(((2S,3S)-1-Amino-3-(((2S,3R,4S,5R)-5-(aminomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-3-((2S,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-1-oxopropan-2-yl)amino)-N-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl) butanamide (1): To a stirred solution of **10** (2.64 g, 1.35 mmol) in CH₂Cl₂ (15 mL) was added TFA (10 mL). The reaction mixture was stirred for 3 h at rt, and all volatile were evaporated *in vacuo*. To a stirred solution of the crude mixture in H₂O (5 mL) was added TFA (20 mL). The reaction mixture was stirred for 2 days at rt, and all volatile were evaporated *in vacuo*. The crude mixture was purified by DOWEX (50W x 4) ion exchange resin. The resin was washed with MeOH/H₂O (4:1) and MeOH. The crude product (TFA salt) was dissolved in MeOH (10 mL) and absorbed on DOWEX (50W x 4): the crude **1** was not detected by TLC (CHCl₃/MeOH/H₂O/50% aqueous ammonia 56:42:7:3). The resins were washed with MeOH and eluted with MeOH/50% aqueous ammonia (10:1). The eluate was concentrated under reduced pressure and the resultant aqueous solution was lyophilized. The resulted mixture was purified by C18 reverse-phase HPLC [column: HYPERSIL GOLD™ (175 Å, 12 μm,

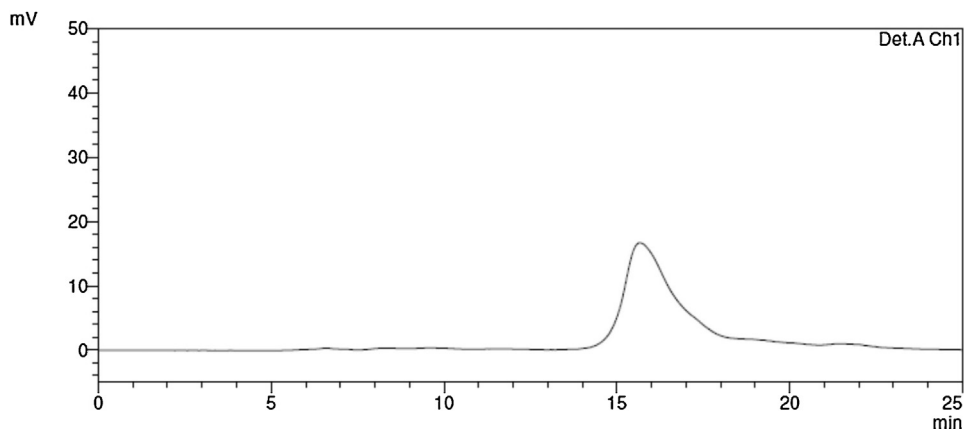


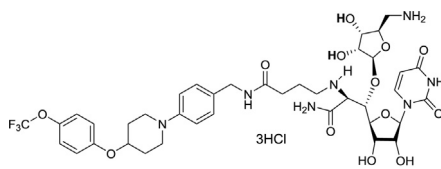
Fig. 1. HPLC analysis of **1**.

Area % purity: 96.8%.

Conditions: column: Phenomenex Kinetex 5 μm XB-C18 100 \AA 250 \times 4.60 mm column, solvents: 85:15 MeOH:0.05M NH_4HCO_3 in water, UV: 254 nm, flow rate: 0.5 mL/min.

150 \times 20 mm), solvents: 80:20 MeOH:0.05M NH_4HCO_3 in H_2O , flow rate: 6.0 mL/min, UV: 254 nm, retention time: 14 min] to afford **1** (1.05 g, 1.19 mmol, 88%): TLC (*n*-butanol/ethanol/ CHCl_3 /28% aqueous ammonia 4:7:2:7) R_f =0.50; $[\alpha]_D^{21}$ +0.375 (c =0.30, methanol); IR (thin film) ν_{max} = 3352 (br), 2932, 1677, 1505, 1243, 1201, 1136, 801, 722 cm^{-1} ; ^1H NMR (400 MHz, CD_3OD) δ 7.78 (d, J =8.1 Hz, 1H), 7.18 (dd, J =9.0, 3.5 Hz, 4H), 7.00 (dd, J =16.0, 8.6 Hz, 4H), 5.77 (d, J =2.9 Hz, 1H), 5.73 (d, J =8.1 Hz, 1H), 5.14 (s, 1H), 4.57–4.50 (m, 1H), 4.28 (s, 2H), 4.22–4.13 (m, 3H), 4.10 (dd, J =8.6, 4.4 Hz, 1H), 4.07–3.98 (m, 2H), 3.52–3.46 (m, 3H), 3.44 (d, J =8.8 Hz, 1H), 3.17 (d, J =13.0 Hz, 1H), 3.14–3.02 (m, 3H), 2.60 (ddq, J =18.4, 11.8, 6.9 Hz, 2H), 2.29 (td, J =7.3, 2.8 Hz, 2H), 2.12 (dd, J =14.5, 5.6 Hz, 2H), 1.93–1.73 (m, 4H), 1.39–1.25 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 175.6, 166.2, 157.6, 152.0, 142.6, 131.2, 129.6 (2C), 123.6 (2C), 118.11 (2C), 118.07 (2C), 110.5, 102.7, 92.3, 85.3, 81.4, 80.4, 76.5, 75.1, 74.1 (2C), 73.0, 71.3, 64.4, 43.7, 43.6, 34.7, 31.5, 26.9; HRMS (ESI+) m/z calcd for $\text{C}_{39}\text{H}_{51}\text{F}_3\text{N}_7\text{O}_{13}$ $[\text{M}+\text{H}]$ 882.3497, found: 882.3512 (Fig. 1).

Preparation of HCl salt of **1**

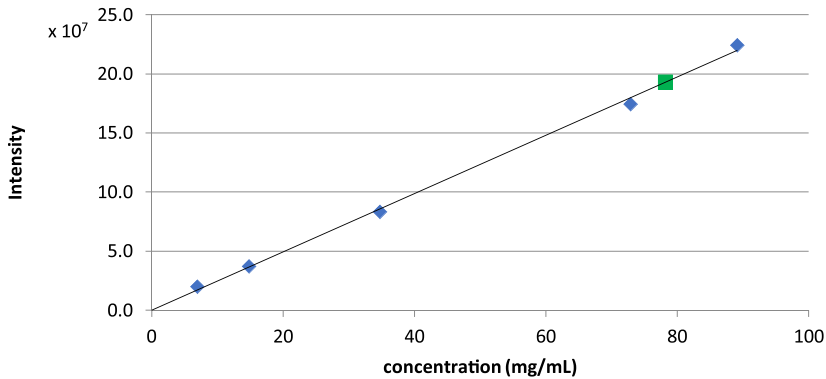


APPB-HCl salt (**1-HCl**)

To a stirred solution of **1** (1.05 g, 1.19 mmol) in MeOH (50 mL) was added ice cold 1N HCl (23.8 mL, 23.8 mmol) dropwise. After being stirred for 1 h at rt, the solution was concentrated under reduced pressure and the resultant aqueous solution was lyophilized to give **1**•HCl salt (Fig. 2).

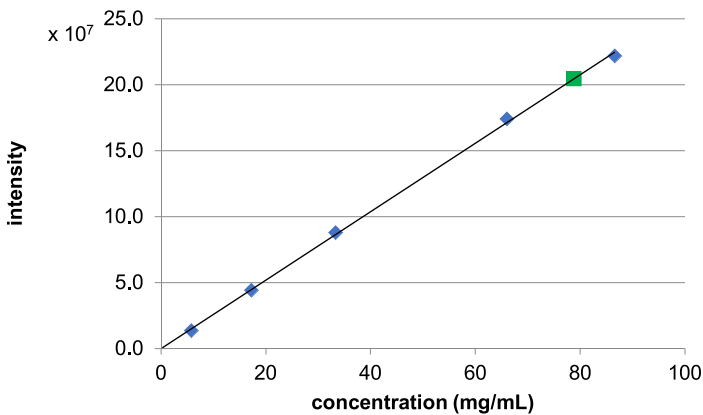
Determination of solubility of **1**•HCl in 0.9% NaCl (saline)

A suspension of **1**•HCl (4.0 mg) in 0.9% NaCl (30 μL) was stirred for 24 h, and the precipitate was separated by centrifugation at 10,000 \times g for 5 min. The upper solution (1 μL) was analyzed via C18 reverse-phase HPLC [column: Kinetex (100 \AA , 5 μm , 250 \times 4.60 mm), solvents: 70:30 MeOH:



Concentration (mg/mL)	6.9	14.8	34.7	72.9	78.2	89.1
Intensity	20096819	37269477	83517041	174550616	192719948	224399521

Fig. 2. Water solubility of **1•HCl** in saline.



Concentration (mg/mL)	5.8	17.2	33.3	66.0	78.8	86.5
Intensity	13645284	44234794	87884914	174012130	204558681	222058666

Fig. 3. Water solubility of **1•HCl** in PBS (pH7.4).

0.05 M NH_4HCO_3 aq., flow rate: 0.5 mL/min, UV: 254 nm, retention time: 12.0 min]. The area of the peak for **1** was quantified. The concentrations were determined *via* the HPLC intensity-concentration curves [7–9].

Determination of solubility of **1•HCl** in PBS (pH7.4) buffer

A suspension of **1•HCl** (3.8 mg) in phosphate buffered saline (pH 7.4, 30 μL) was stirred for 24 h, and the precipitate was separated by centrifugation at $10,000 \times g$ for 5 min. The upper solution (1 μL) was analyzed *via* C18 reverse-phase HPLC [column: Kinetex (100 \AA , 5 μm , 250×4.60 mm), solvents: 70:30 MeOH:0.05 M NH_4HCO_3 aq., flow rate: 0.5 mL/min, UV: 254 nm, retention time: 12.0 min]. The area of the peak for **1** was quantified. The concentrations were determined *via* the HPLC intensity-concentration curves [7–9] (Fig. 3).

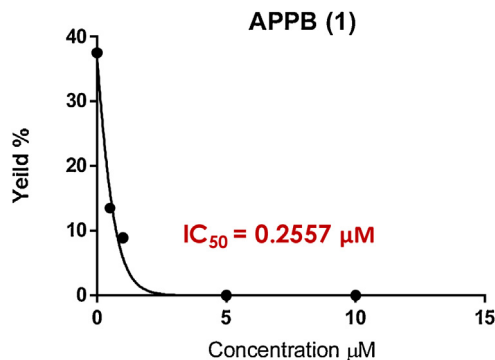


Fig. 6. IC_{50} curve for APPB (1).

reaction mixture, DPAGT1 solution (10 μ L) was added (total volume of reaction mixture: 50 μ L adjust with Tris buffer). The reaction mixture was incubated for 4 h at 37 °C and quenched with *n*-butanol (150 μ L). Two phases were mixed *via* vortex and centrifuged at 10,000 \times *g* for 3 min. The upper organic phase was assayed *via* reverse-phase HPLC. The organic phase (30 μ L) was injected into HPLC (solvent: gradient elution of 85:15–95:5 MeOH/0.05 M aq. NH_4HCO_3 over 20 min; UV: 485 nm; flow rate: 0.5 mL/min; column: Kinetex 5 μ m C8, 100 Å, 150 \times 4.60 mm), and the area of the peak for C₅₅-P-P-glucosamine-C₆-FITC was quantified to obtain the IC_{50} value. The IC_{50} values were calculated from plots of the percentage product inhibition versus the inhibitor concentration (Fig. 6).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.mex.2019.09.031>.

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