MethodsX 6 (2019) 2305–2321

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Protocol Article

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

Katsuhiko Mitachi[a], Shou M. Kurosu[a], Cody D. Gillman[b], Hyun Gi Yun[b], William M. Clemons Jr.[b], Michio Kurosu[a,*]

[a] *Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, 881 Madison Avenue, Memphis, TN 38163, United States*
[b] *Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125, United States*

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*-acetylglucosaminephosphotransferase 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 phenoxypiperidinbenzyl butanamide (APPB) showed DPAGT1 inhibitory activity with the IC_{50} 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 ($IC_{50} > 200$ 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.

* Corresponding author.
E-mail addresses: kmitachi@uthsc.edu (K. Mitachi), kurosu@wisc.edu (S.M. Kurosu), gillmanc@caltech.edu (C.D. Gillman), hyun@caltech.edu (H.G. Yun), clemons@caltech.edu (W.M. Clemons), mkurosu@uthsc.edu (M. Kurosu).

https://doi.org/10.1016/j.mex.2019.09.031

- Remember that the abstract is what readers see first in electronic abstracting & indexing services.
- This is the advertisement of your article. Make it interesting, and easy to be understood.
- Be accurate and specific, keep it as brief as possible.

ARTICLE INFO
Protocol name: A practical synthesis of a novel DPAGT1 inhibitor, aminouridyl phenoxypiperidinbenzyl butanamide (APPB) for *in vivo* studies
Keywords: Selective dolichyl-phosphate, *N*-Acetylglucosaminephosphotransferase 1, DPAGT1 inhibitor, Gram-scale synthesis
Article history: Received 6 December 2018; Accepted 23 September 2019; Available online 27 September 2019

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 in vivo 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*

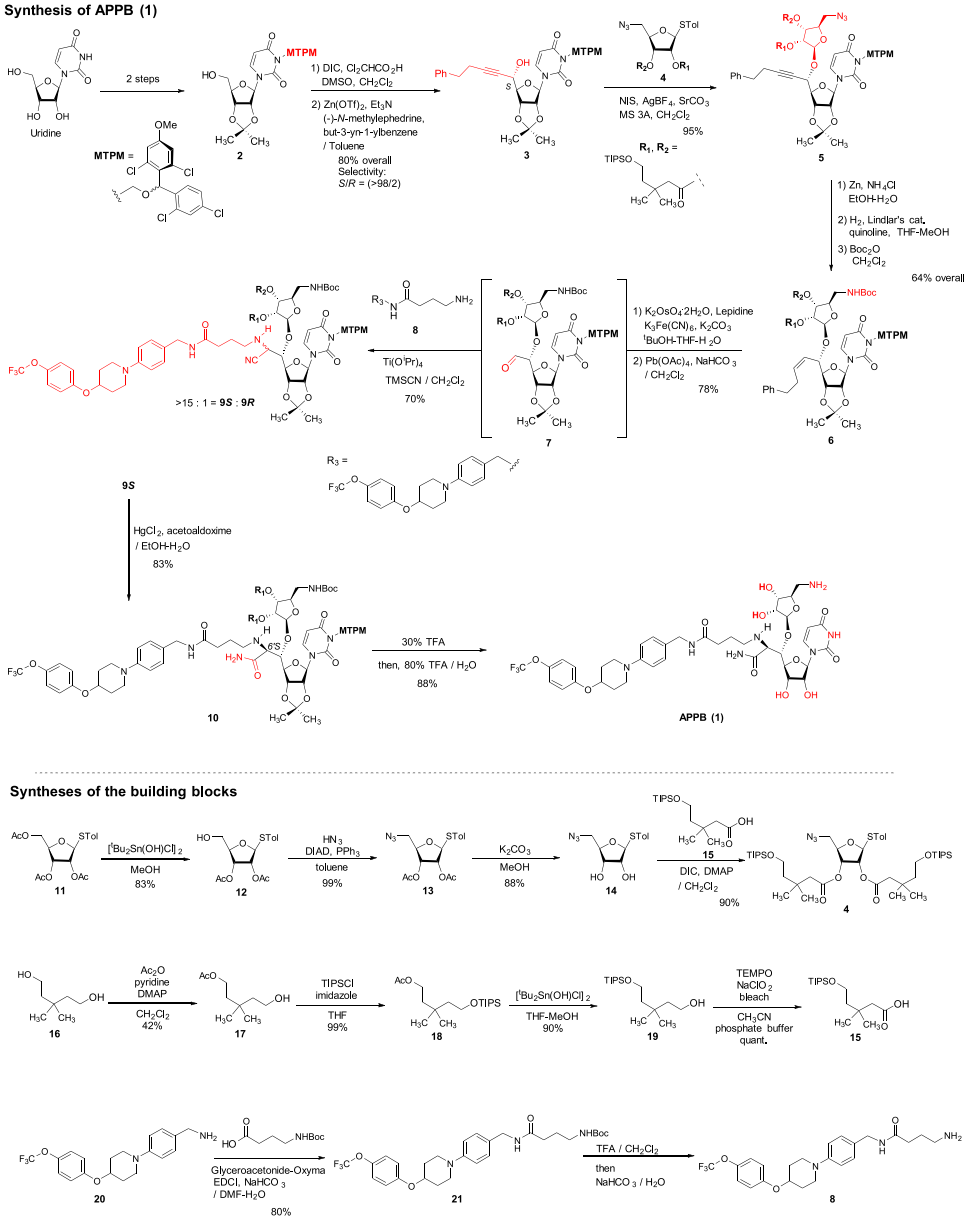
Value of the Protocol

- 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 alkynation reaction using (−)-*N*-methylephedrine [2], yielding the (*S*)-propargyl alcohol **3** in 80% yield with selectivity of >98:2. NIS-$AgBF_4$ 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. NH_4Cl, 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**. $Ti(O^iPr)_4$-mediated Strecker reaction of **7** with the 4-aminobutanamide derivatives **8** provided the *S*-diasteromer **9*S*** in 70% yield with greater than 15:1 *S*/*R* ratio. The desired diastereomer, **9*S*** was subjected to hydration reaction with $HgCl_2$-acetoaldoxime, furnishing the amide **10** in 83% overall yield. Global deprotection of **10** was performed in one-pot two step reaction using 30% TFA in CH_2Cl_2



Scheme 1. Synthesis of APPB (**1**).

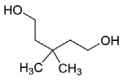
followed by 80% TFA in H_2O; the crude product was purified by DOWEX 50W x 4 ion exchange resin followed by preparative HPLC to furnish **1** in 88% overall yield (Scheme 1).

General

All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. THF, CH_2Cl_2, and DMF were purified *via* Innovative Technology's Pure-Solve System. All reactions were performed under nitrogen atmosphere. Reactions were monitored by TLC

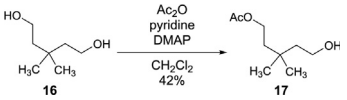
using 0.25 mm coated commercial silica gel plates (EMD, Silica Gel 60F_{254}). TLC spots were visualized by UV light at 254 nm, or developed with ceric ammonium molybdate or anisaldehyde or copper sulfate or ninhydrin solutions by heating on a hot plate. Reactions were also monitored by using SHIMADZU 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). ^{1}H NMR spectral data were recorded on 400, and 500 MHz instruments. ^{13}C 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. ^{1}H and ^{13}C NMR spectra were calibrated with residual undeuterated solvent ($CDCl_3$: δH = 7.26 ppm, δC = 77.16 ppm; CD_3CN: δH = 1.94 ppm, δC = 1.32 ppm; CD_3OD: δH = 3.31 ppm, δC = 49.00 ppm; DMSO-d_6: δH = 2.50 ppm, δC = 39.52 ppm; D_2O: 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***

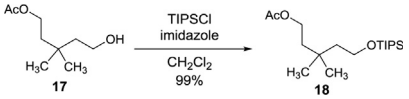


16

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}; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) δ 59.60 (2C), 44.06 (2C), 31.67, 28.08 (2C); HRMS (ESI+) *m/z* calcd for $C_7H_{16}O_2$ 132.1150, found 132.1144.

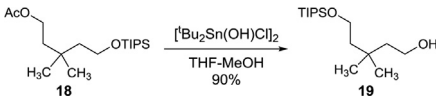


5-Hydroxy-3,3-dimethylpentyl acetate (17): To a stirred solution of **16** (47.5 g, 359.3 mmol) in CH_2Cl_2 (500 mL) were added pyridine (31.8 mL, 395.2 mmol), Ac_2O (33.9 mL, 359.3 mmol) and DMAP (0.44 g, 3.59 mmol) at 0 °C. The reaction mixture was stirred for 12 h at rt, and all volatiles were evaporated *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; ^{1}H 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 $C_9H_{18}O_3$ 174.1256, found 174.1249.

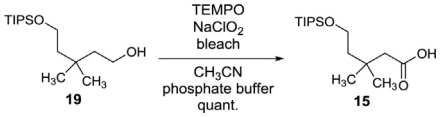


3,3-Dimethyl-5-((triisopropylsilyl)oxy)pentyl acetate (18): To a stirred solution of **17** (26.3 g, 150.9 mmol) and imidazole (20.6 g, 301.8 mmol) in dry CH_2Cl_2 (500 mL) were added TIPSCl (48.4 mL, 226.4 mmol) and DMAP (0.18 g, 1.51 mmol) at 0 °C. The reaction mixture was warmed to rt and stirred for 16 h. The reaction was quenched with saturated $NaHCO_3$ (aq.) and extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 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; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.12 (t, *J* = 7.6 Hz, 2H),

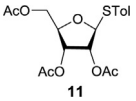
3.74 (t, J = 7.2 Hz, 2H), 2.03 (s, 3H), 1.59 (t, J = 7.6 Hz, 2H), 1.53 (t, J = 7.2 Hz, 2H), 1.11–1.02 (m, 21H), 0.94 (s, 6H); HRMS (ESI+) m/z calcd for $C_{18}H_{39}O_3Si$ [M+H] 331.2668, found 331.2685.



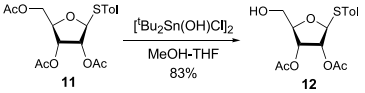
3,3-Dimethyl-5-((triisopropylsilyl)oxy)pentan-1-ol (19): To a stirred solution of **18** (49.4 g, 149.4 mmol) in MeOH/THF (4:1, 300 mL) was added $[^tBu_2Sn(OH)Cl]_2$ (0.86 g, 1.50 mmol). After 20 h at rt, all volatiles were evaporated *in vacuo*. The crude mixture was purified by silica gel column chromatography (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) ν_{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.



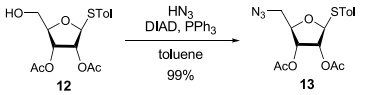
3,3-Dimethyl-5-((triisopropylsilyl)oxy)pentanoic acid (15): To a stirred solution of **19** (38.8 g, 134.5 mmol) and TEMPO (1.05 g, 6.73 mmol) in MeCN (135 mL) an phosphate buffer (pH = 6.8, 135 mL) were added $NaClO_2$ (14.6 g, 141.4 mmol) and bleach (8.25%, 65 mL) at 35 °C. After being stirred for 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) ν_{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.



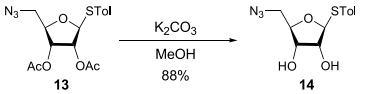
(2*R*,3*S*,4*S*,5*S*)-2-(Acetoxymethyl)-5-(*p*-tolylthio)tetrahydrofuran-3,4-diyl diacetate (10): The title compound was synthesized according to the reported procedure [1]: TLC (hexanes/EtOAc 50:50) R_f = 0.60; $[\alpha]^{20}_D$ −0.411 (c = 0.51, $CHCl_3$); IR (thin film) ν_{max} = 1742, 1371, 1214, 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.



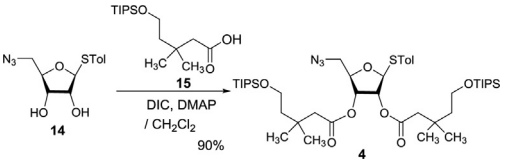
(2*R*,3*S*,4*S*,5*S*)-2-(Hydroxymethyl)-5-(*p*-tolylthio)tetrahydrofuran-3,4-diyl diacetate (12): To a stirred solution of **10** (24.3 g, 62.8 mmol) in MeOH/THF (4:1, 300 mL) was added $[^tBu_2Sn(OH)Cl]_2$ (0.72 g, 1.26 mmol). After 20 h at rt, all volatiles were evaporated *in vacuo*. The crude mixture 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; $[\alpha]^{21}_D$ −0.298 (c = 1.37, $CHCl_3$); 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, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{16}H_{21}O_6S$ [M+H] 341.1059, found 341.1075.



(2*R*,3*S*,4*S*,5*S*)-2-(Azidomethyl)-5-(*p*-tolylthio)tetrahydrofuran-3,4-diyl diacetate (13): To a stirred solution of **12** (17.9 g, 52.7 mmol) and PPh_3 (27.6 g, 105.1 mmol) in dry toluene (100 mL) were added HN_3 (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; $[\alpha]^{21}_D$ −0.899 (c = 3.93, $CHCl_3$); 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, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{16}H_{20}N_3O_5S$ [M+H] 366.1124, found: 366.1133.

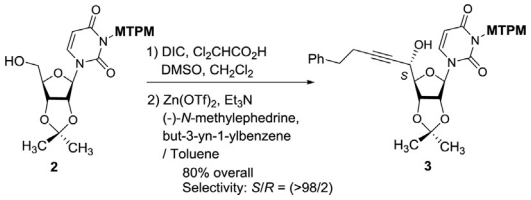


(2*R*,3*R*,4*S*,5*S*)-2-(Azidomethyl)-5-(*p*-tolylthio)tetrahydrofuran-3,4-diol (13): To a stirred solution of **13** (19.0 g, 52.0 mmol) in MeOH (200 mL) was added K_2CO_3 (10.0 g, 72.5 mmol). After being stirred for 30 min, the reaction mixture was filtered and concentrated *in vacuo*. The crude mixture 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; $[\alpha]^{21}_D$ −0.152 (c = 0.34, $CHCl_3$); IR (thin film) ν_{max} = 3385 (br), 2923, 2103, 1493, 1437, 1399, 1286, 1117, 1065, 1042, 1017, 809 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{12}H_{16}N_3O_3S$ [M+H] 282.0912, found: 282.0928.

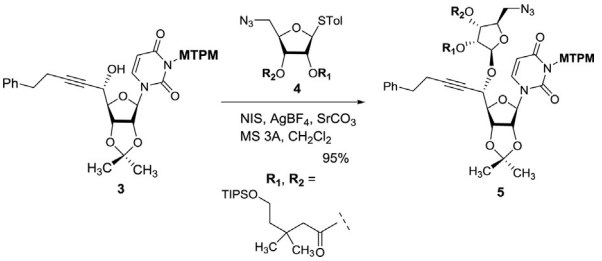


(2*R*,5*S*)-2-(Azidomethyl)-5-(*p*-tolylthio)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (4): To a stirred solution of **14** (12.9 g, 45.9 mmol) and **15** (34.7 g, 114.8 mmol) in CH_2Cl_2 (231 mL) were added DMAP (1.12 g, 9.17 mmol) and DIC (18.0 mL, 114.8 mmol) at 0 °C. The reaction mixture was stirred for 16 h at rt and concentrated *in vacuo*. The crude mixture

was purified by silica gel column chromatography (hexanes/EtOAc 95:5) to afford **4** (35.1 g, 41.2 mmol, 90%): TLC (hexanes/EtOAc 90:10) R_f = 0.60; $[\alpha]^{21}_D$ −0.293 (c = 1.39, $CHCl_3$); IR (thin film) ν_{max} = 2792, 2892, 2866, 2102, 1745, 1464, 1390, 1367, 1282, 1254, 1219, 1190, 1100, 1071, 1054, 1013, 998, 882, 809, 772, 742, 681 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 5.26 (d, J = 5.3 Hz, 1H), 5.18 (t, J = 5.3 Hz, 1H), 5.11 (t, J = 5.0 Hz, 1H), 4.13 (q, J = 4.7 Hz, 1H), 3.76 (dt, J = 10.6, 6.9 Hz, 4H), 3.42 (d, J = 4.7 Hz, 2H), 2.34 (s, 3H), 2.31 (d, J = 10.6 Hz, 2H), 2.26 (d, J = 4.9 Hz, 2H), 1.61 (dtd, J = 17.4, 6.9, 2.1 Hz, 4H), 1.08–1.00 (m, 54H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.91, 170.54, 138.67, 133.89 (2C), 129.81 (2C), 127.88, 88.58, 81.48, 73.52, 71.70, 60.02, 59.97, 52.70, 46.15, 46.03, 44.64, 44.55, 32.68, 32.60, 27.51, 27.47, 27.38, 21.17, 18.06 (6C), 18.05 (6C), 11.93 (3C), 11.92 (3C); HRMS (ESI+) m/z calcd for $C_{44}H_{79}N_3NaO_7SSi_2$ [M+Na] 872.5075, found: 872.5088.

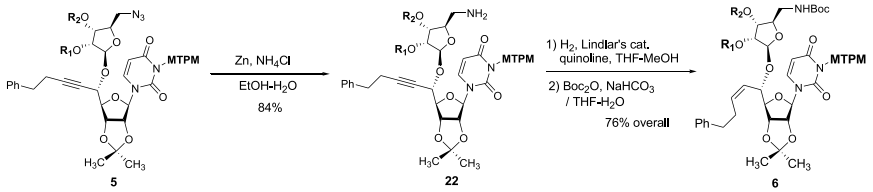


3-(((2,6-Dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-1-((3a*R*,4*R*,6-*R*,6a*R*)-6-((*S*)-1-hydroxy-5-phenylpent-2-yn-1-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione (3): Title compound was synthesized according to the reported procedure [1]: TLC (hexanes/EtOAc 50:50) R_f = 0.30; $[\alpha]^{22}_D$ −0.116 (c = 2.17, $CHCl_3$); IR (thin film) ν_{max} = 3387 (br), 2981, 2937, 1664, 1454, 1276, 1065, 1039, 856, 733, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 (ddd, J = 20.4, 8.5, 0.7 Hz, 1H), 7.35–7.27 (m, 4H), 7.24–7.15 (m, 4H), 6.85 (d, J = 5.1 Hz, 2H), 6.51 (d, J = 5.4 Hz, 1H), 5.68 (dd, J = 8.1, 4.1 Hz, 1H), 5.60–5.50 (m, 3H), 4.89–4.78 (m, 2H), 4.57 (ddt, J = 12.0, 4.3, 2.0 Hz, 1H), 4.24 (dd, J = 4.4, 3.1 Hz, 1H), 3.78 (d, J = 3.3 Hz, 3H), 2.83 (t, J = 7.5 Hz, 2H), 2.53 (td, J = 7.4, 2.0 Hz, 2H), 1.57 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.11, 162.08, 159.5, 150.87, 150.85, 141.1, 140.8, 140.30, 140.27, 136.9, 135.4, 135.3, 133.99, 133.95, 133.8, 133.6, 131.2, 129.4, 129.3, 128.41, 128.39, 126.4, 126.21, 126.18, 125.5, 125.4, 115.34, 115.32, 114.3, 114.2, 101.8, 101.7, 96.7, 96.4, 89.23, 89.19, 86.8, 86.7, 84.1, 84.0, 80.9, 69.5, 63.02, 62.99, 55.7, 34.72, 34.70, 27.2, 25.3, 20.87, 20.85; HRMS (ESI+) m/z calcd for $C_{37}H_{34}N_2O_8NaCl_4$ [M+Na] 797.0967, found: 797.0994.



(2*R*,3*R*,4*R*,5*R*)-2-(Azidomethyl)-5-(((1*S*)-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-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-((triisopropylsilyl)oxy)pentanoate) (5). To a stirred suspension of **3** (5 g, 6.44 mmol), **4** (6.57 g, 7.73 mmol), MS3A (7.56 g) and $SrCO_3$ (4.75 g, 32.2 mmol) in CH_2Cl_2 (260 mL) were added $AgBF_4$ (0.63 g, 3.22 mmol) and NIS (1.88 g, 8.37 mmol) at 0 °C. After 24 h, the reaction mixture was added Et_3N (2 mL) and passed through a silica gel pad (hexanes/EtOAc 1:1). The combined organic phase was concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 90:10 to 80:20 to 70:30) to afford 5 (9.19 g, 6.12 mmol, 95%): TLC (hexanes/EtOAc 67:33) R_f = 0.70; $[\alpha]^{21}_D$ +0.100 (c = 2.09, $CHCl_3$); IR (thin film) ν_{max} = 2942, 2866, 2102, 1743, 1724, 1675, 1456, 1278, 1218, 1099, 1070, 882, 772 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.54

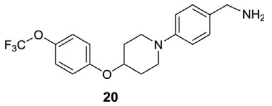
(dd, J = 23.1, 8.5 Hz, 1H), 7.32–7.27 (m, 4H), 7.24–7.16 (m, 4H), 6.84 (d, J = 7.3 Hz, 2H), 6.51 (d, J = 3.7 Hz, 1H), 5.71–5.64 (m, 2H), 5.60–5.49 (m, 2H), 5.20–5.16 (m, 3H), 4.79 (ddd, J = 7.5, 6.5, 3.1 Hz, 1H), 4.64 (td, J = 5.9, 2.6 Hz, 1H), 4.57 (ddt, J = 11.4, 6.3, 1.9 Hz, 1H), 4.28 (dt, J = 6.2, 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 $C_{74}H_{106}Cl_4N_5O_{15}Si_2$ [M+H] 1500.5978, found: 1500.5992.



(2*R*,3*R*,4*R*,5*R*)-2-(Aminomethyl)-5-(((1*S*)-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-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-((triisopropylsilyl)oxy)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 EtOH/H_2O (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 $CHCl_3$/MeOH 96:4) to afford the primary amine **22** (5.80 g, 3.93 mmol, 84%): TLC ($CHCl_3$/MeOH 90:10) R_f = 0.60; $[\alpha]^{21}{}_D$ −0.013 (c = 1.35, $CHCl_3$); IR (thin film) ν_{max} = 2941, 2866, 1742, 1721, 1675, 1600, 1556, 1461, 1382, 1278, 1215, 1099, 1070, 1050, 999, 882 cm^{-1}; ^{1}H 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 $C_{74}H_{108}Cl_4N_3O_{15}Si_2$ [M+H] 1474.6073, found: 1475.6091.

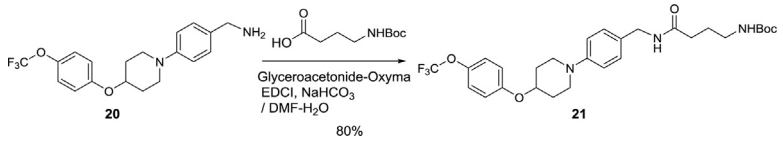
(2*R*,3*R*,4*R*,5*R*)-2-(((*tert*-Butoxycarbonyl)amino)methyl)-5-(((1*S*,*Z*)-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-5-phenylpent-2-en-1-yl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (6): To a stirred solution of **22** (5.80 g, 3.93 mmol) and quinoline (10 mL) in THF-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 1*N* HCl and extracted with EtOAc. The combined organic solution was washed with 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 80:20 to 70:30). to afford 6 (4.71 g, 2.99 mmol, 76%): TLC (hexanes/EtOAc 75:25) R_f = 0.40; $[\alpha]^{21}{}_D$ −0.015 (c = 0.86, $CHCl_3$); 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, $CDCl_3$) 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, 51H), 0.99 (dd, J = 9.6, 4.0 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{79}H_{118}Cl_4N_3O_{17}Si_2$ [M+H] 1576.6754, found: 1576.6771.



20

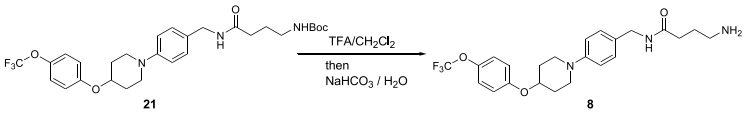
(4-(4-(4-(Trifluoromethoxy)phenoxy)piperidin-1-yl)phenyl)methanamine (20): The title compound was synthesized according to the reported procedure [5]: ^1H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 8.2 Hz, 2H), 7.14 (d, J = 8.6 Hz, 2H), 6.97–6.87 (m, 4H), 4.43 (tt, J = 7.7, 3.8 Hz, 1H), 3.79 (s, 2H), 3.49 (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); ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{19}H_{22}F_3N_2O_2$ [M+H] 367.1633, found 367.1628.



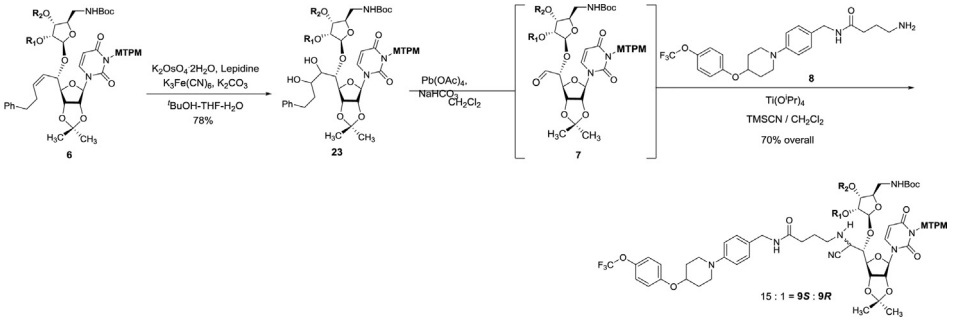
20 **21**

***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 $NaHCO_3$ (6.00 g, 72.0 mmol) in THF-H_2O (1:1, 24 mL) was added Boc_2O (5.76 g, 26.4 mmol). After being stirred for 8 h at rt, the reaction mixture was quenched with 1*N* HCl and extracted with $CHCl_3$. The combined organic solution was dried over Na_2SO_4 and concentrated *in vacuo*. To a stirred solution of the crude mixture, **20** (4.45 g, 12.14 mmol), $NaHCO_3$ (5.10 g, 60.7 mmol) and Glyceroacetonide-Oxyma (5.54 g, 24.3 mmol) in DMF-H_2O (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 H_2O and extracted with EtOAc. The combined organic solution was washed with 1*N* HCl (aq.), saturated $NaHCO_3$ (aq.), dried over Na_2SO_4 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 $C_{28}H_{37}F_3N_3O_5$ [M+H] 552.2685, found: 552.2701.



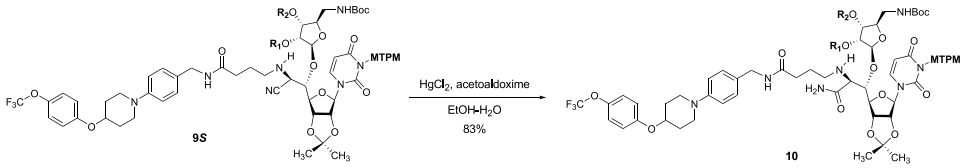
4-Amino-*N*-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)butanamide (8): To a stirred solution of **22** (3.81 g, 6.99 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL). The reaction mixture was stirred for 3 h at rt, and all volatile were evaporated *in vacuo*. The residue was neutralized with 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.



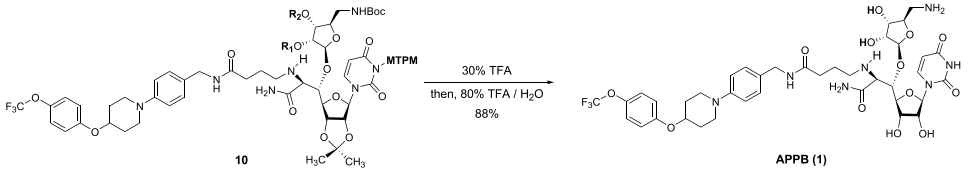
(2*R*,3*R*,4*R*,5*S*)-2-(((*tert*-Butoxycarbonyl)amino)methyl)-5-(((1*S*)-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-2,3-dihydroxy-5-phenylpentyl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)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-BuOH/THF/H_2O (1:1:1, 180 mL) were added K_2CO_3 (2.06 g, 14.9 mmol), $K_3Fe(CN)_6$ (4.91 g, 14.9 mmol) and $K_2OsO_4{\cdot}2H_2O$ (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), $K_3Fe(CN)_6$ (4.91 g, 14.9 mmol) and $K_2OsO_4{\cdot}2H_2O$ (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 1*N* 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; $[\alpha]^{22}_D$ 0.210 (*c* = 1.62, $CHCl_3$); IR (thin film) ν_{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}; ^{1}H 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 $C_{79}H_{120}Cl_4N_3O_{19}Si_2$ [M+H] 1610.6809, found: 1610.6827. Data for polar diastereomer: TLC (hexanes/EtOAc 67:33) R_f = 0.20; $[\alpha]^{22}_D$ 0.071 (*c* = 1.08, $CHCl_3$); IR (thin film) ν_{max} = 3413 (br), 2941, 2866, 1719, 1675, 1457, 1367, 1278, 1248, 1219, 1160, 1099, 1070, 1049, 882, 772 cm^{-1}; ^{1}H 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 $C_{79}H_{120}Cl_4N_3O_{19}Si_2$ [M+H] 1610.6809, found: 1610.6831.

(2*R*,3*R*,4*R*,5*S*)-2-(((*tert*-Butoxycarbonyl)amino)methyl)-5-((1*S*,2*R*)-2-cyano-1-((3a*R*,4*R*,6-*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-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 $Pb(OAc)_4$ (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 $Ti(OiPr)_4$ (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 **9*S*** (3.15 g, 1.63 mmol, 70% for 2 steps): TLC (hexanes/EtOAc 50:50) R_f = 0.40; $[\alpha]^{21}_D$ +0.102 (*c* = 0.75, $CHCl_3$); IR (thin film) ν_{max} = 3342 (br), 2941, 2866, 1718, 1675, 1505, 1464, 1243, 1164, 1101, 1071, 883, 772, 688 cm^{-1}; ^{1}H 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, 125.2, 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 $C_{94}H_{135}Cl_4F_3N_7O_{20}Si_2$ [M+H] 1934.8007, found: 1934.8021.



(2*S*,3*R*,4*R*,5*R*)-2-((1*S*,2*S*)-3-Amino-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[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 **9*S*** (3.15 g, 1.63 mmol) in EtOH/H_2O (9:1, 10 mL) were added $HgCl_2$ (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. $NaHCO_3$, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH 99.5:0.5–99.2:0.8–98.8:1.2) to afford **10** (2.64 g, 1.35 mmol, 83%): TLC ($CHCl_3$/MeOH 95:5) R_f = 0.30; $[\alpha]^{21}_D$ +0.144 (*c* = 0.53, $CHCl_3$); 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, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{94}H_{137}Cl_4F_3N_7O_{21}Si_2$ [M+H] 1952.8112, found: 1952.8098.



4-(((2*S*,3*S*)-1-Amino-3-(((2*S*,3*R*,4*S*,5*R*)-5-(aminomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-3-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-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 CH_2Cl_2 (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 H_2O (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/H_2O (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 ($CHCl_3$/MeOH/H_2O/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^{TM} (175 Å, 12 μm,

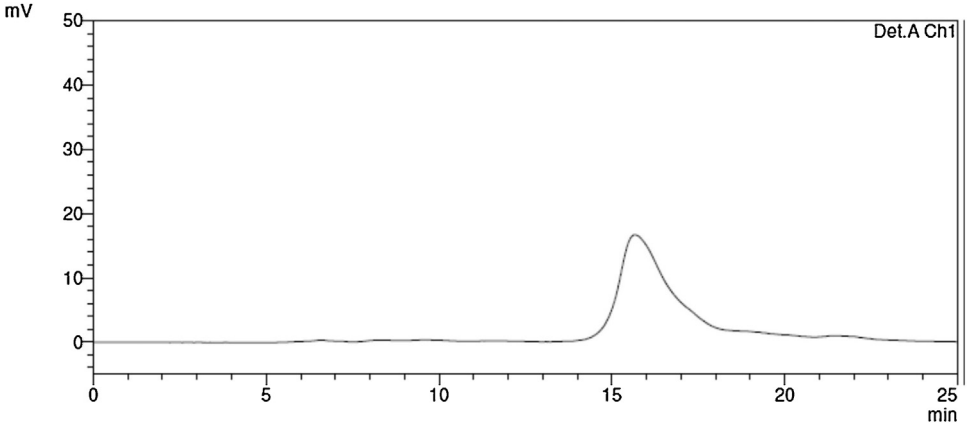
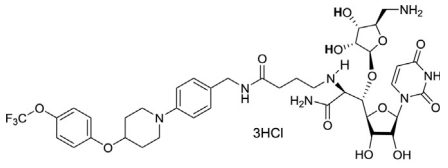


Fig. 1. HPLC analysis of **1**.
Area % purity: 96.8%.
Conditions: column: Phenomenex Kinetex 5 μm XB-C18 100 Å 250 × 4.60 mm column, solvents: 85:15 MeOH:0.05M NH_4HCO_3 in water, UV: 254 nm, flow rate: 0.5 mL/min.

150 × 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; $[\alpha]^{21}{}_D$ +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 $C_{39}H_{51}F_3N_7O_{13}$ [M+H] 882.3497, found: 882.3512 (Fig. 1).

*Preparation of HCl salt of **1***

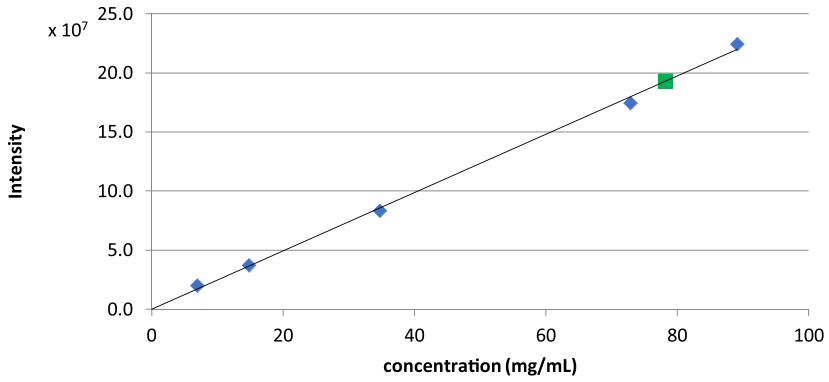


APPB-HCl salt (1-HCl)

To a stirred solution of **1** (1.05 g, 1.19 mmol) in MeOH (50 mL) was added ice cold 1*N* 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).

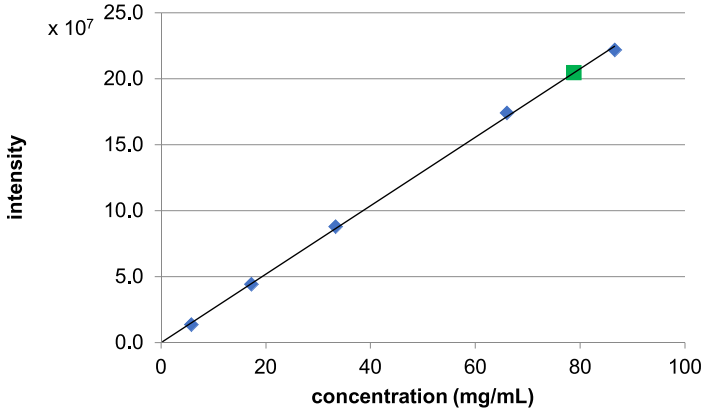
*Determination of solubility of **1•HCl** in 0.9% NaCl (saline)*

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 × *g* for 5 min. The upper solution (1 μL) was analyzed *via* C18 reverse-phase HPLC [column: Kinetex (100 Å, 5 μm, 250 × 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 × *g* for 5 min. The upper solution (1 μL) was analyzed *via* C18 reverse-phase HPLC [column: Kinetex (100 Å, 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).

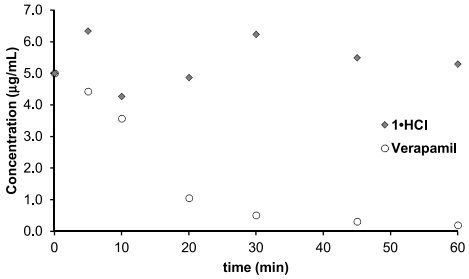


Fig. 4. Microsomal stability of **1**.

Microsomal stability

Pooled Sprague-Dawley rat liver microsomes were purchased from Corning Life Sciences (Oneonta, NY, USA). Microsomes (20 mg/mL) were thawed on ice and diluted with PBS, potassium phosphate buffer (100 mM, pH: 7.4) at a 1:8 ratio in 1.5 mL Eppendorf tubes. Stock solutions of **1•HCl** and verapamil (positive control) were made by diluting 10 mg/mL solutions. From the drug stock solution, 10 μL was diluted with 390 μL of buffer (0.1 mg/400 μL). The diluted microsomes (390 μL) were reacted with 10 μL of the diluted drug solution and allowed to equilibrate for 5 min while shaking at 440 rpm. NADPH (10 mg/200 μL; 1000× drug concentration) was used as a co-factor for this reaction, and 100 μL was added to the solution after equilibration. Ice cold methanol (200 μL) was used to quench the reaction mixture (50 μL aliquots) at 0, 5, 10, 20, 30, 45 and 60 min. The samples containing methanol was lyophilized to remove all volatiles. The residue was dissolved in 1*N* HCl aq. (10 μL) and MeOH (40 μL). The resulting solution (20 μL) was injected to LC–MS. MS solvent 90:10 acetonitrile/0.05% formic acid in water. Flow rate: 0.5 mL/min (Fig. 4).

DPAGT1 assay

The enzymatic substrate, UDP-Glucosamine-C_6-FITC was chemically synthesized according to the reported procedures [10]. DPAGT1 was expressed in suspended Expi293 cells for 36 h. The cells were lysed by drawing through a 26 g needle (10 times) and membrane protein was extracted using buffer containing 1% DM (decyl β-D-maltopyranoside) detergent. DPAGT1 was purified using HA (hemagglutinin)-agarose resin and a superdex 200 size exclusion column (Fig. 5).

UDP-Glucosamine-C_6-FITC (2 mM stock solution, 0.56 μL), $MgCl_2$ (0.5 M, 4 μL), β-mercaptoethanol (50 mM, 5 μL), CHAPS (20%, 2.5 μL), Tris buffer (pH 8.0, 50 mM), C_{55}-dolichyl phosphate (4 mM, 1.68 μL), and **1•HCl** (0–50 μg/mL in Tris buffer) were place in a 500 μL Eppendorf tube. To a stirred

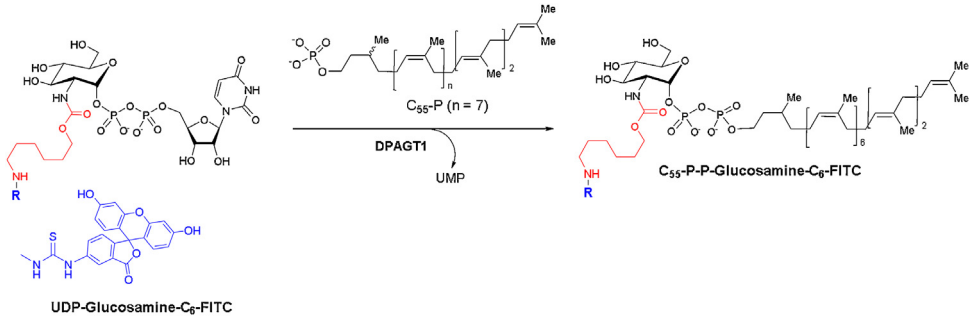


Fig. 5. DPAGT1-catalyzed reactions.

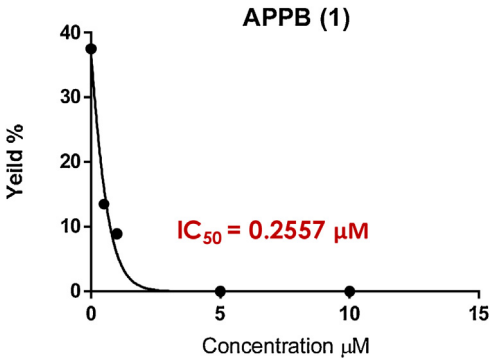


Fig. 6. IC_{50} curve for APPB (**1**).

reaction mixture, DPAGT1 solution (10 μL) was added (total volume of reaction mixture: 50 μL adjust with Tris buffer). The reaction mixture was incubated for 4 h at 37 °C and quenched with *n*-butanol (150 μL). Two phases were mixed *via* vortex and centrifuged at 10,000 × *g* for 3 min. The upper organic phase was assayed *via* reverse-phase HPLC. The organic phase (30 μL) 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 μm C8, 100 Å, 150 × 4.60 mm), and the area of the peak for C_{55}-P-P-glucosamine-C_6-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).

Acknowledgments

The National Institutes of Health is greatly acknowledged for financial support of this work (Grant GM114611). MK also thank University of Tennessee Health Science Center for generous financial support (CORNET award). NMR data were obtained on instruments supported by the NIH Shared Instrumentation Grant.

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.

References

[1] K. Mitachi, B.A. Aleiwi, C.M. Schneider, S. Siricilla, M. Kurosu, Stereocontrolled total synthesis of muraymycin D_1 having a dual mode of action against Mycobacterium tuberculosis, J. Am. Chem. Soc. 138 (2016) 12975–12980.
[2] K. Mitachi, H.-G. Yun, S.M. Kurosu, S. Eslamimehr, M.R. Lemieux, L. Klaić, W.M. Clemons, M. Kurosu, Novel FR-900493 analogs that inhibit outgrowth of Clostridium difficile spores, ACS Omega 3 (2018) 1726–1739.
[3] Y. Wang, M. Kurosu, A new protecting group and linker for uridine ureido nitrogen, Tetrahedron 68 (2012) 4797–4804.
[4] (a) Q. Wang, Y. Wang, M. Kurosu, A new oxyma derivative for nonracemizable amide-forming reactions in water, Org. Lett. 14 (2012) 3372–3375;
(b) Y. Wang, B.A. Aleiwi, Q. Wang, M. Kurosu, Selective esterifications of primary alcohols in a water-containing solvent, Org. Lett. 14 (2012) 4910–4913;
(c) B.A. Aleiwi, K. Mitahi, M. Kurosu, Mild and convenient *N*-formylation protocol in water-containing solvents, Tetrahedron Lett. 54 (2013) 2077–2081.
[5] S. Kang, R.Y. Kim, M.J. Seo, S. Lee, Y.M. Kim, M. Seo, J.J. Seo, Y. Ko, I. Choi, J. Jang, J. Nam, S. Park, H. Kang, H.J. Kim, J. Kim, S. Ahn, K. Pethe, K. Nam, Z. No, J. Kim, Lead optimization of a novel series of imidazo[1,2-*a*]pyridine amides leading to a clinical candidate (Q203) as a multi- and extensively-drug-resistant anti-tuberculosis agent, J. Med. Chem. 57 (2014) 5293–5305.
[7] K.J. Box, G. Völgyi, E. Baka, M. Stuart, K. Takács-Novák, J.E.A. Comer, Equilibrium versus kinetic measurements of aqueous solubility, and the ability of compounds to supersaturate in solution—a validation study, J. Pharm. Sci. 95 (2006) 1298–1307.

[8] E. Baka, J.E.A. Comer, K. Takács-Novák, Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound, J. Pharm. Biomed. Anal. 46 (2008) 335–341.
[9] G. Völgyi, E. Baka, K.J. Box, J.E.A. Comer, K. Takács-Novák, Study of pH-dependent solubility of organic bases. Revisit of Henderson-Hasselbalch relationship, Anal. Chim. Acta 673 (2010) 40–46.
[10] K. Mitachi, S. Siricilla, D. Yang, Y. Kong, K. Skorupinska-Tudek, E. Swiezewska, M. Kurosu, Fluorescence-based assay for polyprenyl phosphate-GlcNAc-1-phosphate transferase (WecA) and identification of a new WecA inhibitor that kills non-replicating Mycobacterium tuberculosis, Anal. Biochem. 512 (2016) 78–90.