

Supporting Information

Identification of novel carbocyclic pyrimidine cyclic dinucleotide STING agonists for antitumor immunotherapy using systemic intravenous route

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Cloning, expression, and purification of STING

Human STING (accession number GB:BC047779) DNA encoding residues 140-379 (H232R) was cloned into pET28 as *N*-terminal HIS and C-terminal Avi tagged fusion protein, and a rTEV cleavage site between HIS tag and STING. The sequence was verified before recombinant protein expression using E.coli expression system.

For expression, STING expression vector was transformed into BL21(DE3) cells. The expression was induced by adding IPTG and expressing in 16 °C for 20 hours.

For purification, STING was purified by Nickel affinity resin (Thermo Scientific #25215), followed by TEV enzyme cleavage and Superdex 200 column (GE Healthcare #28-9893-35) purification. For site directed in vitro biotinylation, purified Avi-tagged STING protein was incubated with Biotin (SUPELCO #47868), ATP (SIGMA# A-7699) and BirA enzyme, followed by further purification through a Superdex 200 column (GE Healthcare #28-9893-35). Biotinylation was confirmed by Mass Spectrometry analysis.

Human STING TR-FRET assay

40 nL of test compounds in DMSO were added to wells in a white, 384 well microtitre plate (Greiner 784075). 2 uL STING assay buffer (PBS and 0.01% BSA) containing fluorescein labeled ligand (c[G(2',5')p-2'-Fluo-AHC-A(3',5')p]- Biolog C 195, 100 nM final) and Tb labeled Streptavidin (Streptavidin-Tb cryptate- CisBio 610SATLB) were added. Then 2 uL STING assay buffer containing STING protein (100 nM final) was added and the mixture was incubated at rt for 60 minutes. The plates were then read on a BMG PheraStar Plus reader (LanthaScreen module).

For the assay method described above, test compound percent inhibition, at various concentrations, was calculated relative to untreated and DMSO only treated samples. Compound concentration versus percent inhibition curves were fitted to generate IC₅₀ values.

Reporter cellular assays

STING agonists trigger the production of type I IFNs and the induction of ISGs through IRFs. The potency of the STING agonist was evaluated in reporter assays using the ISRE_Nanoluc HEK293T cells, and the more physiologically relevant human monocyte derived THP1-Dual™ cells and the mouse macrophage RAW-Lucia™ ISG cells. These cell lines that are of mouse or human origin allow measurement of STING agonist activity using the nanoluc luciferase (HEK293T) or IRF-inducible Lucia luciferase as read-outs. (THP1 and RAW).

THP1-Dual™ Cells

THP1-Dual™ cells (InvivoGen, ID thpd-nfis), THP1-Dual™ cGAS K/O cells (InvivoGen, ID thpd-kocgas), THP1-Dual™ KI-R232 cells and the THP1-Dual™ STING K/O cells (InvivoGen, ID thpd-kost) were obtained from InvivoGen (San Diego, CA, US). These cell lines were derived at InvivoGen from the human THP-1 acute myelocytic leukemia (AML) cell line by stable integration of the Lucia luciferase gene, a secreted luciferase reporter gene, under the control of an ISG54 (interferon-stimulated gene) minimal promoter in conjunction with five interferon (IFN)-stimulated response elements. On the day of the experiment, the cells were plated to a black, 384-well plates at 7500 cells/25 µL per well density in growth media (RPMI 1640, 2 mM L-glutamine, 25 mM HEPES (4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-*N*-(2-ethanesulfonic acid), 10% heat-inactivated fetal bovine serum (FBS), 100 µg/mL Normocin™, 100 units (U)/mL-100 µg/mL Pen-Strep (Penicillin Streptomycin), 10 µg/mL of blasticidin, and 100 µg/mL of Zeocin). The cell plates were dosed with 62.5 nL of the testing compounds using the Labcyte ECHO 555, and then incubated at 37°C, 5% CO₂ for 20 hours. At the end of the incubation, 15 µL/well of the QUANTI-Luc™ (InvivoGen, ID rep-qlc1) were added, and luminescence was measured immediately using the LEADSeeker™ Imaging System.

ISRE NanoLuc HEK293T Cells

A stable HEK293T cell line expressing pNL[NLucP/ISRE/Hygro]vector (Promega [Fitchburg, WI, US]) was established as per manufacturers protocol. The cells were cultured in growth media (DMEM [Dulbecco's Modified Eagle Medium] with 10% FBS and transiently transfected with STING DNA from human WT (R232), or other variants R232H, R293Q, G230A-R293Q (AQ) and R71H-G230A-R293Q (HAQ) that exist in human populations the day before the assay using forward transfection assisted with Fugene® HD (Promega, Catalog No. E2312). The cells were plated into a white, Corning 384 well plate at 5000 cell/25 µL per well density in growth media. For each compound plate, 2 plates of cells transiently expressing the human STING were plated. On the day of the experiment, the media for one plate of cells was replaced by 25 µL/well digitonin buffer (50 mM HEPES pH 7.0, 100 mM KCl, 3 mM MgCl₂, 0.1 mM dithiothreitol [DTT], 85 mM sucrose, 0.2% bovine serum albumin [BSA], 0.1 mM adenosine triphosphate [ATP], 0.1 mM guanosine triphosphate [GTP], and 10 µg/mL digitonin), followed by dosing with 62.5 nL of test compound. The cells were incubated for 30 minutes at 37°C and 5% CO₂ to permeabilize the cell membranes. The digitonin buffer was then replaced by 25 µL cell growth media, and the cells were incubated at 37°C and 5% CO₂ for 4 hours. At the end of the incubation, 15 µL/well of the Nano-Glo® Detection System N1150 (Promega) were added, and luminescence was measured immediately using the LEADseeker™ Imaging System (General Electric Healthcare). For the other cell plate that had not been permeabilized with digitonin, 62.5 nL of test compound in DMSO were added to each well using the Labcyte ECHO 555. These plates were incubated at 37°C 5% CO₂ for 20 hours

before the Nano-Glo® Detection System was added and the luminescence detection was measured immediately as described above.

RAW-Lucia™ ISG cells

RAW-Lucia™ ISG cells (InvivoGen, ID rawl-isg) were derived by InvivoGen from the mouse RAW 264.7 macrophage cell line by stable integration of the Lucia luciferase gene, a secreted luciferase reporter gene, under the control of an ISG54 (interferon-stimulated gene) minimal promoter in conjunction with five IFN-stimulated response elements. One day before the experiment, the cells were plated to a white, 384-well plate (Corning, Catalog No.356661) at 12500 cells/25 µL per well density in growth media (DMEM, 2 mM L-glutamine, 4.5 g/L glucose, 10% FBS, 100 µg/ml Normocin™, 50 U/mL-50 µg/mL Pen-Strep, and 200 µg/mL of Zeocin). For each compound plate, 2 plates of cells were plated. The cells were incubated at 37°C overnight. On the day of the experiment, cell growth media from one of the 2 cell plates were replaced with 25 µL/well digitonin buffer (50 mM HEPES pH 7.0, 100 mM KCl, 3 mM MgCl₂, 0.1 mM DTT, 85 mM Sucrose, 0.2% BSA, 0.1 mM ATP, 0.1 mM GTP, and 10 µg/mL digitonin), followed by dosing with 62.5 nL of test compound. The cells were incubated for 30 minutes at 37°C to permeabilize the cell membranes. The digitonin buffer was then replaced by 25 µL cell growth media, and the cells were incubated at 37°C 5% CO₂ for 6 hours. At the end of the incubation, 10 µL/well of the QUANTI-Luc™ (InvivoGen, ID rep-qlc1) were added, and luminescence was measured immediately using the LEADseeker. For the cell plate that was not treated with

digitonin. 62.5 nL of the test compound in DMSO were added to the cells, and incubated at 37°C, 5% CO₂ for 20 hours before 10 µL/well of the QUANTI-Luc was added and the luminescence detection was measured immediately as described above.

Statistical Analysis

The EC₅₀ value and the 95% confidence interval were calculated from a dose-response curve generated using Genedata Screener® Condoseo v.11 (Genedata AG [Basel, Switzerland]). The curves were generated using test compound percent luminescence signal induction, at various concentrations relative to DMSO only and positive control treated samples. Compound concentration versus percent signal induction curves are fitted to generate EC₅₀ values that are determined from the inflection point of the curves, representing the concentration that induces a response halfway between baseline and maximum as calculated from normalization of raw data to DMSO controls (0% activation) and positive control (10 µM ADU-S100).

Western blot analysis THP-1 cells were plated in 6-wells dishes in RPMI-1640 medium containing phorbol 12-myristate 13-acetate (PMA, 50 ng/ml) and were incubated for 48 hours at 37°C. Medium was removed from PMA-differentiated THP-1 cells by pipetting. Cells were washed three times in pre-warmed PBS. After washing, RPMI-1640 medium containing 0.0055 mM 2-Mercaptoethanol and 0.1% was added to the cells. Differentiated

THP-1 cells were treated with DMSO (0.1%), compound **2** (10 μ M) or compound **15a** (3.3 and 10 μ M) and were stimulated for 2 hours at 37 °C. Whole-cell extracts were prepared using 1% Triton X-100 lysis buffer (Sigma) with Phosphatase Inhibitor Cocktail Set V (CalBiochem) and Protease Inhibitor Cocktail for use with mammalian cells and tissue extracts (Sigma). The protein concentrations of the lysates were determined by Bradford assay (Bio-Rad). 20 μ g total proteins was fractionated by SDS-PAGE and immunoblotted with the following primary antibodies: Phospho-TBK1 (S172) (Cell Signaling, 5483), total TBK-1 (Cell Signaling, 3504), phospho-STING (S366) (Cell Signaling, 85735), total STING (Cell Signaling, 50494), phospho-IRF3 (S396) (Cell Signaling, 4947), total IRF3 (Cell Signaling, 10949), GAPDH (Cell Signaling, 5174). Alexa Fluor® 680 goat anti-rabbit IgG (Invitrogen, A-21109) or Rabbit IgG HRP Linked Whole Ab from Donkey (GE Healthcare, NA934) were used as secondary antibodies. Detection and quantification were performed using the Li-Cor Odyssey Infrared Imaging system.

Stimulation of human monocyte derived dendritic cells CD14⁺ monocytes were isolated from human peripheral blood mononuclear cells obtained from STEMCELL (Cambridge, US) using CD14 MicroBeads (Miltenyi Biotec). Isolated CD14⁺ monocytes were subsequently cultured for 5 days with GM-CSF (100 ng/mL, Peprotech) and IL-4 (50 ng/mL, Invitrogen) to generate monocyte-derived dendritic cells (Mo-DCs). The differentiated Mo-DCs were plated in 96-wells plate and treated with DMSO, compound

2 or compound 15 for 24 hours at 37 °C. After stimulation, Mo-DCs were stained for expression of CD86 and analyzed by BD LSR Fortessa (BD Biosciences).

Pharmacology Materials and methods

Antibody Anti-mouse CD8 α mAb 2.43 was purchased from Bio-X Cell Inc. (West Lebanon, NH, USA).

Cell culture Human monocyte cell line THP-1 (ATCC® TIB-202™) and murine colon tumor cell line CT-26 (ATCC® CRL-2638™) were purchased from ATCC (Manassas, VA, USA). All cell lines were incubated at 37 °C and maintained in an atmosphere containing 5% CO₂. Cells were grown in RPMI-1640 (Gibco) supplemented with 10% FBS (Gibco).

Microsomal Assay. Liver Microsomal (LM) incubations contained 100 mM Phosphate buffer, 0.5 mg/mL of LM, 2 mM NADPH, 2.5 mM UDPGA, 3 mM MgCl₂, alamethicin and 1 μ M test article totaling 100 μ L in incubation volume. All the reagents, except the LM were mixed together and aliquotted (80 μ L) into 96 well plates. The reaction was initiated with LM (20 μ L). At time 0 and 30 min, the reactions were terminated with the addition of acetonitrile containing carbutamide (as an internal standard). The terminated samples were then centrifuged to pellet the protein and the supernatant was injected on a LCMS. The test article was monitored for loss over time.

Serum Stability Assay. The pooled frozen serum of rat was thawed in a water bath at 37 °C prior to experiment. The human serum was collected at the day of experiment. Serum was centrifuged at 4000 rpm for 5 min and the clots were removed if any. The pH will be adjusted to 7.4 ± 0.1 if required. Blank serum (142.5 μ L) was spiked with of dosing solution (7.5 μ L, 20 μ M or 40 μ M) to achieve 2 μ M or 1 μ M of the final concentration in duplicate and samples were incubated at 37°C in a water bath. At each time point (0,15, 30, 60, 90 and 120 min), stop solution (400 μ L, 200 ng/mL tolbutamide and 200 ng/mL Labetalol in MeOH) was added to precipitate protein and mixed thoroughly. Centrifuge sample plates at 4,000 rpm for 15 min then an aliquot of supernatant (40 μ L) was transferred from each well and mixed with ultrapure water containing 1.5 mM EDTA (120 μ L). The samples were shaken at 800 rpm for about 10 min before submitting to LC-MS/MS analysis. The % remaining of test compound after incubation in serum was then calculated.

All animal experiments performed in the manuscript were conducted in compliance with institutional guidelines

Mice. Female BALB/c mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). All studies were conducted under the approval of the Takeda Oncology Institutional Animal Care and Use Committee.

PK in Rats and Mice. Compounds **14a** and **15a** were administered intravenously at corresponding doses in PBS. Blood samples were collected following a variable collection scheme from 0.0833 to 24 h post dosing. Blood was collected into tubes containing K2EDTA to obtain plasma after centrifugation. After centrifugation (1500 g for 10 min at 4 °C), plasma samples were frozen at –20 °C, and compounds quantified by LC/MS/MS. Quality control samples and calibration samples were prepared by spiking plasma with working solutions prepared from independent weightings. Pooled rat urine and stool samples were also collected and analyzed. Pharmacokinetic modeling and analysis were performed with WinNonLin 5.2 software using noncompartmental analysis.

PK in BDC Rats. For the BDC study of Compound **15a**, bile duct/jugular cannulated and jugular cannulated control male rats were obtained from Hill Top Laboratories (Scottdale, PA). Both groups of rats were dosed intravenously with Compound **15a** in PBS and blood was collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post dose (N = 3-4). Plasma sample preparation and analysis were as described above.

Table S-1. Detailed PK of **15a** in BALB/c mice after single iv dose. Plasma concentrations are shown in nM. Quantification limit is 0.5 nM. BQL = Below Quantification Limit.

Dose	Time (h)	Group 1	Group 2	Group 3	Mean	SD
0.3 mg/kg	0.08333	999.46	1156.54	2279.20	1478.4 0	697.62
	0.25	BQL	151.54	77.31	76.23	75.77

	0.5	27.72	30.34	17.09	25.10	7.01
	1	3.57	4.96	2.06	3.53	1.45
	2	0.61	1.00	BQL	0.54	NA
	4	BQL	BQL	BQL	BQL	NA
	6	BQL	BQL	BQL	BQL	NA
	8	BQL	BQL	BQL	BQL	NA
	24	BQL	BQL	BQL	BQL	NA
	30	BQL	BQL	BQL	BQL	NA
1 mg/kg	0.08333	4342.80	3865.40	3572.80	3927.00	388.08
	0.25	177.10	440.44	434.28	351.12	150.30
	0.5	39.58	172.48	49.43	87.16	74.07
	1	12.17	11.38	16.32	13.29	2.66
	2	11.97	1.51	1.85	5.11	5.94
	4	BQL	BQL	BQL	BQL	NA
	6	BQL	BQL	BQL	BQL	NA
	8	BQL	BQL	BQL	BQL	NA
	24	BQL	BQL	BQL	BQL	NA
	30	BQL	BQL	BQL	BQL	NA
3 mg/kg	0.08333	2833.60	1786.40	12320.00	5647.18	5802.72
	0.25	1333.64	902.44	2864.40	1700.16	1031.80
	0.5	251.02	158.62	287.98	232.54	66.68
	1	254.10	45.89	71.92	123.97	113.50
	2	12.71	34.50	4.54	17.25	15.55
	4	7.13	1.13	18.63	8.96	8.90
	6	0.51	1.00	3.06	1.52	1.36
	8	2.86	0.55	BQL	1.71	NA
	24	BQL	BQL	0.58	NA	NA

	30	BQL	BQL	BQL	BQL	NA
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Table S-2. Detailed rat PK parameters for **15a** in control and bile duct cannulated (BDC) animals following single 1 mg/kg iv dose.

Group	Subject	AUC _{inf} (h.ng/mL)	C _{max} (ng/mL)	t _{1/2} (h)	Cl (L/h/kg)	Vss (L/kg)
Control	1	298	1040	0.28	3.35	0.482
	2	336	1190	0.26	2.98	0.384
	3	305	1100	0.28	3.29	0.39
	Mean	313	1110	0.27	3.21	0.419
	SD	20	76	0.01	0.198	0.055
BDC	1	248	855	0.45	4.03	0.741
	2	200	700	0.23	4.99	0.703
	3	512	1500	0.53	1.95	0.492
	4	205	710	0.25	4.87	0.703
	Mean	291	941	0.37	3.96	0.66
	SD	148	379	0.15	1.40	0.113
Recovered % of 15a dose						
Matrix	Control rats		BDC rats			
Urine	4.53 ± 3.06		10.1 ± 5.36			
Feces	41.7 ± 15		0.20 ± 0.28			
Bile	Not determined		84.3 ± 9.25			

Murine Tumor Models. For syngeneic CT-26 tumor model, approximately 6-week-old, female, BALB/c mice were inoculated subcutaneously with 2 x 10⁵ CT-26 cells in the right

lower flank. Tumor growth was monitored with vernier calipers, and the mean tumor volume was calculated using the formula ($0.5 \times [\text{length} \times \text{width}^2]$).

For the PD study, when the mean tumor volume reached approximately 300-500 mm³, animals were randomized into treatment groups (n = 3/group). PBS or Compound **15a** (1 mg/kg) were administered intravenously, and plasma samples for cytokine analysis were collected at 6 hours after the treatment.

For the primary tumor efficacy study, when the mean tumor volume reached approximately 120 mm³, animals were randomized into treatment groups (n = 8/group) and dosing was initiated on Day 0 of the study. Tumor size and body weight were measured three times weekly. During the observation period, animals bearing oversized tumor exceeding 2,000 mm³ were sacrificed. PBS or Compound **15a** were administered intravenously on day 0, 3 and 6.

For re-challenge study, anti-CD8 depletion antibody was administered intraperitoneally on day 0, and naïve BALB/c mice or tumor-free mice previously treated with 3 mg/kg of Compound **15a** were inoculated subcutaneously with 2×10^5 CT-26 cells in the right lower flank on day 1.

Plasma Cytokine Analysis. Simultaneous determination of multiple cytokine concentrations was carried out using Cytokine & Chemokine 36-Plex Mouse

ProcartaPlex™ Panel 1A (Invitrogen) on a Bio-Rad Bio-Plex Array Reader. The kit was used according to the manufacturer's instructions.

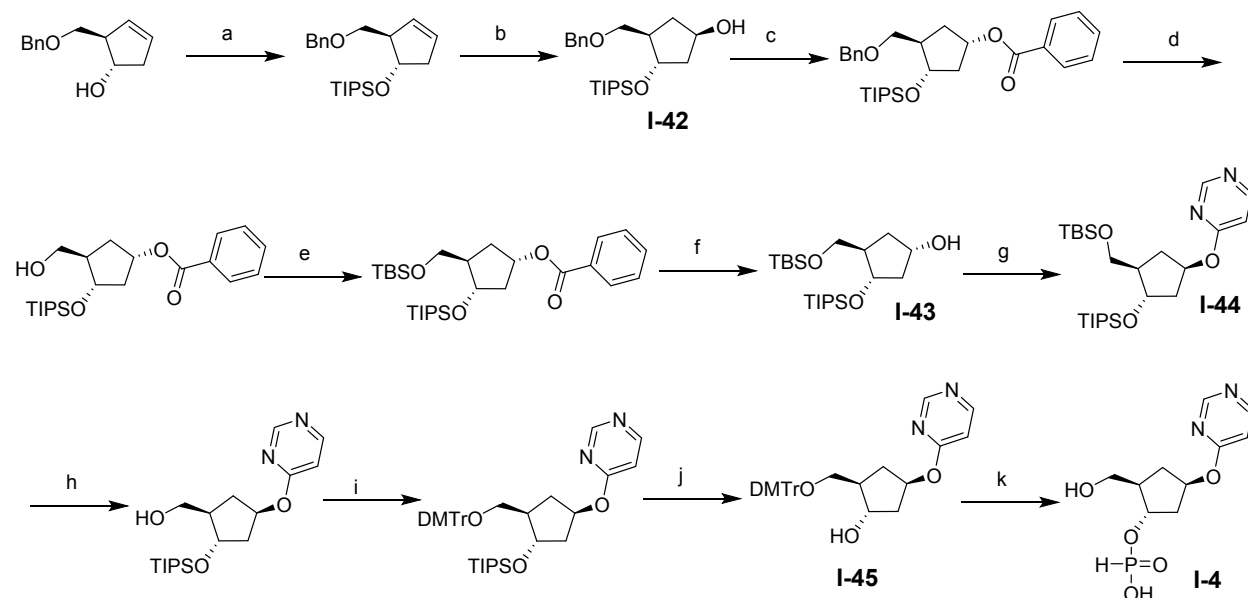
Statistical Analysis Statistical analyses were done using unpaired t-test or Dunnett's multiple comparisons test with significance at $p < 0.05$ with GraphPad Prism 8 (GraphPad Software).

Table S-3. Percent of tumor growth inhibition (TGI), growth rate inhibition, tumor-free mice and lowest body weight change of compound **15a** in CT-26 efficacy studies

Dose of 15a [mg/kg]	% of TGI	% of GRI	% of tumor-free mice	Lowest % of BWC
0.5	50 (Day 14)	28 (Day 14)	0	-0.7 (Day 3)
1.0	91 (Day 13)	75 (Day 13)	13	-3.5 (Day 10)
2.0	90 (Day 14)	93 (Day 14)	20	-7.9 (Day 10)

Synthetic protocols:

Scheme S-1: Synthesis of I-4, I-42, I-43, I-44 and I-45



Reagents and conditions: (a) TIPS-Cl, imidazole, DMAP, DMF, 18 h; (b) *i*) 9-BBN, THF, rt, 18 h; *ii*) oxone, rt, 2 h; (c) PPh₃, DIAD, 0 °C, 30 min, benzoic acid; (d) Pd(OH)₂/C, H₂, rt, 18 h; (e) TBSCl, imidazole, DCM, rt, 16 h; (f) K₂CO₃, MeOH, rt, 16 h; (g) PPh₃, DEAD, pyrimidin-4(3*H*)-one, THF, 0 °C to rt, 1 h; (h) HCl, EtOH, 40 °C, 1 h; (i) DMTrCl, DBU, DCM, 0 °C to rt, 16 h; (j) TBAF, THF, rt, 16 h; (k) *i*) diphenylphosphite, pyridine, H₂O, 0 °C to rt, 20 min; *ii*) AcOH, H₂O, rt 1 h.

Step 1: (((1*S*,2*R*)-2-((benzyloxy)methyl)cyclopent-3-en-1-yl)oxy)triisopropylsilane To a solution of (1*S*,2*R*)-2-((benzyloxy)methyl)cyclopent-3-enol (60.0 g, 293 mmol) in DMF (600 mL) was added with TIPS-Cl (84.6 g, 439 mmol), imidazole (79.6 g, 1.17 mol) and DMAP (1.78 g, 14.6 mmol). The resulting mixture was allowed to stir at rt for 16 h under a nitrogen atmosphere. The reaction mixture was quenched with saturated NaHCO₃ (500 mL), then extracted with EtOAc (3 x 250 mL). The combined organic phases were washed with brine (2 x 250 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under vacuum. The resulting residue was purified by silica gel chromatography (100% petroleum ether) to give the desired product (((1*S*,2*R*)-2-((benzyloxy)methyl)cyclopent-3-en-1-yl)oxy)triisopropylsilane (70.0 g, 66%) as colorless liquid. ¹H NMR (CDCl₃) δ 7.29-7.38 (m, 5H), 5.68-5.77 (m, 2H), 4.54 (s, 2H), 4.40 (dt, *J* = 6.7, 3.3 Hz, 1H), 3.43 (ddd, *J* = 16.8, 9.2, 6.0 Hz, 2H), 2.86-2.93 (m, 1H), 2.69 (ddq, *J* = 16.7, 6.4, 1.9 Hz, 1H), 2.33 (dsxt, *J* = 16.7, 1.9 Hz, 1H), 1.05-1.08 (m, 21H).

Step 2: (1*R*,3*R*,4*S*)-3-((benzyloxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentan-1-ol A solution of 9-BBN (440 mL, 220 mmol, 0.500 M in THF) was added dropwise at -10 °C under a nitrogen atmosphere to a solution of (((1*S*,2*R*)-2-((benzyloxy)methyl) cyclopent-3-en-1-yl)oxy) triisopropylsilane (40.0 g, 110 mmol) in THF (800 mL). The reaction mixture was allowed to warm slowly to rt and stir for 18 h. The reaction mixture was cooled to 0 °C, treated with a solution of oxone (338 g, 550 mmol) in water (3.50 L) and allowed to stir for 2 h. The aqueous phase was extracted with EtOAc (3 x 1.0 L) and the combined organic phases were washed with saturated NaHCO₃ (1.0 L), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (PE:EtOAc:NH₃·H₂O, 50/1/0.1 to 10/1/0.1) to afford (1*R*,3*R*,4*S*)-3-((benzyloxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentan-1-ol (**I-42**) (14.6 g, 35%) as a colorless oil. ¹H NMR (CDCl₃) δ 7.29-7.39 (m, 5H), 4.51-4.62 (m, 2H), 4.42 (ddd, *J* = 6.4, 4.4 Hz, 1H), 4.33 (spt, *J* = 3.0 Hz, 1H), 3.59 (dd, *J* = 8.9, 4.3 Hz, 1H), 3.51 (dd, *J* = 8.9, 4.1 Hz, 1H), 2.36 (ddd, *J* = 13.8, 10.0, 6.0 Hz, 1H), 2.13-2.24 (m, 1H), 1.97-2.06 (m, 1H), 1.79-1.90 (m, 1H), 1.64-1.77 (m, 1H), 1.41-1.62 (m, 1H), 1.02-1.08 (m, 21H).

Step 3: (1*S*,3*R*,4*S*)-3-((benzyloxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl benzoate To a mixture of **I-42** (25.0 g, 66.0 mmol), benzoic acid (9.70 g, 79.2 mmol) and triphenylphosphine (20.7 g, 79.2 mmol) in THF (250 mL) was added DIAD (16.0 g, 79.2 mmol) drop-wise at 0 °C under nitrogen. The reaction mixture was allowed to stir at 0 °C for 30 min. After the solvent was removed under reduced pressure, the residue was diluted with EtOAc (350 mL) and washed with brine (2 x 150 mL). The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated in vacuum. The crude residue was purified by silica gel chromatography (petroleum ether:EtOAc, 99:1 to 9:1) to give (1*S*,3*R*,4*S*)-3-[(benzyloxy)methyl]-4-[(triisopropylsilyl)oxy]cyclopentyl benzoate (28 g, 88%) as a light yellow oil. ¹H NMR (CDCl₃) δ 8.05 (d, *J* = 7.2 Hz, 2H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.35-7.20 (m, 5H), 5.34 (sept, *J* = 3.6 Hz, 1H), 4.51 (d, *J* = 2.0 Hz, 2H), 4.22 (q, *J* = 6.4 Hz, 1H), 3.50 (ddd, *J* = 5.2, 9.6, 26.4 Hz, 2H), 2.42 (m, 2H), 2.15 (m, 1H), 1.95 (m, 2H), 1.00 (m, 21 H).

Step 4: (1*S*,3*R*,4*S*)-3-(hydroxymethyl)-4-((triisopropylsilyl)oxy)cyclopentyl benzoate To a solution of (1*S*,3*R*,4*S*)-3-[(benzyloxy)methyl]-4-[(triisopropylsilyl)oxy]cyclopentyl benzoate (28.0 g, 58.0 mmol) in MeOH (560 mL) was added Pd(OH)₂/C (20% w/w, 4.10 g, 5.80 mmol). The resulting mixture was allowed to stir at rt under 40 psi of hydrogen for 18 h. The reaction mixture was filtered through Celite and washed with MeOH (3 x 150 mL). The filtrate was

concentrated under vacuum to give the desired product (1*S*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl benzoate (22.0 g, 97%) as a colorless oil, which was taken on directly without purification.

Step 5: (1*S*,3*R*,4*S*)-3-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl benzoate To a solution of (1*S*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl benzoate (44.0 g, 112 mmol) in DCM (600 mL) was added TBS-Cl (21.8 g, 145 mmol) and imidazole (11.4 g, 168 mmol). The reaction mixture was allowed to stir at rt for 4 h. The reaction mixture was quenched with water (500 mL) and extracted with DCM (2 x 600 mL). The combined organic phases were washed with brine (800 mL), dried over anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (PE:EtOAc, 99:1 to 7:3) to give (1*S*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl benzoate (50 g, 88%) as a colorless oil. ¹H NMR (CDCl₃) δ 8.00-7.94 (m, 2H), 7.48-7.42 (m, 1H), 7.36-7.31 (m, 2H), 5.26 (tt, *J* = 7.0, 3.7 Hz, 1H), 4.23-4.14 (m, 1H), 3.62-3.46 (m, 2H), 2.35 (dt, *J* = 13.9, 6.8 Hz, 1H), 2.21 (tt, *J* = 9.8, 4.9 Hz, 1H), 2.04-1.93 (m, 1H), 1.91-1.76 (m, 2H), 0.97 (d, *J* = 1.9 Hz, 21H), 0.81 (s, 9H), -0.04 (d, *J* = 1.0 Hz, 6H).

Step 6: (1*S*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentanol To a solution of (1*S*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl benzoate (50.0 g, 98.6 mmol) in MeOH (650 mL) was added K₂CO₃ (20.3 g, 147 mmol). The reaction mixture was allowed to stir at 20 °C for 16 h. The reaction mixture was quenched with water (600 mL). The mixture was then extracted with DCM (3 x 600 mL) and the combined organic phases were dried over Na₂SO₄, filtered and concentrated. The residue was purified by silica gel chromatography (PE:EtOAc, 99:1 to 19:1) to provide (1*S*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentanol (**I-43**) (25.2 g, 63%) as a light yellow oil. LCMS (FA): *m/z* = 403.5 (M+H).

Step 7: *tert*-butyl-dimethyl-[[*(1R,2*S*,4*R*)-4-pyrimidin-4-yloxy-2-triisopropylsilyloxy-cyclopentyl]methoxy]silane* Compound **I-43** (5.00 g, 12.4 mmol), triphenylphosphine (3.74 g, 14.3 mmol) and 4(3*H*)-pyrimidone (1.46 g, 14.9 mmol) were dissolved in THF (100 mL) under an atmosphere of argon. The solution was cooled to 0 °C and DEAD (2.15 mL, 13.7 mmol) was added. The reaction mixture was allowed to stir at rt for 1 h, then concentrated to dryness. The crude product was adsorbed onto Celite and purified by silica gel chromatography (0-15% EtOAc in hexanes) to provide *tert*-butyl-dimethyl-[[*(1R,2*S*,4*R*)-4-pyrimidin-4-yloxy-2-*

triisopropylsilyloxy- cyclopentyl]methoxy]silane (**I-44**) (5.18 g, 83%). LCMS (FA): m/z = 481.3 (M+H), ^1H NMR (DMSO- d_6) δ 8.75 (s, 1H,) 8.48 (d, J = 5.8 Hz, 1H,) 6.85 (d, J = 5.9 Hz, 1H), 5.46 (m, 1H), 4.34 (m, 1H), 3.58 (m, 2H), 2.31 - 2.44 (m, 1H), 1.94 - 2.11 (m, 3H,) 1.44 - 1.53 (m, 1H), 1.04 (s, 21H) 0.84 (s, 9H,) 0.00 (d, J = 3.1 Hz, 6H).

Step 8: {(1*R*,2*S*,4*R*)-4-(pyrimidin-4-yloxy)-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol
Compound **I-44** (5.98 g, 12.4 mmol) was taken up in EtOH (50.0 mL). A solution of HCl (12.0 mol/L, 2.07 mL, 24.9 mmol) in EtOH (50.0 mL) was added. The reaction mixture was allowed to stir at 40 °C for 1 h. After most EtOH was removed under vacuum, the aqueous residue was extracted with EtOAc and the combined organic phases were washed with water, brine, dried with Na_2SO_4 and concentrated. The crude compound was purified by silica gel chromatography (0-60% EtOAc in hexanes) to provide {(1*R*,2*S*,4*R*)-4-(pyrimidin-4-yloxy)-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol (3.03 g, 66%). LCMS (FA): m/z = 367.2 (M+H).

Step 9: 4-({(1*R*,3*R*,4*S*)-3-[[bis(4-methoxyphenyl)(phenyl)methoxy]methyl]-4-[(triisopropylsilyl)oxy]cyclopentyl}oxy)pyrimidine
To a solution of {(1*R*,2*S*,4*R*)-4-(pyrimidin-4-yloxy)-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol (3.03 g, 8.26 mmo) in DCM (100 mL) was added DBU (3.70 mL, 24.8 mmol). The reaction mixture was cooled to 0 °C and DMTrCl (5.60 g, 16.5 mmol) was added. The reaction mixture was then warmed to rt and allowed to stir overnight. Water was added and the mixture was extracted with DCM. The combined organic phases were washed with water, brine, dried with Na_2SO_4 and concentrated. The crude compound was purified by silica gel chromatography (0-50% EtOAc in hexanes) to provide 4-({(1*R*,3*R*,4*S*)-3-[[bis(4-methoxyphenyl)(phenyl)methoxy]methyl]-4-[(triisopropylsilyl)oxy]cyclopentyl}oxy)pyrimidine (5.30 g, 96%). LCMS (FA): m/z = 669.4 (M+H).

Step 10: (1*S*,2*R*,4*R*)-2-[[bis(4-methoxyphenyl)(phenyl)methoxy]methyl]-4-(pyrimidin-4-yloxy)cyclopentanol
To a solution of 4-({(1*R*,3*R*,4*S*)-3-[[bis(4-methoxyphenyl)(phenyl)methoxy]methyl]-4-[(triisopropylsilyl)oxy]cyclopentyl}oxy)pyrimidine (5.30 g, 7.90 mmol) in THF (25 mL) was added TBAF (1.00 mol/L, 9.50 mL, 9.50 mmol) at rt. The reaction mixture was allowed to stir at rt overnight. Brine was added and the mixture was extracted with EtOAc. The combined organic phases were dried with Na_2SO_4 and concentrated. The crude compound was purified by silica gel chromatography (0-70% EtOAc in hexanes) to provide

(1*S*,2*R*,4*R*)-2 {[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrimidin-4-yloxy)cyclopentanol **I-45** (2.80 g, 69%). LCMS (FA): m/z = 513.3 (M+H).

Step 11: provide [(1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-pyrimidin-4-yloxy-cyclopentoxy]phosphinic acid (I-4**)** Compound **I-45** (1.44 g, 2.81 mmol) was dissolved in pyridine (12.0 mL). The solution was cooled to 0 °C and diphenyl phosphite (1.08 mL, 5.62 mmol) was slowly added over 1 min. The reaction mixture was warmed to rt and allowed to stir for 20 min. The reaction mixture was cooled back to 0 °C and water (2.77 mL, 154 mmol) was added. The reaction mixture was warmed to rt and allowed to stir for 30 min. The reaction mixture was concentrated and concentrated from toluene (2 x 50 mL). The residue was taken up in acetic acid (6.45 mL, 112 mmol) and water (1.70 mL) and the reaction mixture was allowed to stir at rt for 1 h. The reaction mixture was concentrated. The crude compound was purified by silica gel chromatography (0-100% MeOH in DCM) to provide [(1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-pyrimidin-4-yloxy-cyclopentoxy]phosphinic acid (**I-4**) (600 mg, 78%). LCMS (FA): m/z = 275.2 (M+H). ¹H NMR (MeOD) δ 8.84 (s, 1H), 8.49 (dd, J = 6.24, 0.7 Hz, 1H), 7.62 (s, 0.5H), 6.98 (dd, J = 6.2, 1.0 Hz, 1H), 5.99 (s, 0.5H), 5.65 (ddd, J = 6.7, 4.8, 2.0 Hz, 1H), 4.72 (dd, J = 9.4, 6.2 Hz, 1H), 3.58 - 3.69 (m, 2H), 2.44 - 2.59 (m, 1H), 2.23 - 2.37 (m, 3H), 1.63 - 1.76 (m, 1H). ³¹P NMR (MeOD) δ 4.37 (s, 1P).

Table S-4. The compound **I-5** listed below was prepared as described as in the synthesis of compound **I-4** starting with Step 7, substituting the starting material shown in the table for pyrimidin-4(3*H*)-one.

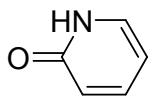
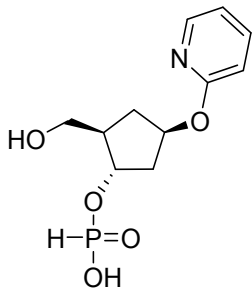
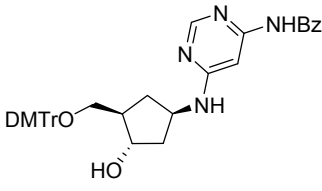
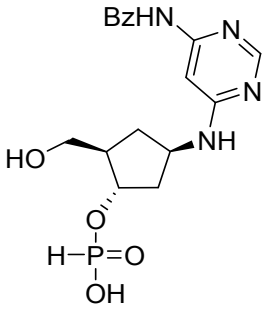
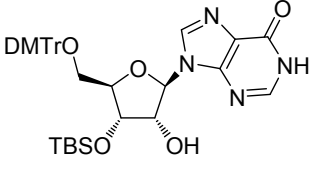
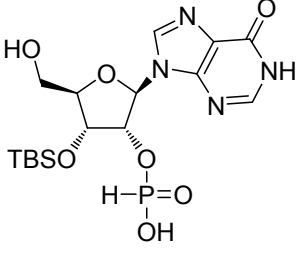
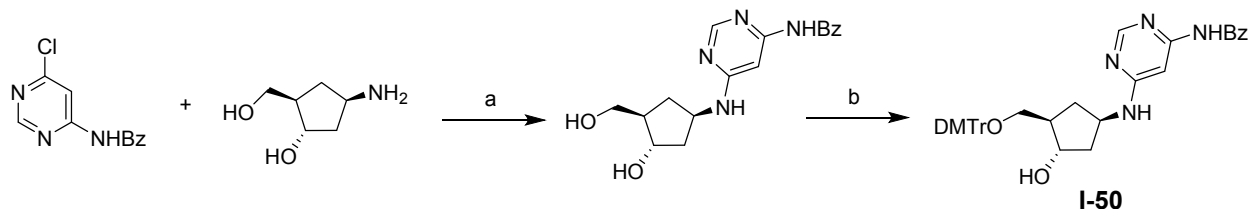
Starting material	Product	LCMS and NMR data
	 I-5	LCMS (FA): m/z = 274.1 (M+H); ¹ H NMR (DMSO- <i>d</i> ₆) δ 1.50 (ddd, J =13.51, 7.64, 5.50 Hz, 1 H) 2.00 - 2.24 (m, 3 H) 2.28 - 2.40 (m, 1 H) 3.36 - 3.51 (m, 2 H) 4.57 - 4.68 (m, 1 H) 5.39 (quin, J =6.02 Hz, 1 H) 5.92 (s, 0.5 H) 6.76 (d, J =8.31 Hz, 1 H) 6.95 (dd, J =6.60, 5.38 Hz, 1 H) 7.58 (s, 0.5 H) 7.65 - 7.71 (m, 1 H) 8.10 - 8.20 (m, 1 H) ; ³¹ P NMR (DMSO- <i>d</i> ₆) δ 3.84 (s, 1 P).

Table S-5. The compounds listed below was prepared as described as in the synthesis of compound **I-4** starting with Step 11, substituting the starting material shown in the table for **I-45**.

Starting material	Product	LCMS and NMR data
 <p>I-50</p>	 <p>I-1</p>	<p>LCMS (FA): $m/z = 393.2$ (M+H); ^1H NMR (DMSO-d_6) δ 0.95 - 1.19 (m, 1 H), 1.63 - 1.76 (m, 1 H), 1.89 - 2.02 (m, 2 H), 2.12 (m, 1 H), 3.28 - 3.36 (m, 2 H), 4.22 - 4.46 (m, 2 H), 5.87 (s, 0.5 H), 5.99 - 6.08 (m, 1 H), 7.30 (s, 0.5 H), 7.33 (s, 1 H), 7.49 (m, 3 H), 7.58 (m, 1 H), 7.98 (d, $J=7.40$ Hz, 2 H), 8.24 (s, 1 H), 10.52 (br s, 1 H) ; ^{31}P NMR (DMSO-d_6) δ 1.54 (s, 1 P).</p>
 <p>I-51</p>	 <p>I-31</p>	<p>LCMS (FA): $m/z = 447.2$ (M+H). ^1H NMR (DMSO-d_6) δ 12.30 (br s, 1H), 8.19 (s, 1H), 7.89 (s, 1H), 7.02 (s, 0.5 H), 5.79 (d, $J = 6.8$ Hz, 1H), 5.61 (s, 0.5 H), 4.97 (t, $J = 5.5$ Hz, 1H), 4.90 (ddd, $J = 11.0, 6.7, 5.0$ Hz, 1H), 4.25 (dd, $J = 4.6, 2.2$ Hz, 1H), 3.78 (q, $J = 2.1$ Hz, 1H), 3.43-3.53 (m, 1H), 3.33-3.42 (m, 1H), 0.76 (s, 9H), 0.00 (s, 6H). ^{31}P NMR (DMSO-d_6) δ -0.51 (s, 1P).</p>

Scheme S-2: Synthesis of I-50

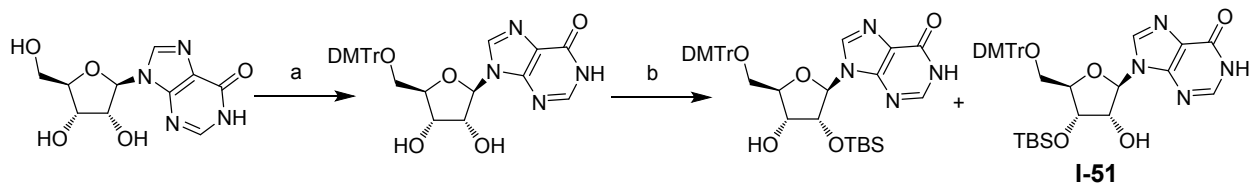


Reagents and conditions: (a) NMP, DIPEA, 150 °C, 6 h; (b) DMTrCl, pyridine, rt.

Step 1: *N*-(6-{[(1*R*,3*S*,4*R*)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-4-yl)benzamide *N*-(6-chloro-4-pyrimidinyl)-benzamide (2.10 g, 9.01 mmol) was dissolved in NMP (10 mL). DIPEA (6.75 mL, 38.7 mmol) was added, followed by (1*S*,2*R*,4*R*)-4-amino-2-(hydroxymethyl)cyclopentanolhydrochloride (1.30 g, 7.76 mmol). The reaction mixture was heated under microwave irradiation at 150 °C for 6 h. The reaction mixture was diluted with EtOAc and concentrated to give *N*-(6-{[(1*R*,3*S*,4*R*)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-4-yl)benzamide (2.50 g, 98%) which was used without purification.

Step 2: *N*-(6-(((1*R*,3*R*,4*S*)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxycyclopentyl)amino)pyrimidin-4-yl)benzamide (**I-50**) *N*-(6-{[(1*R*,3*S*,4*R*)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-4-yl)benzamide (2.50 g, 7.6 mmol) was dissolved in dry pyridine (3 x 10 mL) and concentrated to dryness, and then taken up in pyridine (20 mL). DMTrCl (3.0 g, 8.8 mmol) was added and the reaction mixture was allowed to stir at rt for 2 h. Pyridine was mostly removed, then the mixture was added water (20 mL) and extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under vacuum. The crude material was purified by flash column chromatography (EtOAc:MeOH, 1:0 to 1:9) to give *N*-(6-(((1*R*,3*R*,4*S*)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxycyclopentyl)amino)pyrimidin-4-yl)benzamide **I-50** (1.9 g, 40.0%). LCMS (FA): m/z = 631.4 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.27 (m, 1 H), 1.63 (m, 1 H), 1.79 - 1.93 (m, 1 H), 1.99 - 2.08 (m, 1 H), 2.32 (m, 1 H), 2.90 (t, J =7.95 Hz, 1 H), 3.08 (dd, J =8.74, 5.56 Hz, 1 H), 3.73 (s, 6 H), 3.90 (m, 1 H), 4.43 (br s, 1 H), 4.64 (m, 1 H), 6.89 (dd, J =8.93, 1.47 Hz, 4 H), 7.19 - 7.61 (m, 14 H), 7.95 - 8.01 (m, 2 H), 8.25 (s, 1 H), 10.52 (s, 1 H).

Scheme S-3: Synthesis of I-51



Reagents and conditions: (a) *i*) DMTrCl, pyridine, rt, 16 h; (b) *ii*) TBSCl, imidazole, pyridine, rt, 16 h.

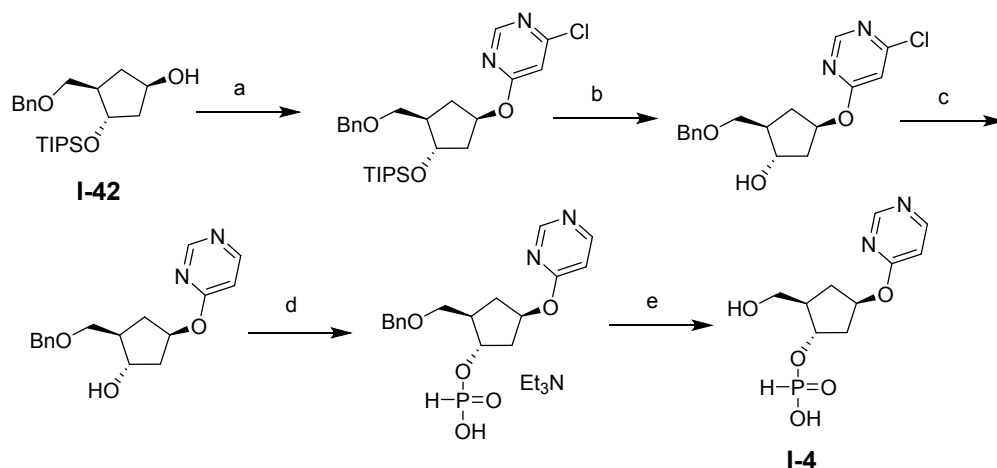
Step 1: 9-[(2R,3R,4S,5R)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-3,4-dihydroxytetrahydrofuran-2-yl]-1,9-dihydro-6H-purin-6-one Inosine (5.40 g, 20.1 mmol) was dried under vacuum for 5 h at 50 °C, and then taken up in pyridine (104 mL) under nitrogen. DMTrCl (4.10 g, 12.1 mmol) was added, and the reaction mixture was stirred at rt for 10 min. Another portion of DMTrCl (4.10 g, 12.1 mmol) was added, and the reaction mixture was stirred at rt for 16 h. MeOH (10 mL) was added, and the reaction mixture was concentrated. The resulting residue was diluted with EtOAc and water. After the phases were separated, the aqueous phase was extracted with EtOAc. The combined organic phases were washed with water, dried over Na₂SO₄ and concentrated. The crude compound was purified by silica gel chromatography (0-10% MeOH in DCM) to provide 9-[(2R,3R,4S,5R)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-3,4-dihydroxytetrahydrofuran-2-yl]-1,9-dihydro-6H-purin-6-one (7.02 g, 62%). LCMS (FA): *m/z* = 571.2 (M+H).

Step 2: 9-[(2R,3R,4S,5R)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[*tert*-butyl(dimethyl)silyl]oxy}-3-hydroxytetrahydrofuran-2-yl]-1,9-dihydro-6H-purin-6-one (I-51)

9-[(2R,3R,4S,5R)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-3,4-dihydroxytetrahydrofuran-2-yl]-1,9-dihydro-6H-purin-6-one (6.80 g, 11.9 mmol), TBS-Cl (2.81 g, 18.5 mmol), and imidazole (2.43 g, 35.3 mmol) were taken up in pyridine (152 mL) under nitrogen. The reaction mixture was stirred at rt for 16 h, then concentrated and diluted with EtOAc and water. After the phases were separated, the aqueous phase was extracted with EtOAc. The combined organic phases were washed with water, dried over Na₂SO₄ and concentrated. The residue was further concentrated from toluene, then purified by silica gel chromatography (0-70% EtOAc in DCM) to provide undesired isomer 9-[(2R,3R,4R,5R)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-3-{[*tert*-butyl(dimethyl)silyl]oxy}-4-hydroxytetrahydrofuran-2-yl]-1,9-dihydro-6H-purin-6-one (3.19 g, 39%) as the first eluting peak LCMS (FA): *m/z* = 685.3 (M+H), ¹H NMR (DMSO-*d*₆) δ 12.40 (s, 1H), 8.23 (s, 1H), 8.01 (s, 1H), 7.39-7.44 (m, 2H), 7.22-7.35 (m,

$J = 8.9, 1.6$ Hz, 7H), 6.88 (dd, $J = 9.0, 2.9$ Hz, 4H), 5.95 (d, $J = 5.0$ Hz, 1H), 5.17 (d, $J = 6.0$ Hz, 1H), 4.72 (t, $J = 5.0$ Hz, 1H), 4.20 (q, $J = 5.5$ Hz, 1H), 4.12 (q, $J = 4.2$ Hz, 1H), 3.76 (s, 6H), 3.29 (d, $J = 4.6$ Hz, 2H), 0.79 (s, 9H), 0.00 (s, 3H), -0.09 (s, 3H), and 9-[(2*R*,3*R*,4*S*,5*R*)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[*tert*-butyl(dimethyl)silyl]oxy}-3-hydroxytetrahydrofuran-2-yl]-1,9-dihydro-6*H*-purin-6-one (**I-51**) (1.77 g, 22%) as the second eluting peak LCMS (FA): $m/z = 685.4$ (M+H), ^1H NMR (DMSO- d_6) δ : 12.39 (br s, 1H), 8.26 (s, 1H), 7.98 (s, 1H), 7.32-7.38 (m, 2H), 7.19-7.29 (m, 7H), 6.81-6.86 (m, 4H), 5.86-5.89 (m, 1H), 5.42-5.45 (m, 1H), 4.65-4.70 (m, 1H), 4.36-4.40 (m, 1H), 3.99-4.03 (m, 1H), 3.73 (s, 6H), 3.25-3.28 (m, 1H), 3.09-3.16 (m, 1H), 0.82 (s, 9H), 0.06 (s, 3H), 0.02 (s, 3H).

Scheme S-4: Alternate synthesis of I-4 (TEA salt)



Reagents and conditions: (a) KOtBu, 4,6-dichloropyrimidine THF, 0 °C to rt; (b) 4.0 M HCl / dioxane; (c) H₂, Pd-C, MeOH, TEA; (d) diphenylphosphite, pyridine, H₂O, TEA; (e) *i*) BCl₃, DCM, 0 °C; *ii*) MeOH, TEA.

Step 1: 4-(((1*R*,3*R*,4*S*)-3-((benzyloxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl)oxy)-6-chloropyrimidine Compound **I-42** (4.00 g, 10.6 mmol) was dissolved in THF (80.0 mL) and the reaction mixture was cooled to 0 °C. Potassium *tert*-butoxide (2.25 g, 19.0 mmol) was added and the reaction mixture was allowed to stir at 0 °C for 10 min. 4,6-Dichloropyrimidine (2.52 g, 16.9 mmol) was added to the solution and the reaction mixture was allowed to stir at 0 °C for 10 min, then allowed to warm to rt and stirred for 2 h. The reaction mixture was quenched with HCl (80 mL, 0.2 M in water) and extracted with EtOAc (2 x 100mL). The combined organic phases were washed with brine, dried over MgSO₄, filtered, and concentrated to give crude 4-(((1*R*,3*R*,4*S*)-3-

((benzyloxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl)oxy)-6-chloropyrimidine as a yellow oil which was used in the next step without purification.

Step 2: (1*S*,2*R*,4*R*)-2-((benzyloxy)methyl)-4-((6-chloropyrimidin-4-yl)oxy)cyclopentan-1-ol

To the crude 4-(((1*R*,3*R*,4*S*)-3-((benzyloxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl)oxy)-6-chloropyrimidine was added a solution of HCl (30.0 mL, 120 mmol, 4.0M in dioxane). The reaction mixture was allowed to stir at rt for 2 h. The solvent was completely evaporated and the residue was purified by silica gel chromatography (20 to 85% EtOAc:hexanes) to provide (1*S*,2*R*,4*R*)-2-((benzyloxy)methyl)-4-((6-chloropyrimidin-4-yl)oxy)cyclopentan-1-ol as a colorless oil (2.32 g, 66 %). LCMS (FA): m/z = 335.1 (M+H).

Step 3: (1*S*,2*R*,4*R*)-2-((benzyloxy)methyl)-4-(pyrimidin-4-yloxy)cyclopentan-1-ol

(1*S*,2*R*,4*R*)-2-((benzyloxy)methyl)-4-((6-chloropyrimidin-4-yl)oxy)cyclopentan-1-ol (1.21 g, 3.62 mmol) and TEA (1.47 g, 14.5 mmol) were added into MeOH (40.0 mL). Palladium (10% on carbon, 192 mg) was added and the mixture was stirred under an atmosphere of H₂ at rt for 3 h. The reaction mixture was filtered and washed with MeOH. The filtrate was concentrated, dissolved in EtOAc (100 mL) and washed with water (2 x 10 mL). The organic phase was dried and concentrated to provide (1*S*,2*R*,4*R*)-2-((benzyloxy)methyl)-4-(pyrimidin-4-yloxy)cyclopentan-1-ol (1.04 g, 96%). LCMS (AA): m/z = 301.2 (M+H).

Step 4: [(1*S*,2*R*,4*R*)-2-(benzyloxymethyl)-4-pyrimidin-4-yloxy-cyclopentoxy]phosphinic acid

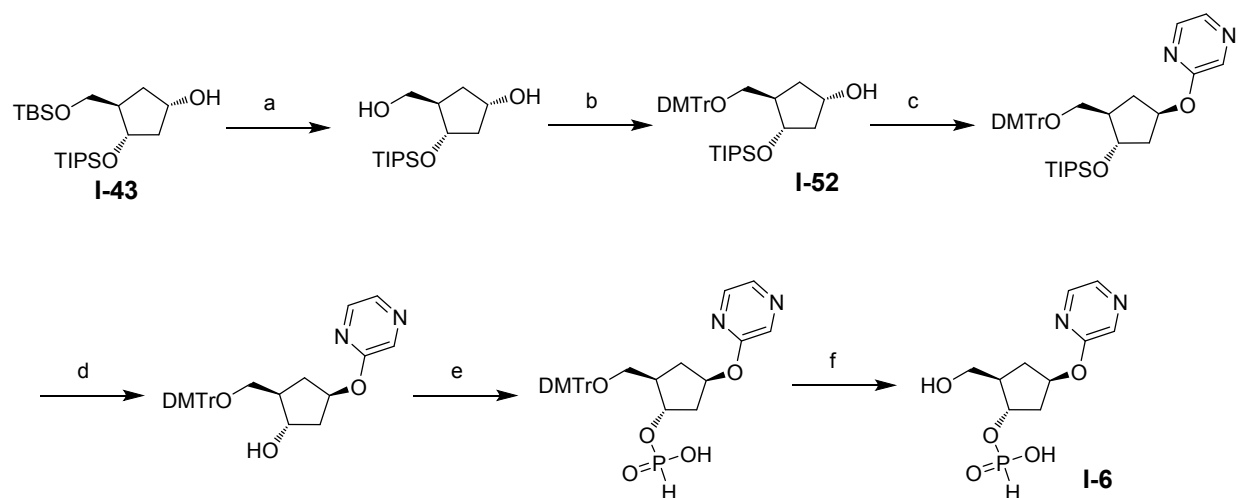
(1*S*,2*R*,4*R*)-2-((benzyloxy)methyl)-4-(pyrimidin-4-yloxy) cyclopentan-1-ol (1.03 g, 3.42 mmol) was dissolved in pyridine (15.0 mL), and diphenyl phosphite (1.31 mL, 6.85 mmol) was added. The reaction mixture was allowed to stir at rt for 30 min. Water (5.00 mL) was added and the reaction mixture was allowed to stir at rt for 30 min. After the solvents were evaporated, the residue was dissolved a mixture of methanol (20 mL) and TEA (5 mL). The solvents were then evaporated and the residue was dried under vacuum. The resulting residue was purified by silica gel chromatography (0% to 60% MeOH in DCM) to provide [(1*S*,2*R*,4*R*)-2-(benzyloxymethyl)-4-pyrimidin-4-yloxy-cyclopentoxy]phosphinic acid as an *N,N*-diethylethanamine salt (1.63 g, 84 %). LCMS (FA): m/z = 165.2 (M+H).

Step 5: [(1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-pyrimidin-4-yloxy-cyclopentoxy] phosphinic acid

(I-4) (1.50 g, 3.22 mmol) was dissolved in DCM (20 mL) and the mixture was cooled to 0 °C. Boron trichloride (1.0 M in DCM, 12.9 mL, 12.9 mmol) was added dropwise via a syringe pump at 0 °C. The mixture was allowed to stir at 0 °C for 30 min, and then quenched with MeOH (10

mL) at 0 °C. The solvents were evaporated and the residue was concentrated from toluene. MeOH (15 mL) and TEA (5 mL) were added to the residue and after stirring for 5 min, the solvents were completely evaporated. The residue was purified by silica gel chromatography (10 to 50% MeOH in DCM) to provide **I-4** as the *N,N*-diethylethanamine salt (699 mg, 58%). ¹H NMR (MeOD) δ 8.72 (s, 1H), 8.42 (d, *J* = 6.0 Hz, 1H), 7.56 (s, 0.5H), 6.86 (dd, *J* = 6.0, 1.0 Hz, 1H), 6.01 (s, 0.5H), 5.54 - 5.62 (m, 1H), 4.49 - 4.68 (m, 1H), 3.63 - 3.72 (m, 1H), 3.55 - 3.63 (m, 1H), 3.20 (d, *J* = 7.4 Hz, 6H), 2.42 - 2.58 (m, 1H), 2.16 - 2.33 (m, 3H), 1.59 - 1.74 (m, 1H), 1.31 (t, *J* = 7.34 Hz, 9H). ³¹P NMR (MeOD) δ 3.52 (s, 1 P).

Scheme S-5: Synthesis of I-6 and I-52



Reagents and conditions: (a) HCl, EtOH, 0 °C to rt, 1 h; (b) DMTrCl, pyr, 0 °C to rt, 2 h; (c) PPh₃, DBAD, **2**-hydroxypyrazine, 0 °C to rt, 1 h; (d) TBAF, THF, rt, 3 h; (e) diphenylphosphite, pyridine, 0 °C to rt, 30 min; then Et₃N, H₂O, rt, 30 min; (f) AcOH, H₂O, rt, 1 h.

Step 1: (1*S*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentanol A solution of HCl (0.750 mL, 9.00 mmol, 12.0 M in water) in EtOH (70.0 mL) was cooled to 0 °C and a solution of **I-43** (1.65 g, 4.10 mmol) in EtOH (70.0 mL) was added slowly. The reaction mixture was warmed to rt and allowed to stir for 1 h. Water (10.0 mL) and sodium bicarbonate (1.80 g, 21.4 mmol) were added and the reaction mixture was allowed to stir at rt for 5 min. The mixture was diluted with toluene and concentrated. The crude compound was purified by silica gel

chromatography (0-95% MeOH in DCM) to provide (1*S*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentanol (870 mg, 74%). LCMS (FA): m/z = 289.2 (M+H).

Step 2: (1*S*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(triisopropylsilyl)oxy]cyclopentanol (I-52) (1*S*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentanol (865 mg, 3.00 mmol) was taken up in pyridine (20.0 mL) and cooled to 0 °C. A solution of DMTrCl (1.13 g, 3.17 mmol) in pyridine (10.0 mL) was added and the reaction mixture was allowed to stir at 0 °C for 2 h. An additional portion of DMTrCl (150 mg, 0.421 mmol) was added and the reaction mixture was allowed to warm to rt and stir for 15 min. MeOH (20.0 mL) was added and the reaction mixture was concentrated. The residue was further diluted with toluene (2 x 50 mL), concentrated and adsorbed onto Celite. The crude compound was purified by silica gel chromatography (0-15% EtOAc in hexanes) to provide (1*S*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(triisopropylsilyl)oxy]cyclopentanol **I-52** (1.77 g, 61%). ¹H NMR (DMSO-*d*₆) δ 7.36 (d, J = 7.5 Hz, 2H), 7.30 (t, J = 7.3 Hz, 2H), 7.21 (d, J = 8.8 Hz, 5H), 6.88 (d, J = 8.8 Hz, 4H), 4.52 (d, J = 4.4 Hz, 1H), 4.03-4.08 (m, 1H), 3.87 (q, J = 7.1 Hz, 1H), 3.73 (s, 6H), 3.14 (dd, J = 8.7, 4.3 Hz, 1H), 2.83 (t, J = 8.1 Hz, 1H), 2.20 (quin, J = 7.0 Hz, 2H), 1.87 (ddd, J = 12.1, 7.8, 3.2 Hz, 1H), 1.64 (ddd, J = 13.3, 8.9, 6.6 Hz, 1H), 1.42 (quin, J = 5.8 Hz, 1H), 0.82-0.96 (m, 21H).

Step 3: 2-({(1*R*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(triisopropylsilyl)oxy]cyclopentyl}oxy)pyrazine (1*S*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(triisopropylsilyl)oxy]cyclopentanol (1.35 g, 2.28 mmol), 2-hydroxypyrazine (330 mg, 3.43 mmol), and triphenylphosphine (752 mg, 2.87 mmol) were taken up in THF (22.8 mL). The reaction mixture was cooled to 0 °C and DBAD (660 mg, 2.87 mmol) was added. The reaction mixture was allowed to warm to rt and stir for 1 h. MeOH was added (10.0 mL) and the mixture was concentrated. The crude compound was purified by silica gel chromatography (0-90% EtOAc in hexanes with 0.5% TEA) to provide 2-({(1*R*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(triisopropylsilyl)oxy]cyclopentyl}oxy)pyrazine (1.53 g, 85%). LCMS (AA): m/z = 669.4 (M+H).

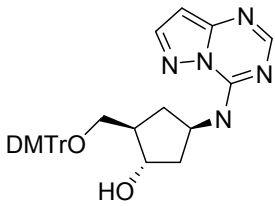
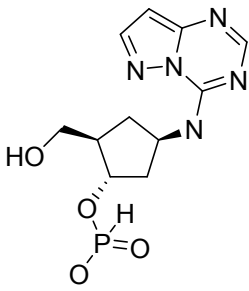
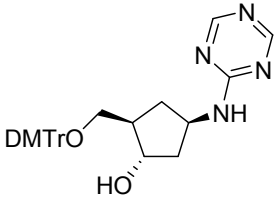
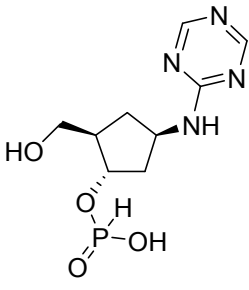
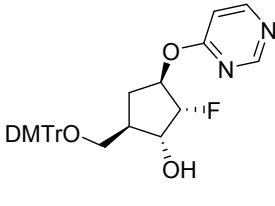
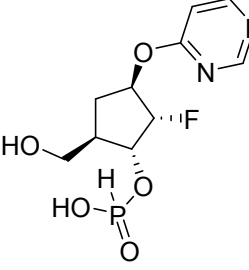
Step 4: (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrazine-2-yloxy)cyclopentanol To a solution of 2-({(1*R*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(triisopropylsilyl)oxy]cyclopentyl}oxy)pyrazine (2.40 g, 3.59 mmol) in THF (29.0 mL) was added TBAF (1.00 M in THF, 4.50 mL, 4.50 mmol). The reaction mixture was allowed to stir at rt for 1 h. An additional portion of TBAF (1.00 M in THF, 4.00 mL, 4.00 mmol)

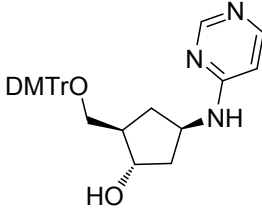
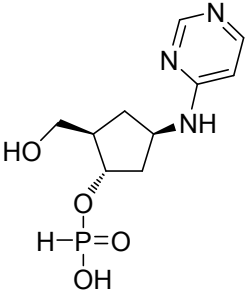
was added and stirring was continued for 2 h. The reaction mixture was concentrated. The crude compound was purified by silica gel chromatography (0-5% MeOH in DCM with 0.5% TEA) to provide (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrazine-2-yloxy)cyclopentanol (1.68 g, 91%). LCMS (AA): m/z = 513.3 (M+H).

Step 5: (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrazine-2-yloxy)cyclopentyl hydrogen phosphonate (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrazine-2-yloxy)cyclopentanol (1.04 g, 2.03 mmol) was taken up in pyridine (8.90 mL) and cooled to 0 °C. Diphenyl phosphite (0.796 mL, 4.14 mmol) was slowly added over 1 min. The reaction mixture was allowed to warm to rt and stir for 30 min. The reaction mixture was cooled to 0 °C and TEA (2.00 mL, 14.2 mmol) was added followed by water (2.00 mL). The reaction mixture was allowed to warm to rt and stir for 30 min. The reaction mixture was concentrated. The residue diluted with toluene (2 x 50 mL) and further concentrated. The crude compound was purified by silica gel chromatography (0-5% MeOH in DCM with 0.5% TEA) to provide (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrazine-2-yloxy)cyclopentyl hydrogen phosphonate (985 mg, 72%) as the *N,N*-diethylethanamine salt. LCMS (AA): m/z = 575.2 (M-H).

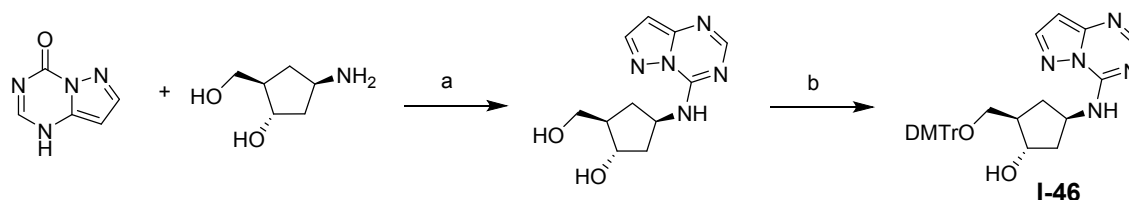
Step 6: (1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-(pyrazine-2-yloxy)cyclopentyl hydrogen phosphonate (I-6) (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrazine-2-yloxy)cyclopentyl hydrogen phosphonate (988 mg, 1.46 mmol) was taken up in water (1.2 mL) and acetic acid (5.6 mL). The reaction mixture was sonicated for 2 min and then the reaction mixture was allowed to stir at rt for 1 h. The reaction mixture was concentrated. The residue was diluted with toluene (2 x 50 mL) and further concentrated. The crude compound was purified by silica gel chromatography (10-80% MeOH in DCM) to provide (1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-(pyrazine-2-yloxy)cyclopentyl hydrogen phosphonate (**I-6**, 348 mg, 64%) as the *N,N*-diethylethanamine salt. LCMS (AA): m/z = 275.1 (M+H), ¹H NMR (DMSO-*d*₆) δ 10.52 (br s, 1H), 8.24 (d, J = 1.1 Hz, 1H), 8.13 - 8.20 (m, 2H), 7.35 (s, 0.5H), 5.88 (s, 0.5H), 5.29 - 5.37 (m, 1H), 4.42 (dq, J = 10.4, 6.7 Hz, 1H), 3.36 - 3.48 (m, 3H), 3.03 (q, J = 7.2 Hz, 6H), 2.28 - 2.37 (m, 1H), 1.97 - 2.11 (m, 3H), 1.40 (m, 1H), 1.18 (t, J = 7.27 Hz, 9H). ³¹P NMR (DMSO-*d*₆) δ 1.41 (s, 1P).

Table S-6. The compounds listed below were prepared as described for the synthesis of intermediate **I-6** starting with Step 5, substituting the starting material shown in the table for (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrazine-2-yloxy) cyclopentanol. Unless otherwise noted the products are *N,N*-diethylethanamine salts.

Starting material	Product	LCMS and NMR data
 <p>I-46</p>	 <p>I-2</p>	<p>LCMS (FA): $m/z = 314.1(M+H)^+$; 1H NMR (DMSO-d_6) δ 1.23 (t, $J=7.27$ Hz, 9 H) 1.31 - 1.45 (m, 1 H) 1.87 - 2.04 (m, 2 H) 2.04 - 2.16 (m, 2 H) 2.30 (s, 1 H) 3.37 (q, $J=7.25$ Hz, 6 H) 3.38 (m, 2 H) 4.38 (m, 1 H) 4.60 - 4.71 (m, 1 H) 5.88 (s, 0.5 H) 6.01 - 6.08 (m, 1 H) 6.44 (d, $J=2.08$ Hz, 1 H) 7.12 - 7.27 (m, 1 H) 7.30 (s, 0.5 H) 8.11 - 8.14 (m, 2 H) 8.81 (br d, $J=8.07$ Hz, 1 H); ^{31}P NMR (DMSO-d_6) δ 1.40 (s, 1P).</p>
 <p>I-47</p>	 <p>I-3</p>	<p>LCMS (AA): $m/z = 275.1 (M+H)^+$; 1H NMR (DMSO-d_6) δ 1.18 (m, 10 H) 1.69 - 1.90 (m, 1 H) 1.90 - 2.13 (m, 3 H) 3.03 (br d, $J=6.85$ Hz, 6 H) 3.17 (s, 3 H) 3.27 - 3.41 (m, 2 H) 4.09 (m, 1 H) 4.28 - 4.39 (m, 2 H) 5.87 (s, 0.5 H) 7.32 (s, 0.5 H) 8.12 (br d, $J=7.46$ Hz, 1 H) 8.42 (s, 1 H) 8.51 (s, 1 H) 10.16 - 10.47 (s, 1 H); ^{31}P NMR (DMSO-d_6) δ 1.29 (s, 1P).</p>
 <p>I-48</p>		<p>LCMS (FA): $m/z = 293.0 (M+H)^+$; 1H NMR (MeOD) δ 1.32 (t, $J=7.28$ Hz, 9 H) 1.62 (m, 1 H) 2.30 - 2.50 (m, 1 H) 2.50 - 2.71 (m, 1 H) 3.14 - 3.24 (m, 6 H) 3.69 (d, $J=7.28$ Hz, 2 H) 4.43 - 4.58 (m, 1 H) 5.06 (dt, $J=48, 6.8$ Hz, 1 H) 5.49 - 5.61 (m, 1 H) 6.09 (s, 0.5 H) 6.93 (dd, $J=5.90,$</p>

	I-25	1.00 Hz, 1 H) 7.66 (s, 0.5 H) 8.49 (d, $J=5.90$ Hz, 1 H) 8.77 (s, 1 H); ^{31}P NMR (MeOD) δ 3.46 (s, 1P).
 I-49	 I-24	LCMS (AA): $m/z = 274.1$ (M+H); ^1H NMR (DMSO- d_6) δ 1.09 (m, 1 H) 1.18 (t, $J=7.27$ Hz, 9 H) 1.61 - 1.74 (m, 1 H) 1.90 - 2.09 (m, 2 H) 2.14 (m, 1 H) 3.04 (q, $J=7.34$ Hz, 6 H) 3.25 - 3.45 (m, 2 H) 4.35 (m, 2 H) 5.87 (s, 0.5 H) 6.41 (dd, $J=5.99, 1.10$ Hz, 1 H) 7.33 (s, 1 H) 7.42 (br d, $J=6.97$ Hz, 1 H) 7.92 - 8.04 (m, 1 H) 8.38 (s, 1 H) 10.19 (br s, 1 H); ^{31}P NMR (DMSO- d_6) δ 1.41 (s, 1P).

Scheme S-6: Synthesis of I-46



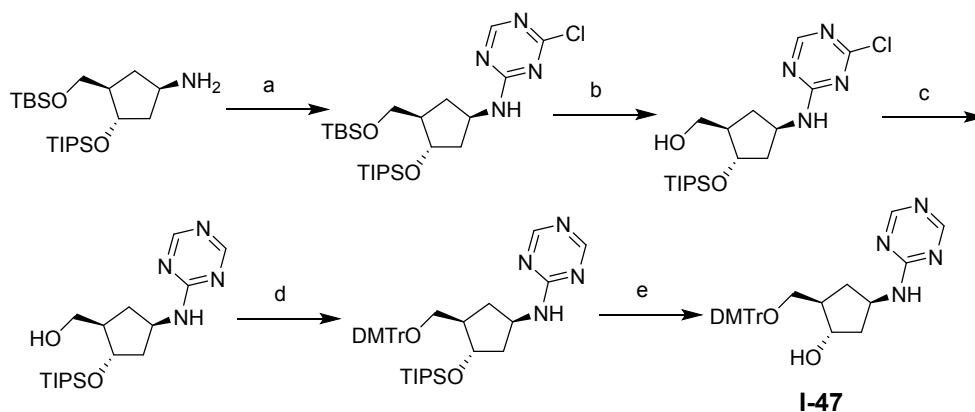
Reagents and conditions: (a) *i*) POCl_3 , benzotriazole, TEA, MeCN, 80 °C; *ii*) THF, DIPEA, 0 °C to rt; (b) DMTrCl, pyridine, 0 °C to rt, 2 h.

Step 1: (1S,2R,4R)-2-(hydroxymethyl)-4-(pyrazolo[1,5-a][1,3,5]triazin-4-ylamino)cyclopentan-1-ol A mixture of 1*H*, 4*H*-pyrazolo[1,5-*a*][1,3,5]triazin-4-one (1.30 g, 9.25 mmol) and benzotriazole (2.76 g, 23.1 mmol) were suspended in ACN (13.0 mL). TEA (3.87 mL, 27.8 mmol) was added followed by dropwise addition of phosphoryl chloride (1.29 mL, 13.9 mmol). The reaction mixture was heated at 80 °C for 6 h, cooled to rt and the solvents were evaporated. The residue was dissolved in THF (95 mL) and cooled to 0 °C. (1*S*,2*R*,4*R*)-4-amino-2-(hydroxymethyl)cyclopentanol (1.21 g, 9.25 mmol) and DIPEA (10.8 mL, 62.0 mmol) were added and the reaction mixture was allowed to stir at rt overnight. The solvents were evaporated and the crude compound was purified by silica gel chromatography (0-15% MeOH in EtOAc) followed by further purification by silica gel chromatography (0-20% MeOH in DCM) to provide

(1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-(pyrazolo[1,5-*a*][1,3,5]triazin-4-ylamino)cyclopentan-1-ol (375 mg, 16%). LCMS (FA): m/z = 250.1 (M+H).

Step 2: (1*S*,2*S*,4*R*)-2-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-4-(pyrazolo[1,5-*a*][1,3,5]triazin-4-ylamino)cyclopentanol (**I-46**) (1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-(pyrazolo[1,5-*a*][1,3,5]triazin-4-ylamino)cyclopentan-1-ol (580 mg, 2.33 mmol) was concentrated from dry pyridine (3 x 20 mL). To the residue was added pyridine (19.4 mL) followed by DMTr-Cl (756 mg, 2.21 mmol) at 0 °C. The reaction mixture was allowed to stir at 0 °C for 20 min, then at rt overnight. Methanol (10 mL) was added and the mixture was allowed to stir for 10 min. The solvents were evaporated and the crude compound was purified by silica gel chromatography (0-6% MeOH in DCM) to provide (1*S*,2*S*,4*R*)-2-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-4-(pyrazolo[1,5-*a*][1,3,5]triazin-4-ylamino)cyclopentanol (**I-46**, 827 mg, 64%). LCMS (FA): m/z = 550.2 (M-H); ¹H NMR (MeOD) δ 1.49 - 1.64 (m, 1 H) 1.94 - 2.04 (m, 1 H) 2.09 (m, 1 H) 2.15 - 2.29 (m, 1 H) 2.40 - 2.52 (m, 1 H) 3.09 - 3.27 (m, 2 H) 3.77 (s, 6 H) 4.18 (m, 1 H) 4.82 (m, 1 H) 6.39 (d, J =2.13 Hz, 1 H) 6.76 - 6.90 (m, 4 H) 7.13 - 7.22 (m, 1 H) 7.22 - 7.36 (m, 6 H) 7.42 - 7.46 (m, 2 H) 8.03 (d, J =2.13 Hz, 1 H) 8.11 (s, 1 H).

Scheme S-7: Synthesis of I-47



Reagents and conditions: (a) THF, DIPEA, 2,4-dichloro-1,3,5-triazine, 0 °C; (b) HCl, EtOH, 0 °C to rt; (c) H₂, Pd/C, MeOH, TEA; (d) DMTr-Cl, DBU, DCM, 0 °C to rt; (e) TBAF, THF, 0 °C to rt.

Step 1: *N*-((1*R*,3*R*,4*S*)-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl)-4-chloro-1,3,5-triazin-2-amine A solution of 2,4-dichloro-1,3,5-triazine (9.68 g, 64.6 mmol) and DIPEA (12.8 g, 99.4 mmol) in THF (200 mL) was cooled to 0 °C and (1*R*,3*R*,4*S*)-3-{{[(tert-butyldimethylsilyl)oxy]methyl}-4-{{[tris(propan-2-yl)silyl]oxy}cyclopentan-1-amine

(20.0 g, 49.7 mmol) in THF (200 mL) was added slowly. The mixture was allowed to stir at 0 °C for 1 h. The reaction mixture was diluted with water (500 mL) and extracted with DCM (100 mL x 3), then washed with brine, dried and evaporated. The residue was purified by silica gel chromatography (petroleum ether:EtOAc, 20:1 to 15:1) to provide *N*-((1*R*,3*R*,4*S*)-3-(((tert-butyl)dimethylsilyl)oxy)methyl)-4-(((triisopropylsilyl)oxy)cyclopentyl)-4-chloro-1,3,5-triazin-2-amine (11.7 g, 45.7%) as a yellow oil. ¹H NMR (CDCl₃) δ 8.10 - 8.36 (m, 1H), 5.89 - 6.18 (m, 1H), 4.37 - 4.55 (m, 1H), 4.24 (quin, *J* = 4.7 Hz, 1H), 3.46 - 3.71 (m, 2H), 2.24 - 2.39 (m, 1H), 2.00 - 2.10 (m, 1H), 1.89 - 2.00 (m, 1H), 1.70 (dq, *J* = 12.6, 6.1 Hz, 1H), 1.21 - 1.31 (m, 1H), 0.96 (m, 21H), 0.82 (s, 9 H), -0.02 - 0.03 (m, 6H).

Step 2: [(1*R*,2*S*,4*R*)-4-[(4-chloro-1,3,5-triazin-2-yl)amino]-2-triisopropylsilyloxy-cyclopentyl]methanol

N-((1*R*,3*R*,4*S*)-3-(((tert-butyl)dimethylsilyl)oxy)methyl)-4-(((triisopropylsilyl)oxy)cyclopentyl)-4-chloro-1,3,5-triazin-2-amine (4.50 g, 8.73 mmol) was dissolved in EtOH (45 mL) and the reaction mixture was cooled to 0 °C. A solution of HCl (1 mL, 12 M in water) in EtOH (15 mL) was added and the reaction mixture was allowed to stir at rt for 1 h. TEA (6 mL) was added and the solvents were evaporated. The crude compound was purified by silica gel chromatography (petroleum ether:EtOAc, 9:1 to 1:1) to provide [(1*R*,2*S*,4*R*)-4-[(4-chloro-1,3,5-triazin-2-yl)amino]-2-triisopropylsilyloxy-cyclopentyl]methanol (3.30 g, 94%). ¹H NMR CDCl₃) δ 8.44 - 8.25 (m, 1H), 6.22 (br d, *J* = 7.1 Hz, 1H), 4.64 - 4.53 (m, 1H), 4.41 - 4.33 (m, 1H), 3.83 - 3.66 (m, 2H), 2.52 - 2.41 (m, 1H), 2.24 - 2.16 (m, 1H), 2.14 - 2.03 (m, 1H), 1.93 - 1.82 (m, 1H), 1.72 (br s, 1H), 1.44 - 1.35 (m, 1H), 1.30 - 1.23 (m, 1H), 1.12 - 1.02 (m, 18H).

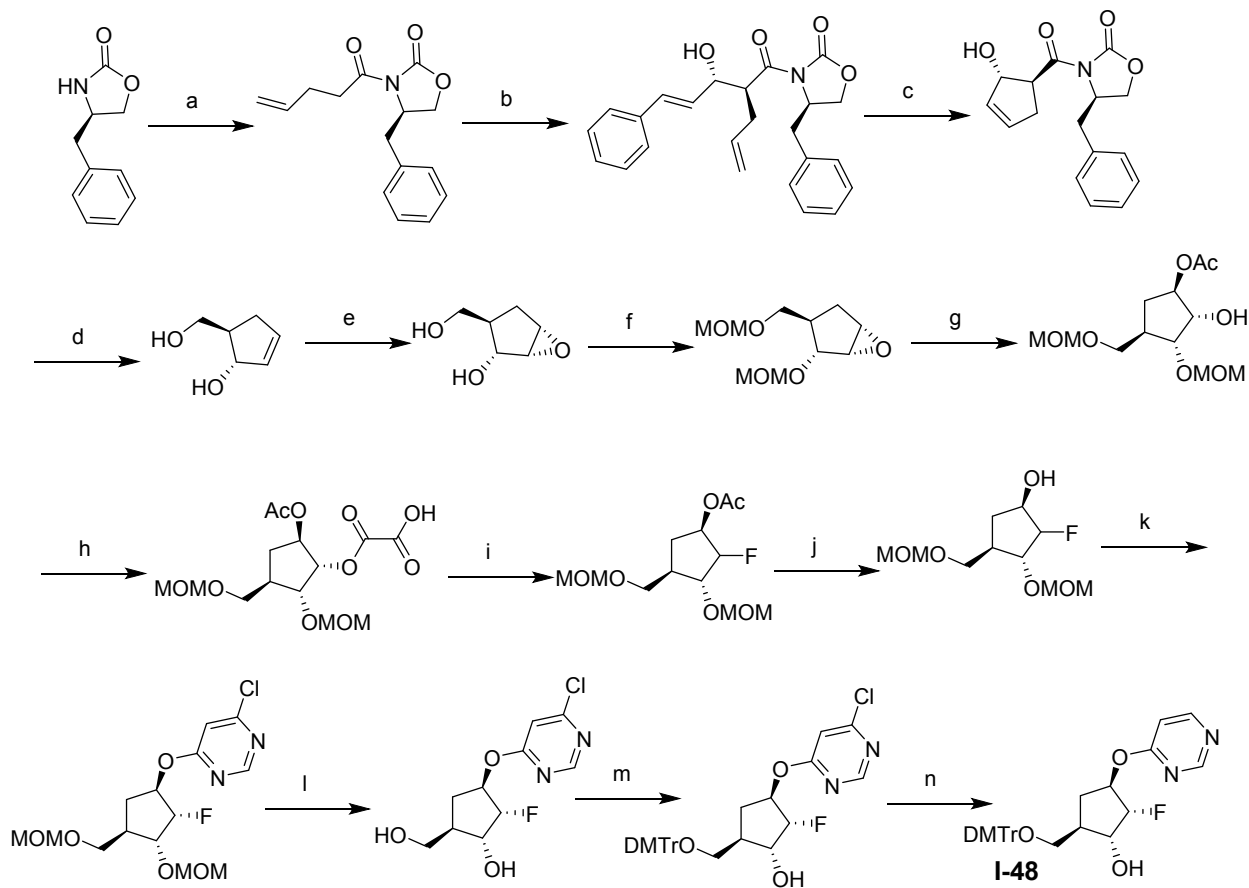
Step 3: [(1*R*,2*S*,4*R*)-4-(1,3,5-triazin-2-ylamino)-2-triisopropylsilyloxy-cyclopentyl]methanol

[(1*R*,2*S*,4*R*)-4-[(4-chloro-1,3,5-triazin-2-yl)amino]-2-triisopropylsilyloxy-cyclopentyl]methanol (3.30 g, 8.22 mmol) was dissolved in MeOH (15 mL) and TEA (997 mg, 9.86 mmol). 10% Palladium on carbon (84 mg) was added and the reaction mixture was stirred under hydrogen (15 psi) at rt for 1 h. The reaction mixture was filtered, and the filtrate was evaporated. The crude compound was purified by silica gel chromatography (petroleum ether:EtOAc, 20:1 to 2:1) to provide [(1*R*,2*S*,4*R*)-4-(1,3,5-triazin-2-ylamino)-2-triisopropylsilyloxy-cyclopentyl]methanol (3.00 g, 95%). ¹H NMR (CDCl₃) δ 8.58 (s, 1H), 8.46 (s, 1H), 5.93 (br d, *J* = 7.7 Hz, 1H), 4.56 (qd, *J* = 7.0, 14.0 Hz, 1H), 4.36 (q, *J* = 5.5 Hz, 1H), 3.81 - 3.67 (m, 2H), 2.47 (td, *J* = 8.5, 13.5 Hz, 1H), 2.23 - 2.14 (m, 1H), 2.14 - 2.05 (m, 1H), 1.92 - 1.84 (m, 1H), 1.79 (t, *J* = 4.2 Hz, 1H), 1.59 (s, 2H), 1.36 (td, *J* = 6.6, 13.4 Hz, 1H), 1.07 (s, 18H).

Step 4: *N*-[(1*R*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[tris(propan-2-yl)silyl]oxy}cyclopentyl]-1,3,5-triazin-2-amine To a solution of [(1*R*,2*S*,4*R*)-4-(1,3,5-triazin-2-ylamino)-2-triisopropylsilyloxy-cyclopentyl]methanol (3.0 g, 8.18 mmol) in DCM (80 mL) was added DBU (3.72 g, 24.5 mmol) followed by DMTrCl (5.52 g, 16.3 mmol) at 0 °C. The reaction was then warmed to rt and stirred for 2 h. To the reaction was added water (100 mL) and the mixture was extracted with DCM (2 x 200 mL). The combined organic layers were washed with water (200 mL), brine (100 mL), dried over Na₂SO₄, filtered and concentrated to dryness. The crude material was purified by gel silica column (petroleum ether:EtOAc, 10:1 to 2:1) to give *N*-[(1*R*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[tris(propan-2-yl)silyl]oxy}cyclopentyl]-1,3,5-triazin-2-amine (5.3 g, 96.8%) as yellow solid. ¹H NMR (CDCl₃) δ 8.57 (s, 1 H) 8.45 (s, 1 H) 7.41 (d, *J*=7.02 Hz, 2 H) 7.27 - 7.32 (m, 6 H) 6.81 (d, *J*=8.77 Hz, 5 H) 5.41 (br d, *J*=7.89 Hz, 1 H) 4.51 - 4.61 (m, 1 H) 4.17 - 4.22 (m, 1 H) 3.79 (s, 7 H) 3.15 (dd, *J*=9.21, 5.70 Hz, 1 H) 3.04 (dd, *J*=8.99, 6.36 Hz, 1 H) 2.50 - 2.58 (m, 1 H) 2.25 (br d, *J*=3.51 Hz, 1 H) 2.06 2.15 (m, 1 H) 2.06 - 2.14 (m, 1 H) 1.57 - 1.64 (m, 5 H) 1.23 - 1.31 (m, 2 H) 0.94 - 1.01 (m, 23 H).

Step 5: (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(1,3,5-triazin-2-yl)amino]cyclopentan-1-ol (I-47) To a solution of *N*-[(1*R*,3*R*,4*S*)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[tris(propan-2-yl)silyl]oxy}cyclopentyl]-1,3,5-triazin-2-amine (5.3 g, 7.92 mmol) in THF (60 mL) was added TBAF (9.50 mL, 1.0 M in THF) at 0 °C. This mixture was warmed to rt and stirred for 16 h. The reaction was quenched by the addition of brine and the mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and concentrated. The crude material was purified by silica gel chromatography eluted with (petroleum ether:EtOAc, 20:1 to 0:1) to give (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(1,3,5-triazin-2-yl)amino]cyclopentan-1-ol (**I-47**, 3.7 g, 91.3%) as a white solid. ¹H NMR (CDCl₃) δ 8.56 (s, 1 H), 8.45 (s, 1H), 7.35 - 7.45 (m, 2H), 7.27 - 7.33 (m, 6H), 7.18 - 7.25 (m, 1H), 6.83 (d, *J* = 8.8 Hz, 4H), 5.40 (br d, *J* = 7.5 Hz, 1H), 4.48 - 4.57 (m, 1H), 4.14 (q, *J* = 6.4 Hz, 1H), 3.79 (s, 6H), 3.34 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.05 (t, *J* = 8.6 Hz, 1H), 2.32 - 2.42 (m, 2H), 2.10 - 2.24 (m, 2H), 1.84 (dt, *J* = 13.7, 7.0 Hz, 1H), 1.08 - 1.17 (m, 1H).

Scheme S-8: Synthesis of I-48



Reagents and conditions: (a) *i)* 4-pentenoic acid, Piv-Cl, TEA, THF; *ii)* *n*-BuLi, THF, -65 °C to rt; (b) *i)* *(2E)*-3-phenylprop-2-enal, MgCl_2 , TEA, TMS-Cl, NaSbF_6 , EtOAc; *ii)* TFA, MeOH; (c) 2nd gen. Grubbs catalyst, DCM; (d) LiBH_4 , THF, MeOH; (e) *m*-CPBA, DCM; (f) MOM-Cl, DIPEA, DCM, 0 °C to rt; (g) $\text{Ti}(\text{OiPr})_4$, KOAc, AcOH, 90 °C; (h) oxalyl chloride, DCM, 0 °C to rt; then water, 0 °C to rt; (i) $\text{Ir}(\text{dF}(\text{CF}_3)\text{ppy})_2(\text{dtbbpy})\text{PF}_6$, Selectfluor, Na_2HPO_4 , acetone, water, blue LED; (j) K_2CO_3 , MeOH; (k) NaH, 4,6-dichloropyrimidine, THF, 0 °C; (l) 6 *N* HCl, THF; (m) DMTrCl, pyridine; (n) H_2 , Pd-C, TEA, MeOH.

Step 1: *(R)*-4-benzyl-3-(pent-4-enyl)oxazolidin-2-one To a solution of *(R)*-4-benzyl-3-(pent-4-enyl)oxazolidin-2-one (125 g, 705 mmol) in dry THF (1.25 L) was added *n*-BuLi (296 mL, 740 mmol) dropwise at -40 °C under nitrogen atmosphere. The reaction mixture was allowed to stir at -40 °C for 30 min, and then cooled to -65 °C. In a separate flask, pivaloyl chloride (118 g, 986 mmol) was added to a solution of 4-pentenoic acid (91.7 g, 916 mmol) and TEA (127 g, 1.26 mol) in dry THF (1.80 L) at -65 °C under nitrogen atmosphere. The reaction mixture was allowed to stir at 0 °C for 1 h, and then cooled to -65 °C. To this reaction mixture was added the *(R)*-4-benzyl-3-(pent-4-enyl)oxazolidin-2-one reaction mixture, and the resulting mixture was stirred at -65 °C for 30 min, warmed to rt and

stirred for 16 h. The reaction mixture was poured into saturated aqueous NH_4Cl solution (2.0 L), extracted with EtOAc (3 x 1.0 L). The combined organic phases were washed with brine (2.0 L), dried over anhydrous Na_2SO_4 , filtered and concentrated. The residue was combined with another reaction carried out on the same scale and purified by silica gel chromatography (petroleum:EtOAc, 20:1 to 5:1) to provide (*R*)-4-benzyl-3-(pent-4-enoyl)oxazolidin-2-one as a yellow oil (250 g, 67%). ^1H NMR (CDCl_3) δ 7.28 - 7.38 (m, 3H), 7.22 (br d, J = 7.1 Hz, 2H), 5.90 (ddt, J = 16.9, 10.3, 6.5, 6.5 Hz, 1H), 5.00 - 5.18 (m, 2H), 4.63 - 4.74 (m, 1H), 4.13 - 4.27 (m, 2H), 3.31 (dd, J = 13.4, 2.9 Hz, 1H), 2.96 - 3.19 (m, 2H), 2.77 (dd, J = 13.2, 9.8 Hz, 1H), 2.47 (q, J = 7.0 Hz, 2H).

Step 2: (4*R*)-3-[(2*S*,3*S*,4*E*)-2-allyl-3-hydroxy-5-phenylpent-4-enoyl]-4-benzyl-1,3-oxazolidin-2-one To a solution of (*R*)-4-benzyl-3-(pent-4-enoyl)oxazolidin-2-one (126.5 g, 485 mmol), MgCl_2 (4.61 g, 48.5 mmol), Et_3N (98.1 g, 970 mmol), TMSCl (78.9 g, 727 mmol), and NaSbF_6 (37.5 g, 145 mmol) in EtOAc (2.53 L) was added (*E*)-3-phenylprop-2-enal (83.2 g, 630 mmol) at rt under nitrogen. The reaction mixture was allowed to stir at rt for 16 h. The reaction mixture was filtered through a pad of Celite. The filter cake was washed with EtOAc (1.0 L x 3). The combined filtrates were concentrated to dryness. MeOH (5.0 L) and TFA (20 mL) were added and the reaction mixture was allowed to stir at rt for 1 h. The reaction mixture was adjusted to pH = 8 with TEA and then concentrated. This reaction mixture was combined with another reaction carried out on the same scale and concentrated. The residue was purified by silica gel chromatography (petroleum:EtOAc, 20:1 to 5:1) to provide (4*R*)-3-[(2*S*,3*S*,4*E*)-2-allyl-3-hydroxy-5-phenylpent-4-enoyl]-4-benzyl-1,3-oxazolidin-2-one (311 g, 81%) as a yellow oil. ^1H NMR (CDCl_3) δ 7.37 - 7.43 (m, 2H), 7.33 (br t, J = 7.3 Hz, 2H), 7.27 (br s, 4H), 7.16 (br d, J = 7.3 Hz, 2H), 6.69 (br d, J = 15.9 Hz, 1H), 6.33 (dd, J = 15.9, 6.1 Hz, 1H), 5.75 - 5.90 (m, 1H), 5.00 - 5.19 (m, 2H), 4.63 - 4.73 (m, 1H), 4.54 (q, J = 6.3 Hz, 1H), 4.30 - 4.39 (m, 1H), 4.13 - 4.20 (m, 2H), 3.26 (br dd, J = 13.4, 2.4 Hz, 1H), 2.93 (br d, J = 8.1 Hz, 1H), 2.42 - 2.68 (m, 3H).

Step 3: (4*R*)-4-benzyl-3-[(1*S*,2*S*)-2-hydroxycyclopent-3-en-1-yl]carbonyl-1,3-oxazolidin-2-one To a solution of (4*R*)-3-[(2*S*,3*S*,4*E*)-2-allyl-3-hydroxy-5-phenylpent-4-enoyl]-4-benzyl-1,3-oxazolidin-2-one (156 g, 395 mmol) in DCM (3.11 L) was added (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium (3.35 g, 3.95 mmol, Grubb's 2nd generation catalyst) at rt under a nitrogen atmosphere. The reaction mixture was allowed to stir at rt for 4 h. The reaction mixture was combined with another reaction

carried out on the same scale and concentrated. The residue was purified by silica gel chromatography (petroleum:EtOAc, 20:1 to 2:1) to provide (4*R*)-4-benzyl-3-[[*(1S,2S)*-2-hydroxycyclopent-3-en-1-yl]carbonyl]-1,3-oxazolidin-2-one (180 g, 79%) as a light yellow solid, ¹H NMR (MeOD) δ 7.21 - 7.37 (m, 5H), 5.83 - 5.90 (m, 1H), 5.76 (dd, *J* = 5.6, 2.2 Hz, 1H), 5.27 (br s, 1H), 4.71 - 4.80 (m, 1H), 4.22 - 4.35 (m, 2H), 4.09 (ddd, *J* = 9.2, 5.9, 4.8 Hz, 1H), 3.17 (dd, *J* = 13.6, 3.1 Hz, 1H), 2.85 - 3.02 (m, 2H), 2.34 - 2.46 (m, 1H).

Step 4: (1*R*,5*S*)-5-(hydroxymethyl)cyclopent-2-en-1-ol To a solution of (4*R*)-4-benzyl-3-[[*(1S,2S)*-2-hydroxycyclopent-3-en-1-yl]carbonyl]-1,3-oxazolidin-2-one (90.0 g, 313 mmol) in THF (1.35 L) was added MeOH (22.0 mL) and LiBH₄ (14.9 g, 688 mmol) at 0 °C under nitrogen. The reaction mixture was allowed to stir at 0 °C for 1 h, then quenched with aqueous NaOH (10%, 500 mL). The reaction mixture was extracted with EtOAc (5 x 1.0 L). The combined organic phases were washed with brine (1.0 L x 2), dried over anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was combined with another reaction carried out on the same scale and purified by silica gel chromatography (petroleum:EtOAc, 20:1 to 1:1). The combined aqueous phases were concentrated and purified by silica gel chromatography eluted with (1:5 to 1:2 EtOAc in PE) The products from the columns were combined to provide (1*R*,5*S*)-5-(hydroxymethyl)cyclopent-2-en-1-ol (58 g, 81%) as a yellow oil. ¹H NMR (CDCl₃) δ 5.91 (td, *J* = 2.3, 1.1 Hz, 1H), 5.78 (dd, *J* = 4.9, 2.0 Hz, 1H), 4.73 (br s, 1 H) 3.62 - 3.82 (m, 2 H) 2.55 - 2.68 (m, 1 H) 2.21 - 2.34 (m, 1 H) 1.95 - 2.04 (m, 1 H).

Step 5: (1*R*,2*R*,3*R*,5*S*)-3-(hydroxymethyl)-6-oxabicyclo[3.1.0]hexan-2-ol To a solution of (1*R*,5*S*)-5-(hydroxymethyl)cyclopent-2-en-1-ol (36.0 g, 315 mmol) in DCM (520 mL) was added *m*-CPBA (101 g, 472 mmol) at 0 °C under nitrogen. The reaction mixture was allowed to stir at 0 °C for 2 h. The suspension was filtered through a pad of Celite and the filter cake was washed with DCM (30 mL x 3). The residue was purified by silica gel chromatography (petroleum:EtOAc:MeOH, 2:1:0 to 0:1:0 to 0:10:1 to provide (1*R*,2*R*,3*R*,5*S*)-3-(hydroxymethyl)-6-oxabicyclo[3.1.0]hexan-2-ol (15.0 g, 36.6%) as a yellow solid. ¹H NMR (DMSO-*d*₆) δ 4.82 (d, *J* = 6.1 Hz, 1H), 4.44 (t, *J* = 5.0 Hz, 1H), 3.81 (t, *J* = 6.4 Hz, 1H), 3.49 - 3.43 (m, 1H), 3.33 (s, 3H), 1.97 - 1.90 (m, 1H), 1.56 - 1.41 (m, 2H).

Step 6: (1*R*,2*S*,3*S*,5*R*)-2-(methoxymethoxy)-3-[(methoxymethoxy)methyl]-6-oxabicyclo[3.1.0]hexane To a solution of (1*R*,2*R*,3*R*,5*S*)-3-(hydroxymethyl)-6-oxabicyclo[3.1.0]hexan-2-ol (17.8 g, 136 mmol) in anhydrous DCM (500 mL) at 0 °C was added DIEA (105 g, 816 mmol),

followed by MOM-Cl (65.6 g, 816 mmol). The reaction mixture was allowed to stir at rt for 15h. Water (100 mL) was added, and the mixture was extracted with DCM (100 mL x 2), washed with water, brine, dried over anhydrous Na₂SO₄ and concentrated. The residue was purified by silica gel chromatography (1:10 to 1:3 EtOAc in PE) and then further purified by silica gel chromatography (1:10 to 1:3 EtOAc in PE) to provide (1*R*,2*S*,3*S*,5*R*)-2-(methoxymethoxy)-3-[(methoxymethoxy)methyl]-6-oxabicyclo[3.1.0]hexane (14.0 g, 38%) as a yellow oil. ¹H NMR (CDCl₃) δ 4.80 - 4.77 (m, 1H), 4.74 - 4.69 (m, 1H), 4.59 (s, 2H), 3.95 (dd, *J* = 1.2, 7.8 Hz, 1H), 3.57 (dd, *J* = 1.4, 2.8 Hz, 1H), 3.54 (d, *J* = 4.4 Hz, 2H), 3.44 - 3.42 (m, 1H), 3.41 (s, 3H), 3.32 (s, 3H), 2.16 (dd, *J* = 7.9, 13.9 Hz, 1H), 2.00 - 1.90 (m, 1H), 1.70 - 1.61 (m, 1H).

Step 7: (1*R*,2*S*,3*R*,4*R*)-2-hydroxy-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate To a solution of (1*R*,2*S*,3*S*,5*R*)-2-(methoxymethoxy)-3-[(methoxymethoxy)methyl]-6-oxabicyclo[3.1.0]hexane (14.0 g, 64.1 mmol) in AcOH (300 mL) was added KOAc (31.3 g, 320 mmol) and Ti(OiPr)₄ (18.2 g, 64.1 mmol). The reaction mixture was heated at 90 °C for 3 h. The mixture was diluted with EtOAc, filtered through Celite and washed with water (500 mL x 2). Sat. solution of NaHCO₃ was added to adjust the pH to 7. The mixture was extracted with EtOAc (500 mL x 2), washed by brine, dried over anhydrous Na₂SO₄ and concentrated. The residue was purified by silica gel chromatography (1:5 to 1:2 EtOAc in PE) then re-purified by silica gel chromatography (1:10 to 1:2 EtOAc in PE) to provide (1*R*,2*S*,3*R*,4*R*)-2-hydroxy-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate as a colorless oil (7.4 g, 34%). ¹H NMR (CDCl₃) δ 4.97 - 4.91 (m, 1H), 4.71 - 4.64 (m, 2H), 4.58 (s, 2H), 4.01 - 3.95 (m, 1H), 3.88 (t, *J* = 5.3 Hz, 1H), 3.50 (d, *J* = 5.4 Hz, 2H), 3.37 (s, 3H), 3.32 (s, 3H), 2.83 (br d, *J* = 4.2 Hz, 1H), 2.42 - 2.26 (m, 2H), 2.02 (s, 3H), 1.39 - 1.29 (m, 1H).

Step 8: ((1*R*,2*S*,3*S*,5*S*)-5-acetoxy-2-(methoxymethoxy)-3-[(methoxymethoxy)methyl]cyclopentyl}oxy)(oxo)acetic acid A solution of (1*R*,2*S*,3*R*,4*R*)-2-hydroxy-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate (992 mg, 3.56 mmol) in Et₂O (35.7 mL) under nitrogen was cooled to 0 °C and oxalyl chloride (2.0M in DCM, 3.56 mL, 7.12 mmol) was added dropwise. The reaction mixture was warmed to rt and stirred overnight. To the reaction mixture at 0 °C added water (6.40 mL, 355 mmol) dropwise. After this mixture was warmed to rt and stirred for 1 h, it was partitioned between water and EtOAc, extracted into Et₂O (2x), washed with brine, dried (Na₂SO₄) and evaporated to provide crude ((1*R*,2*S*,3*S*,5*S*)-5-acetoxy-2-(methoxymethoxy)

-3-[(methoxymethoxy)methyl]cyclopentyl}oxy)(oxo)acetic acid (1.42g) as a clear oil which was used without purification.

Step 9: (1*R*,2*R*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate and (1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate To a 100-mL round bottom flask fitted with a rubber septa was added ((1*R*,2*S*,3*S*,5*S*)-5-acetoxy-2-(methoxymethoxy)-3-[(methoxymethoxy)methyl]cyclopentyl}oxy)(oxo)acetic acid (1.25 g, 3.57 mmol), [4,4'-bis(1,1-dimethylethyl)-2,2'-bipyridine-*NI*,*NI'*]bis[3,5-difluoro-2-[5-(trifluoromethyl)-2-pyridinyl-*N*]phenyl-*C*]iridium(III) hexafluorophosphate (40.0 mg, 0.036 mmol), disodium hydrogen phosphate (1.01 g, 7.14 mmol), *N*-fluoro-*N'*-chloromethyl-triethylenediamine-bis(tetrafluoroborate) (5.69 g, 16.1 mmol), acetone (27.5 mL) and water (7.0 mL). The reaction mixture was degassed with nitrogen and a balloon of argon was attached. The flask was placed between two Kessil® LED illuminators (model H150 blue) and the reaction mixture was allowed to stir for 2 h. Water was added, and the mixture was extracted with Et₂O (3x). The combined organic phase was washed with water, sat. NaHCO₃, brine, dried over Na₂SO₄ and evaporated. The residue was purified by silica gel chromatography (10% to 50% EtOAc in hexanes) to provide (1*R*,2*R*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate and (1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate as a mixture of diastereomers (578 mg, 58%). ¹H NMR (MeOD) δ 5.17 - 5.04 (m, 1H), 4.98 - 4.89 (m, 0.5H), 4.84 - 4.76 (m, 0.5H), 4.74 - 4.66 (m, 2H), 4.65 - 4.60 (m, 2H), 4.13 - 3.99 (m, 0.5H), 3.95 - 3.86 (m, 0.5H), 3.63 - 3.51 (m, 2H), 3.40 - 3.34 (m, 6H), 2.46 - 2.11 (m, 2H), 2.08 - 2.06 (m, 1.5H), 2.06 - 2.03 (m, 1.5H), 1.71 - 1.62 (m, 0.5H), 1.56 - 1.44 (m, 0.5H).

Step 10: (1*R*,2*R*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentanol and (1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentanol To a solution of (1*R*,2*R*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate and (1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl acetate (1.42 g, 5.07 mmol) in methanol (35.0 mL) was added potassium carbonate (135 mg, 0.977 mmol). The reaction mixture was allowed to stir at rt for 2.5 h, after which time more potassium carbonate (233 mg, 1.69 mmol) was added and the reaction mixture was allowed to stir at rt for 2 h. MeOH was evaporated and the residue was partitioned between EtOAc and water. After the layers were separated, the aqueous layer was

further extracted with EtOAc. The combined organic phases were washed with water and brine, dried over Na₂SO₄ and evaporated. The residue was purified by silica gel chromatography (10% to 90% EtOAc in hexanes) to provide (1*R*,2*R*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentanol and (1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentanol as a mixture of diastereomers (475 mg, 39 %). ¹H NMR (DMSO-*d*₆) δ 5.13 (d, *J* = 4.2 Hz, 0.5H), 5.02 (d, *J* = 5.0 Hz, 0.5H), 4.75 - 4.67 (m, 1H), 4.67 - 4.58 (m, 2H), 4.57 - 4.55 (m, 2H), 4.10 - 3.93 (m, 1H), 3.92 - 3.76 (m, 1H), 3.56 - 3.47 (m, 1H), 3.46 - 3.38 (m, 1H), 3.29 - 3.26 (m, 3H), 3.26 - 3.25 (m, 3H), 2.22 - 2.10 (m, 1H), 2.05 - 1.94 (m, 1H), 1.43 - 1.32 (m, 0.5H), 1.32 - 1.20 (m, 0.5H).

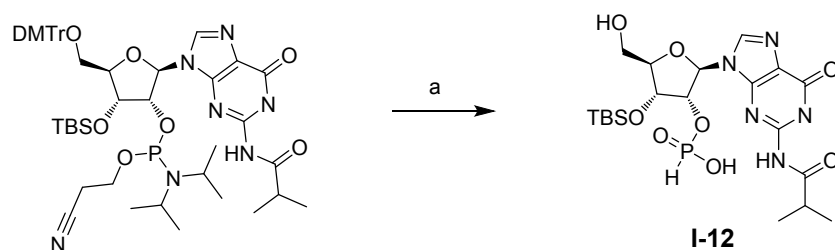
Step 11: 4-chloro-6-((1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl)oxy)pyrimidine To the mixture of (1*R*,2*R*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentanol and (1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentanol (1.19 g, 4.99 mmol) in THF (14.5 mL) was added to a suspension of sodium hydride (392 mg, 15.5 mmol) in THF (43.5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. A solution of 4,6-dichloropyrimidine (1.02 g, 6.85 mmol) in THF (23 mL) was added and the reaction mixture was stirred at 0 °C for 4 h. The reaction mixture was then quenched with a saturated NH₄Cl solution and extracted with EtOAc (3 x). The combined organic phases were washed with brine, dried over anhydrous MgSO₄, filtered and concentrated. The residue was purified by silica gel chromatography (0% to 40% EtOAc in hexanes) to provide 4-chloro-6-((1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl)oxy)pyrimidine as the first eluting diastereomer (584 mg, 33%). LCMS (FA): *m/z* = 351.1 (M+H).

Step 12: (1*R*,2*R*,3*R*,5*R*)-3-[(6-chloropyrimidin-4-yl)oxy]-2-fluoro-5-(hydroxymethyl)cyclopentanol To a solution of 4-chloro-6-((1*R*,2*S*,3*R*,4*R*)-2-fluoro-3-(methoxymethoxy)-4-[(methoxymethoxy)methyl]cyclopentyl)oxy)pyrimidine (270.5 mg, 0.771 mmol) in THF (10.0 mL) was added an HCl solution (6 *N* in water, 2.0 mL). The reaction mixture was heated at 60 °C for 2 h. The mixture was concentrated and the crude compound was purified by C18 flash column chromatography (10-100% CAN with aq. ammonium bicarbonate (5 mM)) to provide (1*R*,2*R*,3*R*,5*R*)-3-[(6-chloropyrimidin-4-yl)oxy]-2-fluoro-5-(hydroxymethyl)cyclopentanol (185 mg, 91 %). LCMS (AA): *m/z* = 263.1 (M+H).

Step 13: (1*R*,2*R*,3*R*,5*R*)-5- $\{[\text{bis(4-methoxyphenyl)(phenyl)methoxy}]\text{methyl}\}$ -3-[(6-chloropyrimidin-4-yl)oxy]-2-fluorocyclopentanol 4,4'-Dimethoxytrityl chloride (543 mg, 1.59 mmol) was added to a solution of (1*R*,2*R*,3*R*,5*R*)-3-[(6-chloropyrimidin-4-yl)oxy]-2-fluoro-5-(hydroxymethyl)cyclopentanol (379 mg, 1.44 mmol) in pyridine (12 mL). The reaction mixture was allowed to stir at rt for 1 h. The solvents were evaporated and the residue was purified by silica gel chromatography (0% to 100% EtOAc in hexanes) to provide (1*R*,2*R*,3*R*,5*R*)-5- $\{[\text{bis(4-methoxyphenyl)(phenyl)methoxy}]\text{methyl}\}$ -3-[(6-chloropyrimidin-4-yl)oxy]-2-fluorocyclopentanol (550 mg, 68 %) as a white powder. ¹H NMR (MeOD) δ 8.58 - 8.54 (m, 1H), 7.43 - 7.38 (m, 2H), 7.30 - 7.20 (m, 6H), 7.19 - 7.13 (m, 1H), 6.91 - 6.86 (m, 1H), 6.83 - 6.77 (m, 4H), 5.52 - 5.40 (m, 1H), 4.86 (td, J = 3.5, 52.0 Hz, 1H), 4.17 - 4.11 (m, 1H), 3.73 (m, sH), 3.22 (d, J = 4.9 Hz, 2H), 2.59 - 2.48 (m, 1H), 2.35 - 2.24 (m, 1H), 1.59 - 1.50 (m, 1H).

Step 14: (1*R*,2*R*,3*R*,5*R*)-5- $\{[\text{bis(4-methoxyphenyl)(phenyl)methoxy}]\text{methyl}\}$ -2-fluoro-3-(pyrimidin-4-yloxy)cyclopentanol (**I-48**) (1*R*,2*R*,3*R*,5*R*)-5- $\{[\text{bis(4-methoxyphenyl)(phenyl)methoxy}]\text{methyl}\}$ -3-[(6-chloropyrimidin-4-yl)oxy]-2-fluorocyclopentanol (548 mg, 0.973 mmol) and triethylamine (0.41 mL, 2.91 mmol) were dissolved in methanol (40 mL). Palladium (10% on carbon, 6.0 mg) was added to the solution and the reaction mixture was stirred under an atmosphere of hydrogen at rt for 2 h. The reaction mixture was filtered through Celite and washed with methanol. The filtrate was evaporated and the residue was dissolved in DCM (50 mL) and then washed with water (2 x 10 mL), dried over Na₂SO₄ and concentrated to provide (1*R*,2*R*,3*R*,5*R*)-5- $\{[\text{bis(4-methoxyphenyl)(phenyl)methoxy}]\text{methyl}\}$ -2-fluoro-3-(pyrimidin-4-yloxy)cyclopentanol (**I-48**, 494 mg, 96 %) as a white solid. LCMS (AA): m/z = 531.2 (M+H). ¹H NMR (MeOD) δ 1.54 (m, 1 H), 2.31 (m, 1 H), 2.46 - 2.59 (m, 1 H), 3.10 - 3.26, (m, 2 H), 3.71 (s, 6 H), 4.01 - 4.21 (m, 1 H), 4.84 (td, J = 3.5, 52.0 Hz, 1H), 5.40 - 5.55 (m, 1 H), 6.72 - 6.85 (m, 5 H), 7.12 - 7.32 (m, 7 H), 7.40 (d, J =7.53 Hz, 2 H), 8.40 (d, J =5.90 Hz, 1 H), 8.71 (s, 1 H); ¹⁹F NMR (MeOD) δ -205.80 (s, 1 F).

Scheme S-9: Synthesis of I-12

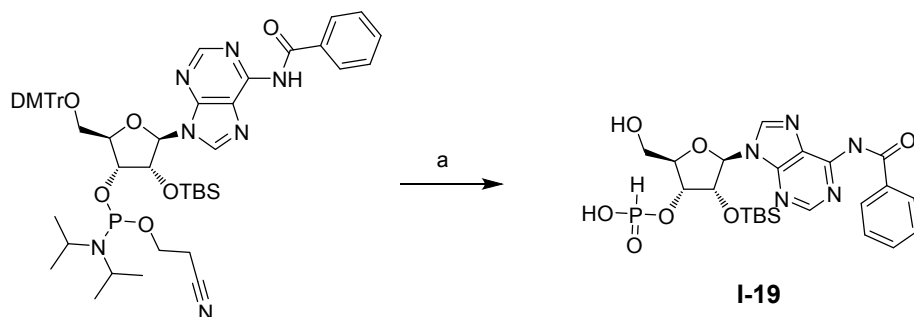


Reagents and conditions: (a) *i*) TFA-pyridine, H₂O, ACN, rt, 1 h; *ii*) *t*BuNH₂, rt, 1 h; *iii*) AcOH, H₂O, rt, 1 h.

(2*R*,3*R*,4*R*,5*R*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-5-(hydroxymethyl)-2-[2-(isobutyrylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]tetrahydrofuran-3-yl hydrogen phosphonate (I-12)

To a solution of (2*R*,3*R*,4*R*,5*R*)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy] methyl}-4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-[2-(isobutyrylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylphosphoramidite (1.00 g, 1.03 mmol) in ACN (5 mL) was added water (40.0 μ L, 2.22 mmol) and pyridine trifluoroacetate (235 mg, 1.22 mmol). The reaction mixture was stirred at rt for 1 h. *tert*-Butylamine (5.00 mL, 47.6 mmol) was added and the reaction mixture was stirred at rt for 1 h. The reaction mixture was concentrated and the residue was dissolved in acetic acid (3.81 mL, 66.6 mmol) and water (800 μ L, 4.44 mmol), and stirred at rt for 1 h. This reaction mixture was then concentrated and further concentrated from ACN (2 x 25 mL) and toluene (2 x 25 mL). The crude compound was purified by silica gel chromatography (0-40% MeOH in DCM) to provide (2*R*,3*R*,4*R*,5*R*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-5-(hydroxymethyl)-2-[2-(isobutyrylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]tetrahydrofuran-3-yl hydrogen phosphonate (**I-12**, 375 mg, 68%) as the *tert*-butylamine salt. LCMS (FA): m/z = 532.2 (M+H). ¹H NMR (DMSO-*d*₆) δ 8.29 (s, 1H), 7.25 (s, 0.5H), 5.86 (d, J = 7.3 Hz, 1H), 5.80 (s, 0.5H), 5.17 - 5.09 (m, 1H), 5.06 - 4.96 (m, 1H), 4.34 (dd, J = 1.4, 4.7 Hz, 1H), 3.92 - 3.87 (m, 1H), 3.61 - 3.46 (m, 2H), 3.17 (s, 1H), 2.81 - 2.72 (m, 1H), 1.23 - 1.19 (m, 9H), 1.12 (d, J = 6.2 Hz, 6H), 0.90 (s, 9H), 0.14 (d, J = 4.5 Hz, 6H). ³¹P NMR (DMSO-*d*₆) δ -0.95 (s, 1P).

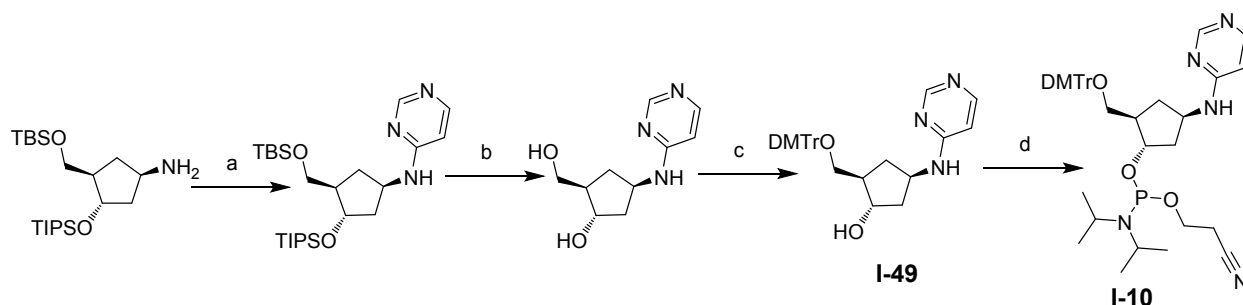
Scheme S-10: Synthesis of I-19



Reagents and conditions: (a) *i*) TFA-pyridine, H₂O, ACN, rt, 1 h; *ii*) *t*BuNH₂, rt, 1 h; *iii*) AcOH, H₂O, rt, 1 h.

(2*R*,3*R*,4*R*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)-2-(hydroxymethyl)tetrahydrofuran-3-yl hydrogen phosphonate (I-19): Intermediate **I-19** was prepared as a *tert*-butylamine salt following the synthesis of intermediate **I-12** starting with (2*R*,3*R*,4*R*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy) tetrahydrofuran-3-yl (2-cyanoethyl) diisopropyl-phosphoramidite. LCMS (FA): m/z = 550.2 (M+H). ¹H NMR (DMSO-*d*₆) δ -0.12 (s, 3 H), -0.01 (s, 3 H), 0.76 (s, 9 H), 1.25 (s, 9H), 3.63 - 3.78 (m, 2 H), 4.13 (m, 1 H), 4.59 - 4.69 (m, 1 H), 4.73 (t, J =4.58 Hz, 1 H), 5.72 (br s, 1 H), 6.01 (s, 0.5 H), 6.04 (d, J =4.77 Hz, 1 H), 7.48 (s, 1 H), 7.52 - 7.58 (m, 2 H), 7.62 - 7.67 (m, 1 H), 7.94 (s, 2 H), 8.05 (d, J =7.40 Hz, 2 H), 8.73 (s, 1 H), 8.75 (s, 1 H), 11.20 (br s, 1 H); ³¹P NMR (DMSO-*d*₆) δ 0.96 (s, 1P).

Scheme S-11: Synthesis of I-49 and I-10



Reagents and conditions: (a) 4-chloropyrimidine, NMP, DIPEA, 150 °C, 4 h; (b) HCl, EtOH, rt to 50 °C; (c) DMTr-Cl, pyridine, 0 °C to rt; (d) 2-cyanoethyl *N,N,N',N'*-tetraisopropyl-phosphordiamidite tetrazole, *N*-Me-imidazole, DMF, MeCN, 0 °C to rt, 16 h.

Step 1: *N*-{(1*R*,3*R*,4*S*)-3-({*tert*-butyl(dimethyl)silyl}oxy)methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl pyrimidin-4-amine To a solution of 4-chloropyrimidine (916 mg, 7.68 mmol) in

NMP (8.00 mL) was added DIPEA (3.35 mL, 19.2 mmol) at rt. (1*R*,3*R*,4*S*)-3-([*tert*-butyl(dimethyl)silyl]oxy)methyl)-4-[(triisopropylsilyl)oxy]cyclopentanamine (2.57 g, 6.40 mmol) was added and the reaction mixture was allowed to stir for 5 min. The reaction mixture was heated under microwave irradiation at 150 °C for 4 h. The reaction mixture was cooled to rt. Water was added and the mixture was extracted with EtOAc. The combined organic phases were washed with brine, dried with Na₂SO₄ and concentrated. The crude compound was purified by silica gel chromatography (0-100% EtOAc in hexanes) to provide *N*-{(1*R*,3*R*,4*S*)-3-([*tert*-butyl(dimethyl)silyl]oxy)methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}pyrimidin-4-amine (870 mg, 28%). LCMS (FA): *m/z* = 480.4 (M+H).

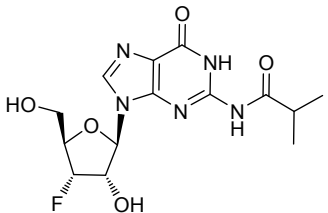
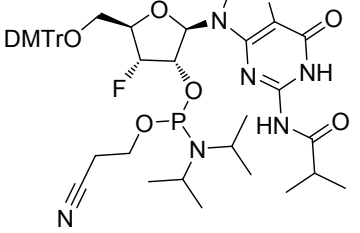
Step 2: (1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-(pyrimidin-4-ylamino)cyclopentanol *N*-{(1*R*,3*R*,4*S*)-3-([*tert*-butyl(dimethyl)silyl]oxy)methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}pyrimidin-4-amine (860 mg, 1.79 mmol) was dissolved in HCl (1.25 M in EtOH, 2.00 mL) and TFA (2.00 mL). The reaction mixture was stirred at rt overnight, then heated at 50 °C for 2 h. The reaction mixture was concentrated and the residue was further concentrated with pyridine (3 x 15 mL) to give crude (1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-(pyrimidin-4-ylamino)cyclopentanol. The crude product was used directly in the next step without purification.

Step 3: (1*S*,2*R*,4*R*)-2-[[bis(4-methoxyphenyl)(phenyl)methoxy]methyl]-4-(pyrimidin-4-ylamino)cyclopentanol (I-49) To a solution of (1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-(pyrimidin-4-ylamino)cyclopentanol (375 mg, 1.79 mmol) in pyridine (10.0 mL) was added DMTrCl (705 mg, 2.06 mmol). The reaction mixture was stirred at rt overnight. The reaction mixture was concentrated and taken up in EtOAc, then washed with brine, dried over Na₂SO₄ and concentrated. The crude compound was purified by silica gel chromatography (0-15% MeOH in EtOAc) to provide (1*S*,2*R*,4*R*)-2-[[bis(4-methoxyphenyl)(phenyl)methoxy]methyl]-4-(pyrimidin-4-ylamino)cyclopentanol (**I-49**, 490 mg, 53%). LCMS (FA): *m/z* = 512.3 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.60 (m, 1 H), 1.79 - 1.94 (m, 2 H), 2.03 (m, 1 H), 2.17 (m, 1 H) 2.33 (m, 1 H) 2.89 (dd, *J*=8.62, 7.40 Hz, 1 H), 3.08 (dd, *J*=8.80, 5.50 Hz, 1 H), 3.73 (s, 6 H), 3.81 - 3.92 (m, 1 H), 4.63 (d, *J*=4.77 Hz, 1 H), 6.39 (d, *J*=5.75 Hz, 1 H), 6.88 (dd, *J*=8.93, 1.34 Hz, 4 H), 7.16 - 7.39 (m, 10 H), 7.92 - 8.05 (br s, 1 H), 8.37 (s, 1 H).

Step 4: (1*S*,2*R*,4*R*)-2-[[bis(4-methoxyphenyl)(phenyl)methoxy]methyl]-4-(pyrimidin-4-ylamino)cyclopentyl 2-cyanoethyl diisopropylphosphoramidite (I-10): (1*S*,2*R*,4*R*)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(pyrimidin-4-ylamino)cyclopentan-1-ol (**I-49**) (870

mg, 1.70 mmol) was dissolved in dry ACN and concentrated to dryness. The residue was taken up in DMF (2.2 mL). *N*-Methylimidazole (83.7 mg, 1.07 mmol) and 1*H*-tetrazole (0.45 M in ACN, 4.50 mL, 2.04 mmol) were added. The reaction mixture was cooled to 0 °C, then 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (1.22 mL, 3.83 mmol) was added dropwise. The reaction mixture was then warmed to rt and allowed to stir for 16 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous NaHCO₃ solution (10 mL). The aqueous phase was extracted with EtOAc (2 x 60 mL). The combined organic phases were washed with brine, dried over MgSO₄ and concentrated. This crude compound was purified by silica gel chromatography (0-100% EtOAc in hexanes, with 0.5% TEA) to provide (1*S*,2*R*,4*R*)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(pyrimidin-4-ylamino)cyclopentyl 2-cyanoethyl diisopropylphosphoramidite (**I-10**, 880 mg, 73%) as a mixture of diastereomers. ¹H NMR (DMSO-*d*₆) δ 8.38 - 8.41 (m, 1H), 7.99 - 8.03 (m, 1H), 7.36 - 7.43 (m, 3H), 7.28 - 7.34 (m, 2H), 7.20 - 7.28 (m, 5H), 6.85 - 6.92 (m, 4H), 6.39 - 6.43 (m, 1H), 4.10 - 4.26 (m, 1H), 3.73 - 3.76 (m, 6H), 3.62 - 3.72 (m, 1H), 3.46 - 3.59 (m, 4H), 3.09 - 3.15 (m, 1H), 2.86 - 3.02 (m, 1H), 2.72 - 2.77 (m, 1H), 2.61 - 2.66 (m, 1H), 2.29 - 2.42 (m, 1H), 2.16 - 2.27 (m, 1H), 2.00 - 2.15 (m, 1H), 1.64 - 1.75 (m, 1H), 1.25 - 1.38 (m, 1H), 1.00-1.22 (m, 12H). ³¹P NMR (DMSO-*d*₆) δ 146.69 (s, 0.5P), 146.39 (s, 0.5P).

Table S-7. The compounds listed below were prepared as described in the synthesis of **I-10** starting with step 3, substituting the starting material shown in the table for (1*S*,2*R*,4*R*)-2-(hydroxymethyl)-4-(pyrimidin-4-ylamino)cyclopentan-1-ol. Unless otherwise noted, the products are a mixture of diastereomers.

Starting material	Product	NMR data
		¹ H NMR (DMSO- <i>d</i> ₆) δ 12.09 (br s, 1H), 11.55 (br s, 1H), 8.07 - 8.10 (m, 1H), 7.19 - 7.38 (m, 9H), 6.81 - 6.88 (m, 4H), 5.98 - 6.03 (m, 1H), 5.05 - 5.43 (m, 2H), 4.27 - 4.47 (m, 1H), 3.71 - 3.78 (m, 7H), 3.34 - 3.63 (m, 5H), 2.66 - 2.84 (m, 2H),

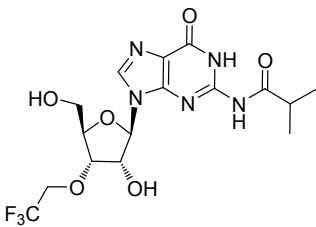
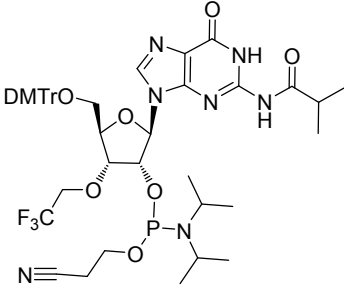
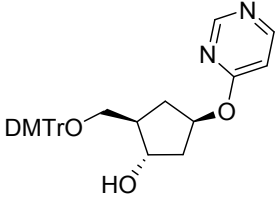
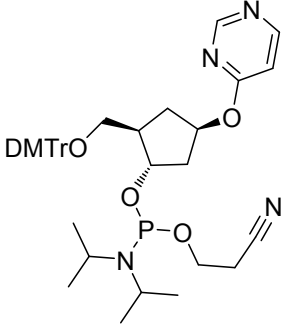
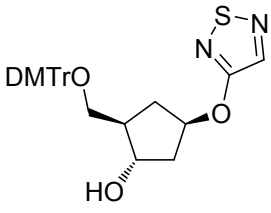
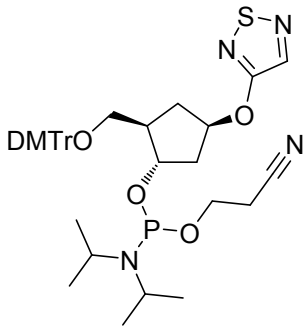
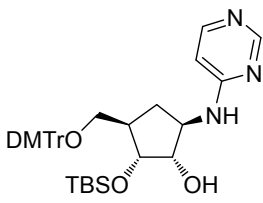
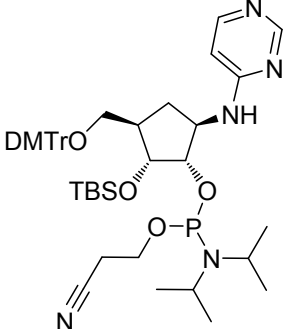
	I-26	2.53 - 2.62 (m, 1H), 0.96 - 1.18 (m, 15H), 0.76 (d, $J = 6.7$ Hz, 3H). ^{31}P NMR (DMSO- d_6) δ 151.56 (br d, $J = 7.8$ Hz, 0.5 P), 151.36 (br d, $J = 3.9$ Hz, 0.5 P).
	 <p style="text-align: center;">I-27</p>	^1H NMR (DMSO- d_6) δ 12.06 (br s, 1H), 11.61 (s, 1H), 8.14 (d, $J = 3.4$ Hz, 1H), 7.17 - 7.35 (m, 9H), 6.80 - 6.86 (m, 4H), 5.89 - 6.05 (m, 1H), 4.98 - 5.19 (m, 1H), 3.94 - 4.47 (m, 5H), 3.73 (d, $J = 1.2$ Hz, 7H), 3.39 - 3.57 (m, 4H), 2.87 - 2.91 (m, 1 H), 2.70 - 2.79 (m, 2H), 0.99 - 1.25 (m, 15H), 0.80 (d, $J = 6.7$ Hz, 3H). ^{31}P NMR (DMSO- d_6) δ 151.15 (s, 0.5P), 150.19 (s, 0.5P).

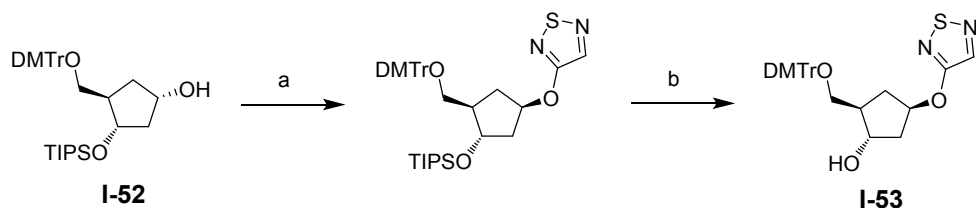
Table S-8. The compounds listed below were prepared as described in the synthesis of **I-10** starting with Step 4, substituting the starting material shown in the table for (1*S*,2*R*,4*R*)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(pyrimidin-4-ylamino)cyclopentan-1-ol (**I-49**).

Unless otherwise noted, the products are a mixture of diastereomers.

Starting material	Product	NMR data
 <p style="text-align: center;">I-45</p>		^1H NMR (DMSO- d_6) δ 8.76 - 8.78 (m, 1H), 8.48 - 8.51 (m, 1H), 7.34 - 7.40 (m, 2H), 7.18 - 7.33 (m, 7H), 6.79 - 6.89 (m, 5H), 5.41 - 5.50 (m, 1H), 4.19 - 4.35 (m, 1H), 3.72 - 3.76 (m, 6H), 3.62 - 3.71 (m, 1H), 3.46 - 3.61 (m, 3H), 3.10 - 3.16 (m, 1H), 2.96 - 3.09 (m, 1H), 2.62 - 2.75 (m,

	 <p style="text-align: center;">I-32</p>	<p>2H), 2.41 - 2.48 (m, 1H), 2.20 - 2.31 (m, 1H), 2.05 - 2.19 (m, 1H), 1.94 - 2.04 (m, 1H), 1.52 - 1.65 (m, 1H), 1.01-1.24 (m, 12H). ³¹P NMR (DMSO-<i>d</i>₆) δ 146.88 (s, 0.5P), 146.49 (s, 0.5P).</p>
 <p style="text-align: center;">I-53</p>	 <p style="text-align: center;">I-11</p>	<p>¹H NMR (DMSO-<i>d</i>₆) δ 8.29 - 8.26 (m, 1H), 7.37 (t, <i>J</i> = 7.0 Hz, 2H), 7.33 - 7.26 (m, 2H), 7.26 - 7.18 (m, 5H), 6.91 - 6.83 (m, 4H), 5.33 - 5.24 (m, 1H), 4.37 - 4.18 (m, 1H), 3.75 - 3.72 (m, 7H), 3.62 - 3.43 (m, 3H), 3.17 - 3.11 (m, 1H), 3.10 - 2.98 (m, 1H), 2.78 - 2.71 (m, 1H), 2.70 - 2.61 (m, 1H), 2.35 - 2.02 (m, 3H), 1.73 - 1.59 (m, 1H), 1.21 - 0.97 (m, 13H). ³¹P NMR (DMSO-<i>d</i>₆) δ 146.95 (s, 0.5P), 146.58 (s, 0.5P).</p>
 <p style="text-align: center;">I-54</p>	 <p style="text-align: center;">I-18</p>	<p>¹H NMR (DMSO-<i>d</i>₆) δ 8.34 (m, 1H), 7.99 (m, 1H), 7.1-7.5 (m, 10H), 6.8-6.9 (m, 4H), 6.3-6.4 (m, 1H), 3.92-3.96 (m, 1H), 3.75-3.88 (m, 1H), 3.4-3.7 (m, 3H), 2.9-3.2 (m, 2H), 2.65-2.75 (m, 2H), 2.2-2.4 (m, 2H), 0.9-1.3 (m, 15H), 0.79 (m, 9H), -0.1-0.0 (m, 6H); ³¹P NMR (DMSO-<i>d</i>₆) δ 147.71 (s, 0.5P), 147.51 (s, 0.5P).</p>

Scheme S-12: Synthesis of I-53

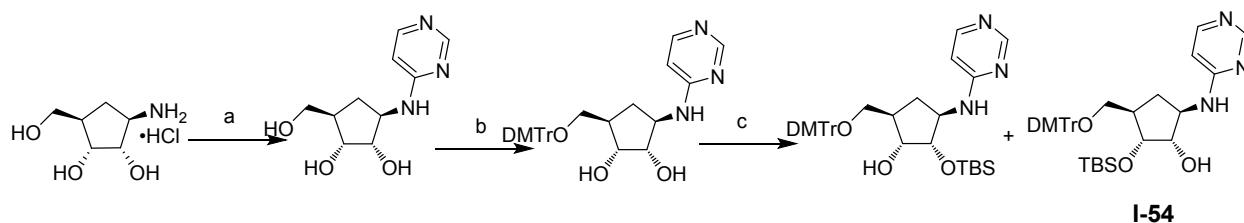


Reagents and conditions: (a) 1,2,5-thiadiazol-3(2*H*)-one, DEAD, PPh₃, THF, 0 °C to rt, 16 h; (b) TBAF, THF, 0 °C to rt, 2 h.

Step 1: **3-(((1*R*,3*R*,4*S*)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl)oxy)-1,2,5-thiadiazole** ((1*S*,3*R*,4*S*)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentanol **I-52** (1.50 g, 2.54 mmol), 1,2,5-thiadiazol-3(2*H*)-one (311 mg, 3.05 mmol), and triphenylphosphine (766 mg, 2.92 mmol) were dissolved in THF (30 mL) and the reaction mixture was cooled to 0 °C. DEAD (0.440 mL, 2.79 mmol) was added dropwise and the reaction mixture was allowed to warm to rt and stirred overnight. The solvents were evaporated, and the crude compound was purified by silica gel chromatography (0% to 15% EtOAc / hexane) to provide 3-(((1*R*,3*R*,4*S*)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl)oxy)-1,2,5-thiadiazole (1.38 g, 81%) as a pale yellow oil. LCMS (FA): *m/z* = 673.3 (M-H).

Step 2: **(1*S*,2*R*,4*R*)-2-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-4-(1,2,5-thiadiazol-3-yloxy)cyclopentanol (**I-53**)** To a solution of 3-(((1*R*,3*R*,4*S*)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((triisopropylsilyl)oxy)cyclopentyl)oxy)-1,2,5-thiadiazole (1.26 g, 1.87 mmol) in THF (9.0 mL) at 0 °C was added tetrabutylammonium fluoride hydrate (626 mg, 2.24 mmol). The reaction mixture was allowed to warm to rt and stirred for 2 h. Brine was added, and the reaction mixture was extracted into EtOAc (2x). The combined organic phases were washed with water, brine, dried (Na₂SO₄) and evaporated. The residue was purified by silica gel chromatography (10% to 50% EtOAc in hexanes) to provide (1*S*,2*R*,4*R*)-2-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-4-(1,2,5-thiadiazol-3-yloxy)cyclopentanol (**I-53**) (866 mg, 90%) as a white solid. LCMS (AA): *m/z* = 517.1 (M-H). ¹H NMR (DMSO-*d*₆) δ 1.52 - 1.62 (m, 1 H), 1.90 - 2.02 (m, 2 H), 2.02 - 2.10 (m, 1 H), 2.46 (m, 1 H), 2.95 (br t, *J*=8.01 Hz, 1 H), 3.10 (m, 1 H), 3.73 (s, 6 H), 3.96 - 4.06 (m, 1 H), 4.79 (d, *J*=5.01 Hz, 1 H), 5.21 - 5.28 (m, 1 H), 6.87 (d, *J*=7.46 Hz, 4 H), 7.18 - 7.37 (m, 9 H), 8.26 (s, 1 H).

Scheme S-13: Synthesis of I-54



Reagents and conditions: (a) NMP, DIPEA, 4-chloropyrimidine, 150 °C, 4 h; (b) DMTrCl, pyridine, rt, 3 h; (c) TBS-Cl, imidazole, pyridine, rt, 16 h.

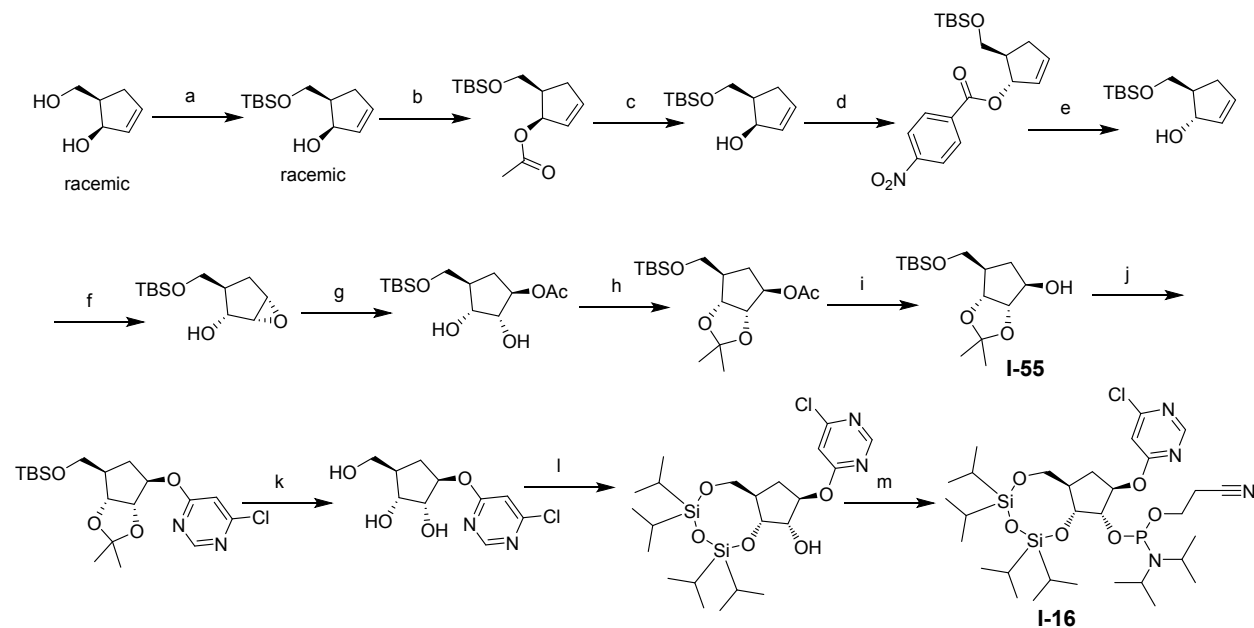
Step 1: (1S,2R,3R,5R)-3-(hydroxymethyl)-5-(pyrimidin-4-ylamino)cyclopentane-1,2-diol
 4-Chloropyrimidine (572 mg, 4.79 mmol) was dissolved in a mixture of NMP (10 mL) and DIPEA (3.04 mL, 17.4 mmol). (1R,2S,3R,4R)-2,3-Dihydroxy-4-(hydroxymethyl)-1-aminocyclopentane hydrochloride (800 mg, 4.36 mmol) was added. The reaction mixture was heated at 150°C under microwave irradiation for 4 h. The reaction mixture was diluted with EtOAc and concentrated to provide (1S,2R,3R,5R)-3-(hydroxymethyl)-5-(pyrimidin-4-ylamino)cyclopentane-1,2-diol. The resulting residue was combined with the product from a second reaction of the same scale. This combined product was used for further elaboration without purification.

Step 2: (1S,2R,3R,5R)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-5-(pyrimidin-4-ylamino)cyclopentane-1,2-diol
 (1S,2R,3R,5R)-3-(hydroxymethyl)-5-(pyrimidin-4-ylamino)cyclopentane-1,2-diol (1.96 g, 8.70 mmol) was concentrated from dry pyridine (20 mL), then dissolved in pyridine (30 mL). DMTrCl (4.47 g, 13.1 mmol) was added and the reaction mixture was allowed to stir at rt for 3 h. The reaction mixture was concentrated and diluted with water. The mixture was extracted with EtOAc and the organic phase was washed with brine, dried with Na₂SO₄ and concentrated. The crude compound was purified by silica gel chromatography (0-100% EtOAc in hexanes, followed by 0-20% MeOH in EtOAc) to provide (1S,2R,3R,5R)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-5-(pyrimidin-4-ylamino)cyclopentane-1,2-diol (1.10 g, 24%). LCMS (FA): *m/z* = 528.3 (M+H).

Step 3: (1S,2R,3R,5R)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2-((tert-butyl dimethylsilyl)oxy)-5-(pyrimidin-4-ylamino)cyclopentane-1-ol (I-54)
 (1S,2R,3R,5R)-3-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-5-(pyrimidin-4-ylamino)cyclopentane-1,2-diol (1.11 g, 2.10 mmol) was concentrated from toluene (3 x 50 mL) and then taken up in pyridine (50.0 mL) at rt. TBS-Cl (1.60 g, 10.5 mmol) and imidazole (578 mg, 8.40 mmol) were added. This reaction was allowed to stir at rt for 4 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic phases were washed with brine, dried with Na₂SO₄ and

concentrated. The crude mixture was purified by silica gel chromatography (40-80% EtOAc in hexanes) to provide undesired isomer (1*R*,2*S*,3*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2-((*tert*-butyldimethylsilyl)oxy)-3-(pyrimidin-4-ylamino)cyclopentan-1-ol (339 mg, 25%) as the first eluting peak. ¹H NMR (DMSO-*d*₆) δ 8.32 (s, 1H), 7.97 (br d, *J* = 5.4 Hz, 1H), 7.34-7.42 (m, 2H), 7.26-7.32 (m, 3H), 7.17-7.26 (m, 6H), 6.87 (d, *J* = 8.7 Hz, 4H), 6.40 (br d, *J* = 4.6 Hz, 1H), 4.11 (br d, *J* = 4.8 Hz, 1H), 3.76 (t, *J* = 5.0 Hz, 1H), 3.72 (s, 6H), 3.64 (q, *J* = 5.0 Hz, 1H), 3.03 (dd, *J* = 8.8, 5.1 Hz, 1H), 2.93 (dd, *J* = 8.9, 6.0 Hz, 1H), 2.03-2.21 (m, 2H), 1.22 (m, 1H), 0.79 (s, 9H), 0.00 (s, 3H), -0.05 (s, 3H); and desired isomer (1*S*,2*R*,3*R*,5*R*)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2-((*tert*-butyldimethylsilyl)oxy)-5-(pyrimidin-4-ylamino)cyclopentan-1-ol (**I-54**, 22 mg, 31%) as the second eluting peak. ¹H NMR (DMSO-*d*₆) δ 8.37 (s, 1H), 8.01 (br s, 1H), 7.19 - 7.40 (m, 10H), 6.85 - 6.91 (m, 4H), 6.45 (br d, *J* = 5.6 Hz, 1H), 4.42 (br d, *J* = 5.8 Hz, 1H), 3.97 - 4.23 (br s, 1H), 3.79 (t, *J* = 5.1 Hz, 1H), 3.73 (s, 6H), 3.61 (q, *J* = 5.9 Hz, 1H), 3.06 (dd, *J* = 8.9, 5.3 Hz, 1H), 2.90 (dd, *J* = 8.8, 7.1 Hz, 1H), 2.28 - 2.40 (m, 1H), 2.14 (m, 1H), 1.02 - 1.21 (m, 1H), 0.78 (s, 9H), -0.02 (s, 3H), -0.07 (s, 3H).

Scheme S-14: Synthesis of **I-16** and **I-55**



Reagents and conditions: (a) TBS-Cl, imidazole, DMAP, rt; (b) *Candida Antarctica*, vinyl acetate, MTBE, rt; (c) NaOMe, MeOH, rt; (d) 4-nitrobenzoic acid, dimethylphenylphosphine, DIAD, toluene, rt; (e) K₂CO₃, MeOH, rt; (f) VO(acac)₂, *t*-BuOOH, DCM, rt; (g) tetramethylammonium

triacetoxyborohydride, NaOAc, THF, reflux; (h) and 2,2-dimethoxypropane, PPTS, acetone, rt; (i) K₂CO₃, MeOH, water, rt; (j) LDA, 4,6-dichloro-pyrimidine, THF, 60 °C; (k) TFA, 3 M HCl in MeOH, 50 °C; (l) 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane, pyridine, DMF, rt; (m) tetrazole, 2-cyanoethyl *N,N,N',N'*-tetraisopropyl-phosphorodiamidite tetrazole, *N*-Me-imidazole, DMF, MeCN, 0 °C to rt.

Step 1: rel-(1*R*,5*R*)-5-([*tert*-butyl(dimethyl)silyl]oxy)methyl)cyclopent-2-en-1-ol To a solution of rel-(1*R*,5*R*)-5-(hydroxymethyl)cyclopent-2-en-1-ol (47.20 g, 0.4135 mol), *N,N*-dimethylaminopyridine (2.52 g, 0.0207 mol) and 1*H*-imidazole (30.97 g, 0.4549 mol) in methylene chloride (800 mL) at 0 °C under an atmosphere of nitrogen was added TBS-Cl (28.0 g, 0.186 mol). The reaction was stirred for at 0 °C for 2.5 h, at which time TBS-Cl (28.0 g, 0.186 mol) was added. The reaction was stirred for 2 additional hours. The reaction was quenched by addition of brine solution (200 mL) and water (200 mL). The layers were separated and the organic layer was washed with water (3 x 200 mL) and brine (200 mL), dried over Na₂SO₄, filtered and concentrated in vacuo to give rel-(1*R*,5*R*)-5-([*tert*-butyl(dimethyl)silyl]oxy)methyl)cyclopent-2-en-1-ol which was used without purification in the next step.

Step 2: (1*R*,5*R*)-5-([*tert*-butyl(dimethyl)silyl]oxy)methyl)cyclopent-2-en-1-yl acetate To a suspension of rel-(1*R*,5*R*)-5-([*tert*-butyl(dimethyl)silyl]oxy)methyl)cyclopent-2-en-1-ol (crude from step 1) and Candida Antartica on acrylic resin (24.9 g; 10,800 units/g) in methyl *tert*-butyl ether (1500 mL) was added acetic acid ethenyl ester (190 mL, 2.05 mol) and the reaction was stirred overnight. Solids were removed by filtration and the volatiles were removed in vacuo to provide a clear colorless oil (143 grams) which was purified by silica gel chromatography (0-10% diethyl ether in hexanes) to afford the desired enantiomer (1*R*,5*R*)-5-([*tert*-butyl(dimethyl)silyl]oxy)methyl)cyclopent-2-en-1-yl acetate (45.6 g, 82%). ¹H NMR (CDCl₃) δ 6.10 - 6.08 (m, 1H), 5.86 - 5.83 (m, 1H), 5.71 - 5.69 (m, 1H), 3.78 (dd, *J* = 10.0, 7.5 Hz, 1H), 3.59 (dd, *J* = 9.9, 7.4 Hz, 1H), 2.58 - 2.49 (m, 1H), 2.45 - 2.38 (m, 1H), 2.26 - 2.17 (m, 1H), 2.01 (s, 3H), 0.88 (s, 9H), 0.44 (d, *J* = 2.8 Hz, 6H).

Step 3: (1*R*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent-2-en-1-ol To a solution of (1*R*,5*R*)-5-([*tert*-butyl(dimethyl)silyl]oxy)methyl)cyclopent-2-en-1-yl acetate (13.5 g, 50.0 mmol) in methanol (100 mL) was added sodium methoxide (6.53 mL, 0.5 M in MeOH) and the reaction mixture was allowed to stir at rt overnight. Acetic acid (186 uL, 3.26 mmol) was added and the mixture was concentrated. The crude mixture was purified by silica gel chromatography (2-10% EtOAc in hexanes) to provide (1*R*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent

-2-en-1-ol (8.45 g, 74%). ¹H NMR (CDCl₃) δ 5.96 - 5.92 (m, 1H), 5.85 - 5.82 (m, 1H), 4.88 - 4.85 (m, 1H), 3.87 (dd, *J* = 10.0, 4.8 Hz, 1H), 3.79 (dd, *J* = 10.3, 7.5 Hz, 1H), 2.97 (d, *J* = 5.5 Hz, 1H), 2.49 - 2.41 (m, 1H), 2.38 - 2.31 (m, 1H), 2.22 - 2.15 (m, 1H), 0.89 (s, 9H), 0.08 (d, *J* = 3.0 Hz, 6H).

Step 4: (1*S*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent-2-en-1-yl 4-nitrobenzoate

To a mixture of (1*R*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent-2-en-1-ol (18.5 g, 81.0 mmol) (Prepared following synthetic procedures in WO 2013028832), dimethylphenylphosphine (14.4 mL, 101 mmol) and 4-nitrobenzoic acid (17.4 g, 104 mmol) in toluene (500 mL) was added DIAD (19.9 mL, 101 mmol) dropwise, and the reaction was stirred at rt for 2 hours. Ethanol (9.46 mL) and EtOAc were added and the mixture was washed with sat. NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and concentrated. The crude mixture was purified by silica gel chromatography (0 to 10% ethyl acetate in DCM/hexanes (5:95)) to provide (1*S*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent-2-en-1-yl 4-nitrobenzoate (20.0 g, 65%). LCMS (AA): *m/z* = 378 (M+H).

Step 5: (1*R*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent-2-en-1-ol To a solution of (1*S*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent-2-en-1-yl 4-nitrobenzoate (20.0 g, 53.0 mmol) in methanol (460 mL) was added potassium carbonate (1.83 g, 13.2 mmol) and the reaction was stirred at rt for 3 h. The reaction was concentrated, and the residue partitioned between ether (250 mL) and water (100 mL). The layers were separated, and the aqueous layer was extracted with ether (100 mL). The ether layers were dried over Na₂SO₄ then concentrated. The crude mixture was purified by silica gel chromatography (0 to 20% ethyl acetate in DCM/hexanes (5:95)) to provide (1*R*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent-2-en-1-ol (9.2 g, 74%). ¹H NMR (CDCl₃) δ 0.07 (s, 6 H), 0.90 (s, 9 H), 1.86 (d, *J* = 5.27 Hz, 1 H), 1.97 (m, 1 H), 2.24 (m, 1 H), 2.53 (m, 1 H), 3.61 (dd, *J* = 9.66, 8.16 Hz, 1 H), 3.73 (dd, *J* = 9.66, 6.15 Hz, 1 H), 4.70 (br s, 1 H), 5.77 (m, 1 H), 5.87 (m, 1 H).

Step 6: (1*R*,2*R*,3*R*,5*S*)-3-([*tert*-butyldimethylsilyl]oxy)methyl)-6-oxabicyclo[3.1.0]hexan-2-ol

To a solution of (1*R*,5*R*)-5-([*tert*-butyldimethylsilyl]oxy)methyl)cyclopent-2-en-1-ol (1.12 g, 4.90 mmol) in DCM (45 mL) was added vanadyl acetylacetonate (26.2 mg, 0.0990 mmol) followed by *tert*-butyl hydroperoxide (5.35 mL, a 5.5 M solution in *n*-decane). The reaction was stirred for 90 min at rt, then concentrated. The crude mixture was purified by silica gel chromatography (0 to 30% ethyl acetate in hexanes) to provide (1*R*,2*R*,3*R*,5*S*)-3-([*tert*-

butyldimethylsilyl]oxy}methyl)-6-oxabicyclo[3.1.0]hexan-2-ol (1.0 g, 84%). (NMR in critchley E1800-34) ¹H NMR (CDCl₃) δ 0.06 (s, 6 H), 0.90 (s, 9 H), 1.53 - 1.57 (m, 1 H), 1.68 - 1.80 (m, 1 H), 2.07 - 2.21 (m, 2 H), 3.46 (d, *J*=2.51 Hz, 1 H), 3.52 - 3.60 (m, 2 H), 3.79 (dd, *J*=9.91, 4.64 Hz, 1 H), 4.09 (br d, *J*=7.53 Hz, 1 H).

Step 7: (1*R*,2*R*,3*R*,4*R*)-4-([*tert*-butyldimethylsilyl]oxy)methyl)-2,3-dihydroxycyclopentyl acetate To a solution of (1*R*,2*R*,3*R*,5*S*)-3-([*tert*-butyldimethylsilyl]oxy)methyl)-6-oxabicyclo[3.1.0]hexan-2-ol (22.0 g, 90.1 mmol) in THF (365 mL) was added sodium acetate (37.9 g, 144 mmol) and tetramethylammonium triacetoxymethylborohydride (14.8 g, 180 mmol). The reaction mixture was heated to reflux for 3 days. The reaction mixture was cooled and poured into sat. NaHCO₃ and stirred for 30 min. Water and ethyl acetate were added, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined ethyl acetate layers were washed with brine, dried over Na₂SO₄, and concentrated. The crude mixture was purified by silica gel chromatography (0 to 60% ethyl acetate in hexanes) to provide (1*R*,2*R*,3*R*,4*R*)-4-([*tert*-butyldimethylsilyl]oxy)methyl)-2,3-dihydroxycyclopentyl acetate (7.6 g, 28%).

Step 8: (3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl acetate To a solution of provide (1*R*,2*R*,3*R*,4*R*)-4-([*tert*-butyldimethylsilyl]oxy)methyl)-2,3-dihydroxycyclopentyl acetate (1.2 g, 3.8 mmol) in acetone (25 mL) was added pyridinium *p*-toluenesulfonate (192 mg, 0.76 mmol) and 2,2-dimethoxypropane (2.34 mL, 19.1 mmol), and the reaction was stirred at rt overnight. The reaction was cooled in an ice bath and basified with sat. NaHCO₃ (10 mL). Water and ethyl acetate were added, and the layers were separated. The aqueous layer was extracted with ethyl acetate twice, and the combined ethyl acetate layers were dried over Na₂SO₄ and concentrated. The crude mixture was purified by silica gel chromatography (0 to 20% ethyl acetate in hexanes) to provide (3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl acetate (406 mg, 30%). ¹H NMR (CDCl₃) δ 0.06 (s, 6 H), 0.91 (s, 9 H), 1.30 (s, 3 H), 1.47 (s, 3 H), 1.65 (m, 1 H), 2.20 - 2.36 (m, 2 H), 3.52 - 3.65 (m, 2 H), 4.47 - 4.55 (m, 2 H), 5.02 - 5.07 (m, 1 H).

Step 9: (3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-ol (I-55) To a solution of (3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl acetate (1.3 g, 3.8 mmol) in MeOH (15 mL) and water (1 mL) was added potassium carbonate (130 mg,

0.94 mmol), and the reaction was stirred at rt for 1 h. The reaction was concentrated, and the residue partitioned between ethyl acetate and brine. The layers were separated, and the aqueous layer extracted with ethyl acetate. The combined ethyl acetate layers were washed with brine, dried over Na₂SO₄ and concentrated to provide (3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-ol (**I-55**, 1.13 g, 98%). ¹H NMR (CDCl₃) δ 0.04 (s, 6 H), 0.84 (s, 9 H), 1.21 (s, 3 H), 1.35 (s, 3 H), 1.48 (br d, *J*=14.18 Hz, 1 H), 2.24 (m, 1 H), 2.35 (m, 1 H), 3.56 (m, 1 H), 3.75 (m, 1 H), 3.99 (m, 1 H), 4.18 (br s, 1 H), 4.32 (d, *J*=5.65 Hz, 1 H), 4.48 (d, *J*=5.52 Hz, 1 H).

Step 10: 4-(((3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)oxy)-6-chloropyrimidine To a solution of (3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-ol **I-55** (800 mg, 2.65 mmol) in dry THF (15 mL) in a dried vial was added LDA (2.64 mL, 2.0 M in THF) followed by 4,6-dichloropyrimidine (493 mg, 3.31 mmol). The reaction was heated in a microwave reactor at 60 °C for 6 h. The reaction was concentrated, and the residue partitioned between ethyl acetate and brine. The ethyl acetate layer was dried over Na₂SO₄ and concentrated. The crude mixture was purified by silica gel chromatography (0 to 30% ethyl acetate in hexanes) to provide 4-(((3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)oxy)-6-chloropyrimidine (928 mg, 85%). LCMS (AA): *m/z* = 415.2 (M+H); ¹H NMR (CDCl₃) δ 0.05 (s, 6 H), 0.89 (s, 9 H), 1.26 (m, 1 H), 1.31 (s, 5 H), 1.49 (s, 3 H), 1.80 (m, 1 H), 2.40 (m, 2 H), 3.60 (m, 2 H), 4.59 (s, 2 H), 5.38 (m, 1 H), 6.72 (s, 1 H), 8.61 (s, 1 H).

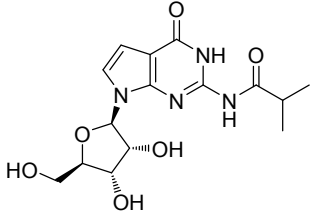
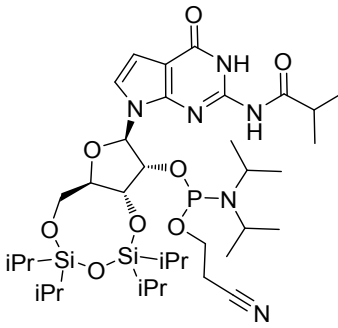
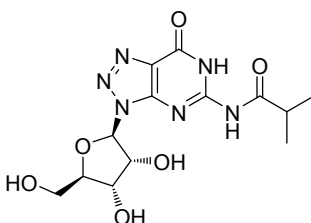
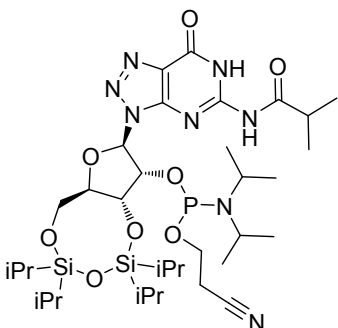
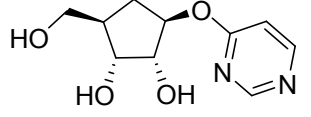
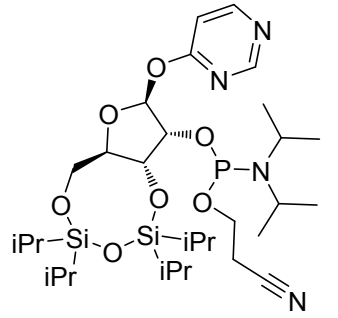
Step 11: (1*R*,2*R*,3*R*,5*R*)-3-((6-chloropyrimidin-4-yl)oxy)-5-(hydroxymethyl)cyclopentane-1,2-diol A solution of 4-(((3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)oxy)-6-chloropyrimidine (700 mg, 1.69 mmol) in HCl solution (8.0 mL, 3 M in MeOH) and TFA (8.0 mL, 106 mmol) was heated in a microwave reactor at 50 °C for 2.5 h. The cooled reaction was concentrated and azeotroped with toluene (3x) and pyridine (3x) to give crude (1*R*,2*R*,3*R*,5*R*)-3-((6-chloropyrimidin-4-yl)oxy)-5-(hydroxymethyl)cyclopentane-1,2-diol (440 mg, 100%) which was used as is in the next reaction.

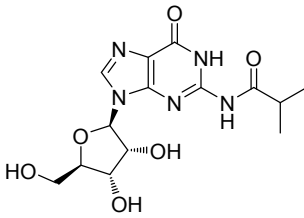
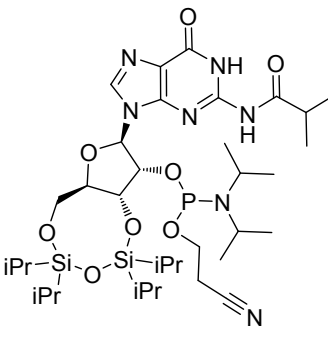
Step 12: (6*aR*,8*R*,9*S*,9*aR*)-8-((6-chloropyrimidin-4-yl)oxy)-2,2,4,4-tetraisopropylhexahydrocyclopenta[f][1,3,5,2,4]trioxadisilocin-9-ol To solution of (1*R*,2*R*,3*R*,5*R*)-3-((6-chloropyrimidin-4-yl)oxy)-5-(hydroxymethyl)cyclopentane-1,2-diol (440 mg, 1.69 mmol) in DMF (13 mL) was

added pyridine (6.9 mL, 84.4 mmol) and 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (666 mg, 2.11 mmol). The reaction was stirred at rt for 24 h. The reaction was taken up in ethyl acetate and washed with brine (3x). The ethyl acetate layer was dried over Na₂SO₄ and concentrated. The crude mixture was purified by silica gel chromatography (0 to 30% ethyl acetate in hexanes) to provide (6a*R*,8*R*,9*S*,9a*R*)-8-((6-chloropyrimidin-4-yl)oxy)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (403 mg, 47%). LCMS (AA): *m/z* = 503.2 (M+H); ¹H NMR (CDCl₃) δ 0.67 - 1.22 (m, 28 H), 1.40 (m, 1 H), 2.17 (m, 1 H), 2.32 (m, 1 H), 3.66 (m, 1 H), 3.89 (m, 1 H), 3.98 (m, 1 H), 4.14 (m, 1 H), 5.14 (br t, *J*=6.02 Hz, 1 H), 6.68 (s, 1 H), 8.48 (s, 1 H).

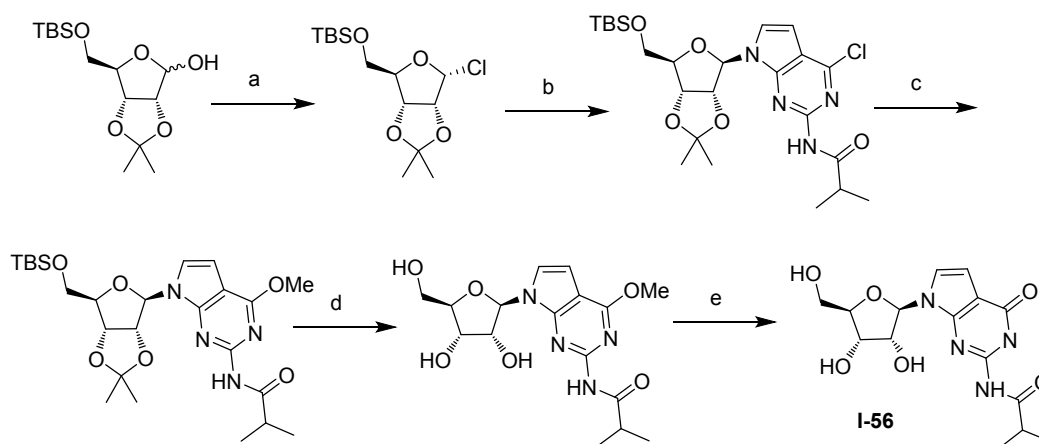
Step 13: (6a*R*,8*R*,9*S*,9a*R*)-8-((6-chloropyrimidin-4-yl)oxy)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl (2-cyanoethyl) diisopropylphosphoramidite (**I-16**) (6a*R*,8*R*,9*S*,9a*R*)-8-((6-chloropyrimidin-4-yl)oxy)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (658 mg, 1.31 mmol) was dissolved in dry toluene and concentrated to dryness (3 x 10 mL). The residue was taken up in DMF (3.0 mL). *N*-Methylimidazole (61.4 mg, 0.785 mmol) and 1*H*-tetrazole (3.50 mL, 0.45 M in ACN) were added. The reaction mixture was cooled to 0 °C, then 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (0.94 mL, 2.94 mmol) was added dropwise. The reaction mixture was then warmed to rt and allowed to stir for 2 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous NaHCO₃ solution (10 mL). The aqueous phase was extracted with EtOAc (2 x 60 mL). The combined organic phases were washed with brine, dried over MgSO₄ and concentrated. This crude compound was purified by silica gel chromatography (0-100% EtOAc in hexanes, with 0.5% TEA) to provide (6a*R*,8*R*,9*S*,9a*R*)-8-((6-chloropyrimidin-4-yl)oxy)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl (2-cyanoethyl) diisopropylphosphoramidite (**I-16**, 484 mg, 53%) as a mixture of diastereomers. ¹H NMR (DMSO-*d*₆) δ 0.81 - 1.25 (m, 40 H), 1.40 (m, 1 H), 2.08 - 2.24 (m, 1 H), 2.40 (m, 1 H), 2.66 - 2.81 (m, 2 H), 3.53 - 3.92 (m, 6 H), 4.12 - 4.23 (m, 2 H), 5.11 - 5.27 (m, 1 H), 6.96 - 7.15 (m, 1 H), 8.62 - 8.73 (m, 1 H); ³¹P NMR (DMSO-*d*₆) δ 149.36 (s, 0.6 P), 150.21 (s, 0.4 P).

Table S-9. The compounds listed below were prepared as described in the synthesis of **I-16** starting with Step 12, substituting the starting material shown in the table for (1*R*,2*R*,3*R*,5*R*)-3-((6-chloro pyrimidin-4-yl)oxy)-5-(hydroxymethyl)cyclopentane-1,2-diol.

Starting material	Intermediate	NMR data
 <p>I-56</p>	 <p>I-36</p>	¹ H NMR (DMSO- <i>d</i> ₆) δ 11.86 (br s, 1H), 11.49 - 11.38 (m, 1H), 7.10 - 7.05 (m, 1H), 6.55 (d, <i>J</i> = 3.7 Hz, 0.5H), 6.52 (d, <i>J</i> = 3.3 Hz, 0.5H), 6.14 - 6.12 (m, 0.5H), 6.09 - 6.06 (m, 0.5H), 4.55 - 4.42 (m, 2H), 4.09 - 4.00 (m, 1H), 3.98 - 3.90 (m, 2H), 3.89 - 3.78 (m, 1H), 3.74 - 3.41 (m, 3H), 2.83 - 2.76 (m, 2H), 2.70 - 2.58 (m, 1H), 1.16-0.82 (m, 46H); ³¹ P NMR (DMSO- <i>d</i> ₆) δ 150.53 (s, 0.5P), 149.27 (s, 0.5P).
 <p>I-57</p>	 <p>I-37</p>	¹ H NMR (DMSO- <i>d</i> ₆) δ 12.47 - 11.75 (m, 2H), 6.13 (s, 0.3H), 6.01 (s, 0.7H), 5.08 (dd, <i>J</i> = 4.6, 8.7 Hz, 0.3H), 5.00 (dd, <i>J</i> = 4.8, 8.8 Hz, 0.7H), 4.89 (dd, <i>J</i> = 4.7, 13.0 Hz, 0.7H), 4.75 (dd, <i>J</i> = 4.7, 9.4 Hz, 0.3H), 4.15 - 4.03 (m, 1H), 4.02 - 3.80 (m, 3H), 3.76 - 3.36 (m, 3H), 2.91 - 2.59 (m, 3H), 1.23 - 0.96 (m, 46H); ³¹ P NMR (DMSO- <i>d</i> ₆) δ 152.20 (s, 0.3P), 149.62 (s, 0.7P)
 <p>I-58</p>	 <p>I-17</p>	¹ H NMR (DMSO- <i>d</i> ₆) δ 8.71 - 8.88 (m, 1 H), 8.71 - 8.88 (m, 1 H), 8.52 (m, 1 H), 6.74 - 7.00 (m, 1 H), 5.12 - 5.32 (m, 1 H), 4.13 - 4.34 (m, 2 H), 3.50 - 3.95 (m, 6 H), 2.67 - 2.82 (m, 2 H), 2.40 (dm, 1 H), 2.12 - 2.28 (m, 1 H), 1.32 - 1.56 (m, 1 H), 0.82 - 1.25 (m, 40 H); ³¹ P NMR (DMSO- <i>d</i> ₆) δ 150.14 (s, 0.3P), 149.43 (s, 0.7P).

	 <p style="text-align: center;">I-59</p>	¹ H NMR (DMSO- <i>d</i> ₆) δ 12.12 (br s, 1H), 11.55 (br d, <i>J</i> = 11.1 Hz, 1H), 8.23 (s, 0.5H), 8.09 (s, 0.5H), 5.95 (s, 0.5H), 5.89 (d, <i>J</i> = 2.1 Hz, 0.5H), 4.78 - 4.66 (m, 1H), 4.58 - 4.49 (m, 1H), 4.11 - 3.49 (m, 9H), 2.84 - 2.76 (m, 2H), 2.72 - 2.57 (m, 1H), 1.19 - 0.90 (m, 46H; ³¹ P NMR (DMSO- <i>d</i> ₆) δ 150.84 (s, 0.5P), 148.98 (s, 0.5P).
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Scheme S-15: Synthesis of I-56



Reagents and conditions: (a) CCl₄, P(NMe₂)₃, toluene, HMPT, -15 °C; (b) KOH, TDA-1, MeCN; (c) NaOMe, MeOH, rt; (d) TFA, water; (e) Me₃SiCl, NaI, MeCN, rt.

Step 1: *tert*-utyl(((3*aR*,4*R*,6*R*,6*aR*)-6-chloro-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methoxy)dimethylsilane To a solution of (3*aR*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (50.0 g, 164 mmol) in toluene (500 mL) was added CCl₄ (50.4 g, 328 mmol) -15°C, then added HMPT (48.1 g, 295 mmol) over 20 min at -15 °C. The reaction mixture was stirred at this temperature for 1 h, then brine (300 mL) was added. The mixture was partitioned, and the organic layer was dried over Na₂SO₄ and

evaporated to give *tert*-butyl(((3*aR*,4*R*,6*R*,6*aR*)-6-chloro-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methoxy)dimethylsilane as colorless oil. This crude product was used directly for next step without purification.

Step 2: *N*-(7-((3*aR*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide

A solution of (3*aR*,6*R*,6*aR*)-6-{[(*tert*-butyldimethylsilyl)oxy]methyl}-2,2-dimethyl-tetrahydro-2*H*-furo[3,4-*d*][1,3]dioxol-4-ol (53.9 g, 167 mmol) in ACN (500 mL) was added to a vigorously stirred suspension of *N*-(4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide (20.0 g, 83.7 mmol), powdered KOH (9.37 g, 167 mmol), and TDA-1 (13.3 mL, 41.8 mmol) in ACN (100 mL). The mixture was stirred for 16 h at rt. The reaction mixture was combined with another reaction carried out on the same scale and was filtered. The organic phase was quenched with sat. NH₄Cl solution (500 mL), and extracted with DCM (1.0 L x 3). The combined organic extracts were dried over Na₂SO₄, filtered, evaporated. The crude residue was purified by silica gel chromatography (petroleum ether:EtOAc, 20:1 to 7:1) to provide *N*-(7-((3*aR*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide (37.5 g, 38.4%) as a yellow oil. LC-MS: 525.4 (M+H). ¹H NMR (DMSO-*d*₆) δ 10.72 - 10.77 (m, 1H), 7.66 - 7.76 (m, 1H), 6.57 - 6.69 (m, 1H), 6.21 - 6.26 (m, 1H), 5.42 - 5.48 (m, 1H), 5.25 - 5.31 (m, 1H), 4.73 - 4.76 (m, 1H), 4.37 - 4.41 (m, 1H), 4.16 - 4.20 (m, 1H), 4.08 - 4.14 (m, 1H), 3.62 - 3.71 (m, 2H), 2.75 - 2.85 (m, 1H), 1.51 - 1.57 (m, 3H), 1.35 (s, 3H), 1.09 - 1.14 (m, 6H).

Step 3: *N*-(7-((3*aR*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide

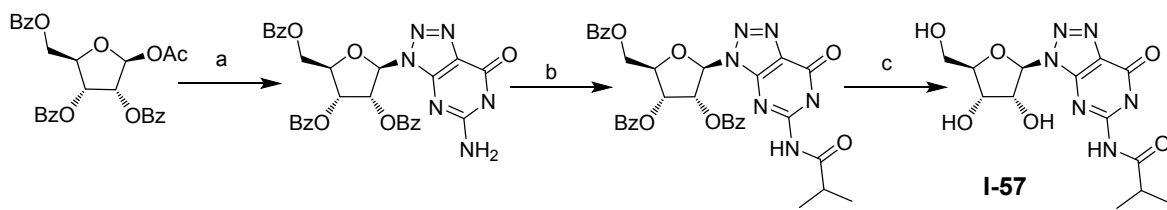
To a mixture of *N*-(7-((3*aR*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide (47.5 g, 90.4 mmol) in MeOH (80 mL) was added NaOMe (24.3 g, 135 mmol, 30% in MeOH) and the reaction mixture was allowed to stir at rt for 3 h. The reaction mixture was adjusted to pH7 with the addition of aqueous HCl (1.0 *N*). Water (60 mL) was added and the mixture was extracted with DCM (600 mL x 3). The combined organic phases were dried over Na₂SO₄, filtered and concentrated. The crude residue was purified by silica gel chromatography (petroleum ether:EtOAc, 20:1 to 4:1) to provide *N*-(7-((3*aR*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)

methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide as colorless oil (37.0 g, 55%). LC-MS: 521.6 (M+H).

Step 4: *N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide *N*-[7-[(3*aR*,4*R*,6*R*,6*aR*)-6-[[*tert*-butyl(dimethyl)silyl]oxymethyl]-2,2-dimethyl-3*a*,4,6,6*a*-tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]-4-methoxy-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2-methyl-propanamide (37.0 g, 49.7 mmol) was added to TFA/H₂O (9:1, 150 mL) at rt. The solution was allowed to stir for 30 min. The volatiles were removed in vacuo, and the residue co-evaporated several times with MeOH. The residue was purified by silica gel chromatography (DCM:MeOH, 20:1 to 5:1) to provide *N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide (18.0 g, 72.5%) as a sticky solid. LC-MS: 366.9 (M+H); ¹H NMR (DMSO-*d*₆) δ 10.22 (s, 1H), 7.48 (d, *J*=3.5 Hz, 1H), 6.49 (d, *J*=3.5 Hz, 1H), 6.07 (d, *J*=6.4 Hz, 1H), 4.40 (t, *J*=5.7 Hz, 1H), 4.10 (dd, *J*=4.9, 3.0 Hz, 1H), 4.03 (s, 3H), 3.87 (br d, *J*=3.1 Hz, 1H), 3.55 (m 2H), 2.80 - 2.92 (m, 1H), 1.06 - 1.12 (m, 6H).

Step 5: *N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide (**I-56**) To a solution of *N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide (18.0 g, 36.0 mmol) in ACN (180 mL) was added NaI (8.09 g, 54.0 mmol) and Me₃SiCl (6.06 g, 55.8 mmol). The mixture was allowed to stir at rt for 16h. The reaction mixture was concentrated and purified by prep-HPLC to provide *N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)isobutyramide as a yellow solid (**I-56**, 5.34 g, 40.9%). ¹H NMR (MeOD) δ 7.29 (d, *J*=3.8 Hz, 1H), 6.62 (d, *J*=3.8 Hz, 1H), 6.16 (d, *J*=5.5 Hz, 1H), 4.41 (t, *J*=5.4 Hz, 1H), 4.28 (dd, *J*=5.1, 4.1 Hz, 1H), 4.03 (q, *J*=3.7 Hz, 1H), 3.80 - 3.86 (m, 1H), 3.71 - 3.78 (m, 1H), 2.67 - 2.78 (m, 1H), 1.24 (d, *J*=6.8 Hz, 6H).

Scheme S-16: Synthesis of I-57



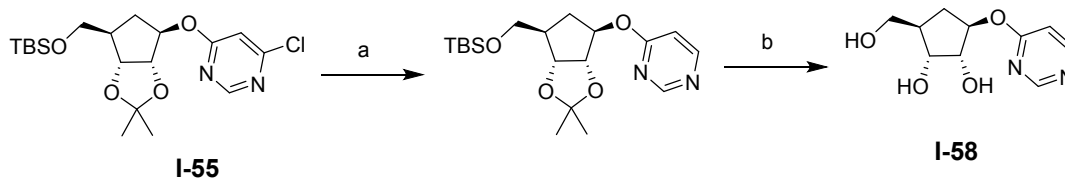
Reagents and conditions: (a) 8-azaguanine, *N,O*-bis(trimethylsilyl)acetamide, trimethylsilyl trifluoromethanesulfonate, MeCN; (b) isobutyric anhydride, 120 °C; (c) NaOH, MeOH, 0 °C.

Step 1: [(2*R*,3*R*,4*R*,5*R*)-5-(5-amino-7-oxo-6*H*-triazolo[4,5-*d*]pyrimidin-3-yl)-3,4-dibenzoyloxy-tetrahydrofuran-2-yl]methyl benzoate To a suspension of 8-azaguanine (980 mg, 6.44 mmol) in dry ACN (27.0 mL) under argon was added *N,O*-bis(trimethylsilyl)acetamide (3.93 g, 19.3 mmol), [(2*R*,3*R*,4*R*,5*S*)-5-acetoxy-3,4-dibenzoyloxy-tetrahydrofuran-2-yl]methyl benzoate (3.56 g, 7.06 mmol) and trimethylsilyl trifluoromethanesulfonate (2.86 g, 12.9 mmol) sequentially. The reaction mixture was allowed to stir at rt for 15 min and then heated at 80 °C for 1 h. The reaction mixture was allowed to cool to rt and the solvents were evaporated. The residue was partitioned between EtOAc and sat. NaHCO₃ solution and the phases were separated. The organic phase was washed with water and brine, dried over Na₂SO₄, filtered and concentrated. The residue was adsorbed onto Celite. The crude mixture was purified by silica gel chromatography (0-7% MeOH in DCM) to provide [(2*R*,3*R*,4*R*,5*R*)-5-(5-amino-7-oxo-6*H*-triazolo[4,5-*d*]pyrimidin-3-yl)-3,4-dibenzoyloxy-tetrahydrofuran-2-yl]methyl benzoate as a yellow solid (2.30 g, 57%). LCMS (AA): *m/z* = 597.2 (M+H).

Step 2: [(2*R*,3*R*,4*R*,5*R*)-3,4-dibenzoyloxy-5-[5-(2-methylpropanoylamino)-7-oxo-6*H*-triazolo[4,5-*d*]pyrimidin-3-yl]tetrahydrofuran-2-yl]methyl benzoate [(2*R*,3*R*,4*R*,5*R*)-5-(5-amino-7-oxo-6*H*-triazolo[4,5-*d*]pyrimidin-3-yl)-3,4-dibenzoyloxy-tetrahydrofuran-2-yl]methyl benzoate (2.30 g, 3.70 mmol) was concentrated from dry toluene (3 x 20 mL), then isobutyric anhydride (1.02 mL, 73 mmol) was added and the reaction mixture was heated at 120 °C for 16 h. Additional isobutyric anhydride (6.10 mL, 37 mmol) was added and heating continued for 4 h. After the mixture was cooled to rt, EtOAc was added and the mixture was washed with 1:1 water / brine, sat. NaHCO₃ and then brine. Organic phases were dried over Na₂SO₄, filtered and concentrated. The crude mixture was purified by silica gel chromatography (0-60% EtOAc in hexanes) to provide [(2*R*,3*R*,4*R*,5*R*)-3,4-dibenzoyloxy-5-[5-(2-methylpropanoylamino)-7-oxo-6*H*-triazolo[4,5-*d*]pyrimidin-3-yl]tetrahydrofuran-2-yl]methyl benzoate as a yellow foam (2.29 g, 89%). LCMS (AA): *m/z* = 667.2 (M+H).

Step 3: *N*-[3-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]-7-oxo-6*H*-triazolo[4,5-*d*]pyrimidin-5-yl]-2-methyl-propanamide (**I-57**) [(2*R*,3*R*,4*R*,5*R*)-3,4-dibenzoyloxy-5-[5-(2-methylpropanoylamino)-7-oxo-6*H*-triazolo[4,5-*d*]pyrimidin-3-yl]tetrahydrofuran-2-yl] methyl benzoate (4.70 g, 7.0 mmol) was dissolved in THF (32 mL) and MeOH (25 mL) then the reaction mixture was cooled to 0 °C. NaOH solution (1.0 *N*, 25 mL, 25 mmol) was added and the reaction mixture was allowed to stir at 0 °C for 30 min. The reaction mixture was concentrated, and further concentrated from toluene (3x) then adsorbed onto Celite. The crude mixture was purified by reverse phase flash column chromatography (0-100% ACN in aqueous ammonium acetate (10 mM)) to provide *N*-[3-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]-7-oxo-6*H*-triazolo[4,5-*d*]pyrimidin-5-yl]-2-methyl-propanamide as a white solid (**I-57**, 2.14 g, 86%). LCMS (AA): *m/z* = 355.1 (*M*+*H*). ¹H NMR (DMSO-*d*₆) δ 11.45 (br s, 1H), 5.97 (d, *J* = 4.5 Hz, 1H), 5.59 (br d, *J* = 5.6 Hz, 1H), 5.22 (br d, *J* = 5.1 Hz, 1H), 4.84 - 4.72 (m, 2H), 4.28 (q, *J* = 4.7 Hz, 1H), 3.96 (q, *J* = 4.8 Hz, 1H), 3.63 - 3.54 (m, 1H), 3.46 (td, *J* = 5.8, 11.8 Hz, 1H), 2.78 (td, *J* = 6.8, 13.8 Hz, 1H), 1.12 (d, *J* = 6.8 Hz, 6H).

Scheme S-17: Synthesis of I-58



Reagents and conditions: (a) Pd(OH)₂-C, H₂, EtOAc; (b) TFA, 3 *N* HCl in MeOH, 50 °C.

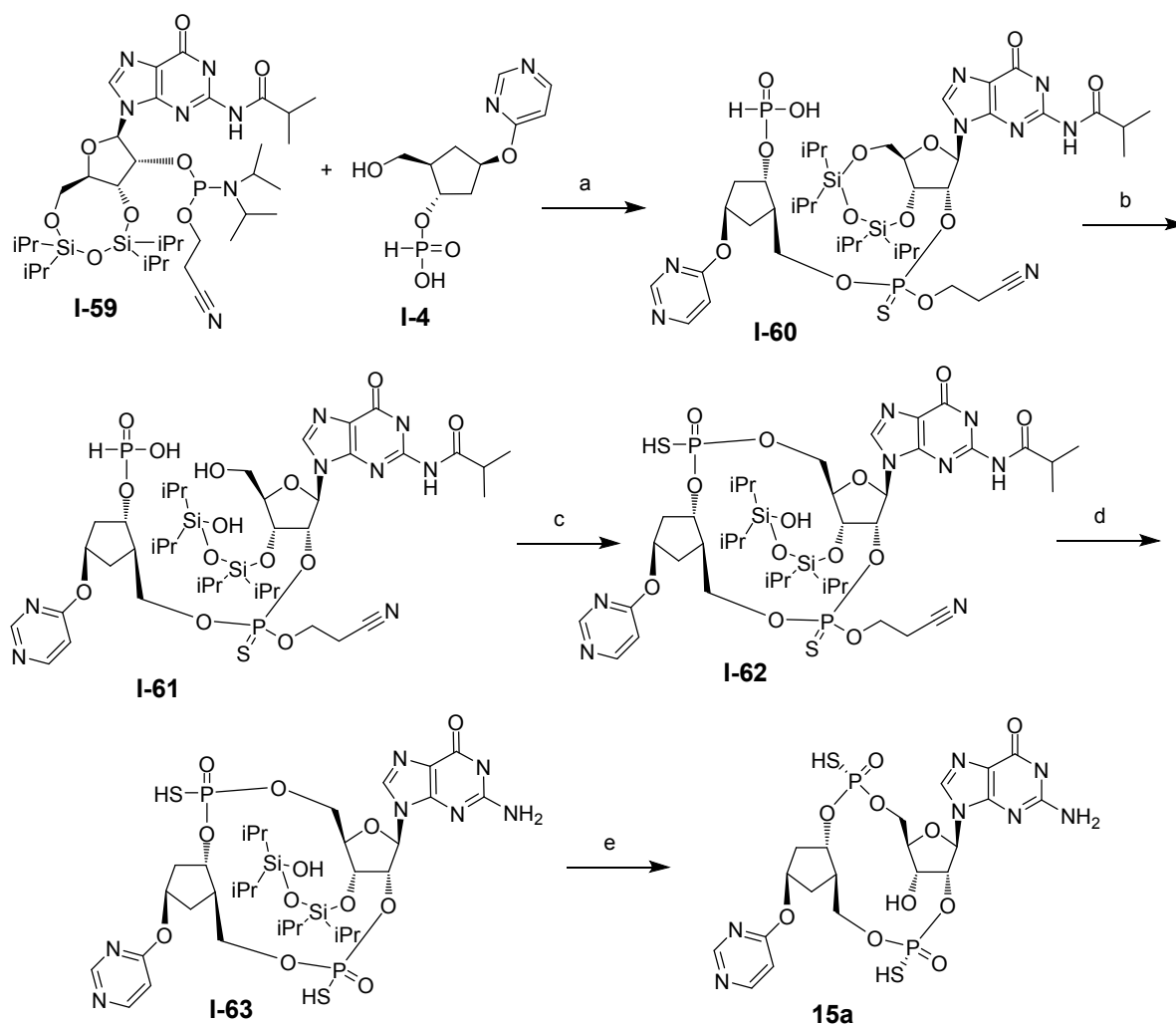
Step 1: 4-(((3a*S*,4*R*,6*R*,6a*R*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)oxy)pyrimidine To a solution of 4-(((3a*S*,4*R*,6*R*,6a*R*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)oxy)-6-chloropyrimidine **I-55** (1.86 g, 4.47 mmol) in ethyl acetate (40 mL) was added triethylamine (1.87 mL, 13.4 mmol) and Pd(OH)₂ (237 mg, 20% on charcoal). The reaction was stirred under a hydrogen filled balloon for 24 h. More

Pd(OH)₂ (200 mg, 20% on charcoal) was added and the reaction was stirred under a hydrogen balloon for another 24 h. The catalyst was filtered through a Celite bed and washed with methanol. The filtrate was concentrated, and the residue partitioned between ethyl acetate and water. The ethyl acetate layer was washed with water (2x), dried over Na₂SO₄ and concentrated. The crude mixture was purified by silica gel chromatography (0 to 30% ethyl acetate in hexanes) to provide 4-(((3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)oxy)pyrimidine (972 mg, 57%). LCMS (AA): *m/z* = 381.3 (M+H); ¹H NMR (CHLOROFORM-*d*, 400 MHz) δ 8.58 (s, 1H), 6.69 (s, 1H), 5.3-5.4 (m, 1H), 4.56 (s, 2H), 3.5-3.7 (m, 2H), 2.3-2.4 (m, 2H), 1.78 (br d, 1H, *J*=11.2 Hz), 1.46 (s, 3H), 1.2-1.3 (m, 4H), 0.8-0.9 (m, 11H), 0.03 (s, 6H).

Step 2: (1*R*,2*R*,3*R*,5*R*)-3-((6-chloropyrimidin-4-yl)oxy)-5-(hydroxymethyl)cyclopentane-1,2-diol (I-58) A solution of 4-(((3*aS*,4*R*,6*R*,6*aR*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl tetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)oxy)pyrimidine (1.04 g, 2.73 mmol) in HCl solution (15 mL, 3 M in MeOH) and TFA (15 mL, 198 mmol) was heated in a microwave reactor at 50 °C for 2.5 h. The cooled reaction was concentrated. THF (6 mL) and HCl solution (15 mL, 3 M in MeOH) were added and the reaction was heated again in a microwave reactor at 50 °C for 30 minutes. The cooled reaction was concentrated and azeotroped with toluene (3x) and pyridine (3x) to give (1*R*,2*R*,3*R*,5*R*)-3-((6-chloropyrimidin-4-yl)oxy)-5-(hydroxymethyl) cyclopentane-1,2-diol **I-58** which was used for further elaboration without purification. LCMS (AA): *m/z* = 227.1 (M+H).

Scheme S-18: Alternative synthesis of compound 15a

2-amino-9-[(2*R*,5*R*,7*R*,8*R*,10*R*,12*aR*,14*R*,15*aS*,16*R*)-16-hydroxy-2,10-dioxido-14-(pyrimidin-4-yloxy)-2,10-disulfanyldecahydro-5,8-methanocyclopenta[1][1,3,6,9,11,2,10]pen-taoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (15a)



Reagents and conditions: (a) *i*) 4,5-dicyanoimidazole, DCE, DMF; *ii*) bis(phenylacetyl)disulfide; (b) TFA, THF, water, 0 °C; (c) *i*) 2-chloro-5,5-dimethyl-1,2,3-dioxaphosphorinane 2-oxide, pyridine; *ii*) H₂O, 3*H*-1,2-benzodithiol-3-one 1,1-dioxide; (d) MeNH₂, EtOH; (e) 3HF-Et₃N, pyridine, Et₃N, 50 °C.

Step 1: (1*S*,2*R*,4*R*)-2-([(*R*)-(2-cyanoethoxy)((6*aR*,8*R*,9*R*,9*aR*)-8-[2-(isobutyrylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-9-yl]oxy)phosphorothioyl]oxy)methyl)-4-(pyrimidin-4-yloxy)cyclopentyl hydrogen phosphonate or

(1*S*,2*R*,4*R*)-2-((*S*)-(2-cyanoethoxy)((6*aR*,8*R*,9*R*,9*aR*)-8-[2-(isobutrylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-9-yl}oxy)phosphorothioyl]oxy)methyl)-4-(pyrimidin-4-yloxy)cyclopentyl hydrogen phosphonate, I-60

A mixture of **I-4** ammonium salt (3.40 g, 11.7 mmol) and **I-59** (13.0 g, 16.4 mmol) were dissolved in dry ACN and concentrated (3 x 100mL). This was repeated with dry toluene (100 mL) and the mixture was finally dried under vacuum for 30 min. DMF (19 mL) and DCE (77 mL) were added to provide a suspension. In a separate flask 4,5-dicyanoimidazole (2.90 g, 24.5 mmol) was dissolved in dry ACN and concentrated to dryness (2 x 50 mL); this was repeated with dry toluene (50 mL) and the resulting mixture was then dissolved in DMF (4.4 mL) and DCE (17.6 mL) and added to the reaction mixture under an atmosphere of nitrogen. The reaction mixture was allowed to stir at rt for 3 h. Bis(phenylacetyl)disulfide (3.89 g, 12.9 mmol) was added as a solid and the reaction mixture was allowed to stir for 90 min. The DCE was evaporated and the mixture was diluted with EtOAc (240 mL), THF (120 mL) and 1:1 aq. sat. NaHCO₃ solution/ water (120 mL). The organic phase was separated and to the aqueous phase was added EtOAc (240 mL), THF (120 mL), and brine (40 mL). The organic phase was separated, and the aqueous phase was extracted with EtOAc (250 mL). The combined organic phases were washed with brine (60 mL), dried over Na₂SO₄ and concentrated. A mixture of methanol and DCM (10% MeOH, 100 mL) was added to the oily mixture and the precipitate was filtered and washed with DCM (5 x 5 mL). The filtrate was evaporated, and the crude product was adsorbed onto Celite and purified by silica gel chromatography in two portions (5-40% MeOH in DCM) to give **I-61** as the first eluting peak (4.95, 40%) LCMS (AA): *m/z* = 1001.1 (M+H).

Step 2: (1*S*,2*R*,4*R*)-2-((*R*)-(2-cyanoethoxy)((2*R*,3*R*,4*R*,5*R*)-5-(hydroxymethyl)-4-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2-[2-(isobutrylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]tetrahydrofuran-3-yl}oxy)phosphorothioyl]oxy)methyl)-4-(pyrimidin-4-yloxy)cyclopentyl hydrogen phosphonate or

(1*S*,2*R*,4*R*)-2-((*S*)-(2-cyanoethoxy)((2*R*,3*R*,4*R*,5*R*)-5-(hydroxymethyl)-4-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2-[2-(isobutrylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]tetrahydrofuran-3-yl}oxy)phosphorothioyl]oxy)methyl)-4-(pyrimidin-4-yloxy)cyclopentyl hydrogen phosphonate (I-61)

I-60 (4.95 g, 4.94 mmol) was taken up in THF (55.0 mL) and water (14.0 mL) and cooled to 0 °C. TFA (14.0 mL, 180 mmol) was added drop-wise and the reaction mixture was allowed to stir at 0 °C for 2 h. Sodium bicarbonate (22.5 g, 267.0 mmol) was added portion-wise, followed by water

(200 mL) and EtOAc (200 mL). The reaction mixture was allowed to warm to rt and extracted with EtOAc (2 × 250 mL). The combined organic phases were washed with brine (85 mL), dried with Na₂SO₄ and concentrated. The crude compound was purified by silica gel chromatography (5-40% MeOH in DCM) to provide **I-61** (4.07 g, 76.7%) as a mixture of diastereomers. LCMS (AA): *m/z* = 1019.2 (M+H).

Step 3: *N*-{9-[(2*S*,5*R*,7*R*,8*R*,10*R*,12*aR*,14*R*,15*aS*,16*R*)-10-(2-cyanoethoxy)-16-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2-oxido-14-(pyrimidin-4-yloxy)-2-sulfanyl-10-sulfidodecahydro-5,8-methanocyclopenta[1][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl}-2-methylpropanamide or

N-{9-[(2*R*,5*R*,7*R*,8*R*,10*R*,12*aR*,14*R*,15*aS*,16*R*)-10-(2-cyanoethoxy)-16-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2-oxido-14-(pyrimidin-4-yloxy)-2-sulfanyl-10-sulfidodecahydro-5,8-methanocyclopenta[1][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl}-2-methylpropanamide or

N-{9-[(2*S*,5*R*,7*R*,8*R*,10*S*,12*aR*,14*R*,15*aS*,16*R*)-10-(2-cyanoethoxy)-16-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2-oxido-14-(pyrimidin-4-yloxy)-2-sulfanyl-10-sulfidodecahydro-5,8-methanocyclopenta[1][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl}-2-methylpropanamide or

N-{9-[(2*R*,5*R*,7*R*,8*R*,10*S*,12*aR*,14*R*,15*aS*,16*R*)-10-(2-cyanoethoxy)-16-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2-oxido-14-(pyrimidin-4-yloxy)-2-sulfanyl-10-sulfidodecahydro-5,8-methanocyclopenta[1][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl}-2-methylpropanamide (**I-62**)

I-61 (5.36g, 5.26 mmol) was dissolved in dry ACN and concentrated to dryness (3 x 30 mL), dried under vacuum for 10 min, and then dissolved in pyridine (105 mL) under a nitrogen atmosphere. 2-Chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (3.40g, 18.4 mmol) was added. The reaction mixture was allowed to stir at rt for 45 min. Water was added (3.32 mL, 184 mmol) followed by 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (1.60 g, 7.89 mmol), and the reaction mixture was allowed to stir at rt under nitrogen for 1 hr 15 min. The mixture was concentrated and diluted with EtOAc (240 mL) and 5% NaHCO₃ (80 mL) then extracted. The phases were separated and the aqueous phase was diluted with brine (25 mL) then extracted with EtOAc (240 mL then 200 mL). The combined organic phases were washed with brine (60 mL) then dried over Na₂SO₄ and

evaporated to give the crude product as a single major diastereomer containing trace amount of a minor diastereomer. The crude material was adsorbed onto Celite and purified by silica gel chromatography (0-10% MeOH in DCM) to give **I-62** (2.01g, 37%). LCMS (AA): $m/z = 1033.2$ (M+H).

Step 4: 2-amino-9-[(2*S*,5*R*,7*R*,8*R*,10*S*,12*aR*,14*R*,15*a**S*,16*R*)-16-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2,10-dioxido-14-(pyrimidin-4-yloxy)-2,10-disulfanyldecahydro-5,8-methanocyclopenta[*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one or**

2-amino-9-[(2*S*,5*R*,7*R*,8*R*,10*R*,12*aR*,14*R*,15*a**S*,16*R*)-16-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2,10-dioxido-14-(pyrimidin-4-yloxy)-2,10-disulfanyldecahydro-5,8-methanocyclopenta[*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one or**

2-amino-9-[(2*R*,5*R*,7*R*,8*R*,10*S*,12*aR*,14*R*,15*a**S*,16*R*)-16-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2,10-dioxido-14-(pyrimidin-4-yloxy)-2,10-disulfanyldecahydro-5,8-methanocyclopenta[*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one or**

2-amino-9-[(2*R*,5*R*,7*R*,8*R*,10*R*,12*aR*,14*R*,15*a**S*,16*R*)-16-[(3-hydroxy-1,1,3,3-tetraisopropylidisiloxanyl)oxy]-2,10-dioxido-14-(pyrimidin-4-yloxy)-2,10-disulfanyldecahydro-5,8-methanocyclopenta[*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (**I-63**)**

I-62 (2.01g, 1.95 mmol) was dissolved in a solution of methylamine (33% in EtOH, 58.4 mL, 469 mmol) under an atmosphere of nitrogen and the reaction mixture was allowed to stir at rt for 3 h. After this time, another portion of methylamine (33% in EtOH, 15.0 mL, 120 mmol) was added and the reaction mixture was allowed to stir at rt for 1 h. The reaction mixture was concentrated and starting material was seen by LCMS. More methylamine (33% in EtOH, 10.0 mL, 80.3 mmol) was added and the reaction mixture was allowed to stir at rt for 1h when another portion of methylamine (33% in EtOH, 10.0 mL, 80.3 mmol) was added and the reaction mixture was allowed to stir for 18h. The reaction mixture was concentrated then placed under vacuum for 10 minutes. The crude material was adsorbed onto Celite and purified by silica gel chromatography (2-40% MeOH in DCM) to give **I-63** (1.07g, 60.4%). LCMS (AA): $m/z = 910.1$ (M+H).

Step 5: 2-amino-9-[(2*R*,5*R*,7*R*,8*R*,10*R*,12*aR*,14*R*,15*a**S*,16*R*)-16-hydroxy-2,10-dioxido-14-(pyrimidin-4-yloxy)-2,10-disulfanyldecahydro-5,8-methanocyclopenta[*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (15a)**

I-63 (553 mg, 0.608 mmol) was taken up in pyridine (3.04 mL, 37.6 mmol) to give a suspension. Triethylamine trihydrofluoride (0.505 mL, 3.04 mmol) was added, followed by TEA (7.60 mL, 54.0 mmol). The reaction mixture was sealed in a propylene tube and allowed to stir at 50 °C overnight. The reaction mixture was diluted with water (11.4 mL, 632 mmol). Then a solution of CaCl₂ (1.05 g, 9.12 mmol) in water (11.4 mL) was added. The cloudy white mixture was allowed to stir at rt for 60 min. The suspension was filtered through Celite, and the Celite was washed with water (7 x 5 mL). The slightly cloudy aqueous filtrate was filtered through celite again then concentrated to a solid residue. No HF was observed by ¹⁹F NMR. The crude compound was adsorbed onto Celite and purified by reverse phase flash column chromatography (0-15% ACN in aqueous triethylammonium acetate (10 mM)) to provide **15a** as the bis-*N,N*-diethylethanamine salt (430 mg, 83.1%). LCMS (AA): *m/z* = 650.0 (M+H). ¹H NMR (D₂O) δ 1.28 (t, *J*=7.34 Hz, 18 H) 1.62 - 1.77 (m, 1 H) 2.26 - 2.42 (m, 1 H) 2.43 - 2.64 (m, 3 H) 3.20 (q, *J*=7.34 Hz, 12 H) 3.82 - 3.97 (m, 1 H) 4.03-4.05 (m, 1 H) 4.14 (br dd, *J*=11.68, 2.75 Hz, 1 H) 4.31 - 4.43 (m, 1 H) 4.48 (br s, 1 H) 4.76 - 4.80 (m, 1 H) 4.96 - 5.10 (m, 1 H) 5.44 (m, 1 H) 5.51 (td, *J*=8.96, 3.97 Hz, 1 H) 6.03 (d, *J*=8.44 Hz, 1 H) 6.80 (d, *J*=5.99 Hz, 1 H) 8.07 (s, 1 H) 8.44 (d, *J*=5.99 Hz, 1 H) 8.68 (s, 1 H). ³¹P NMR (D₂O) δ 54.12 (s, 1P), 52.59 (s, 1P).

Molecular Modeling Methods

Molecular docking of virtual compounds was performed using MOE¹ software. A public crystal structure of STING (R232) has been used as the docking target (PDB code 4KSY). The structure was prepared using the standard protein preparation protocol of MOE. Since the compounds were designed within CDN framework, we employed template docking to position the compounds into ligand binding site of STING. Due to the complexity of the binding site and CDN framework, the protein was treated as a rigid body during docking. To account for any potential flexibility, the docking models were subsequently subjected to conformation search allowing flexibility of the ligand and all protein residues within 5 Å radius. LowModeMD was chosen as the search method along with other default parameters. The resulting top-ranked complex models were utilized to guide rational design of novel STING ligands. All calculations used the AMBER10:EHT forcefield.

References/Notes

1. Molecular Operating Environment (MOE), 2019.0101; Chemical Computing Group
ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC Canada, H3A 2R7, 2021.

Cloning, Expression and Purification of hSTING for Crystallography Analysis

For structure determination, the carboxy terminal domain (CTD) of hSTING (residues 140-379, H232R) was amplified from cDNA by PCR and cloned into the pSX70 vector. The human STING CTD was over-expressed in fusion with an N-terminal 6x poly-histidine tag and TEV cleavage site. Large scale production of recombinant protein was carried out in *E. coli*/BL21 cells utilizing 5 L shake flasks.

Purification of hSTING protein was carried out from cell pellets re-suspended in lysis buffer consisting of 50 mM Tris-HCl (pH 7.6), 200 mM NaCl, 20mM imidazole, 0.25 mM TCEP, 3 Roche Complete tablets, and further lysed via polytron for 2-4 minutes. The lysate was centrifuged at 4200xg for 60 minutes and clarified supernatant was batch bound with 5 ml of Probond Ni resin (Invitrogen). The resin slurry was washed then eluted with buffer containing and additional 200 mM imidazole. Subsequent cleavage of the 6x poly-histidine tag was initiated by the addition of 1 mg of TEV followed by dialysed into buffer containing only 20 mM imidazole. TEV cleavage was confirmed by mass spectroscopy and the sample was incubated with an additional 5 ml of Probond Ni resin.

The resin slurry was removed by centrifugation and the protein sample was further purified by size-exclusion chromatography utilizing a Superdex 200 column equilibrated in 25 mM Tris-HCl (pH 7.9), 150 mM NaCl, 1 mM DTT. Fractions containing the protein of interest were pooled and concentrated to 25 mg/ml utilizing YM10 centricon (Millipore) and flash-frozen in liquid nitrogen for storage at -80°C.

Crystallization, Data Collection and Structure Solution of hSTING

Crystals of hSTING (residues 140-379, H232R) in complex with compounds **14a** and **15a** were obtained by co-crystallography utilizing Takeda California's automated nanovolume crystallization platform. Samples for crystallization were prepared by mixing 50ul of hSTING (15mg/ml) with 1ul of each compound from a 50mM stock solution solubilized in DMSO. Following a 2hr incubation on ice, the samples were centrifugation at 10,000g at 4 °C and subsequently used in co-crystallization trials. STING crystals suitable for data collection were obtained by mixing 50nl protein with 50nl precipitant from a reservoir solution containing 25% PEG 3350, 0.200 M Ammonium Sulfate, 0.1M bis-tris pH 6.5. Crystals selected for data collection were flash frozen in mother liquor containing 25% ethylene glycol with liquid nitrogen in an ALS compatible crystal mounting cassette. Diffraction data were collected from single cryo-cooled crystals at the Advanced Light Source (ALS) beamlines 5.0.2 and 5.0.3. The data reduction was performed using the HKL2000 software package,¹ and the structures were determined by molecular replacement using the programs MOLREP² and PHASER³ from the CCP4 program suite.

Subsequent structure refinement and model re-building was conducted utilizing REFMAC⁴ XtalView⁵ and Coot⁶ software packages. Data collection and refinement statistics are included in Table Supplemental Table P4, and figures were generated using PyMol (<http://www.pymol.org>). The coordinates and structure factors have been deposited in Protein Data Bank with accession codes 7K VX and 7KW1.

Table S-10. Data reduction and refinement statistics for the X-ray structures of STING with compounds **14a** and **15a**.

PDB accession code / Compound	7KVX Compound 14a	7KW1 Compound 15a
Wavelength (Å)	0.98	0.98
Resolution range (Å)	35.78 - 2.48 (2.569 - 2.48)	44.32 - 1.8 (1.864 - 1.8)
Space group	C 1 2 1	C 1 2 1
Unit cell (Å)	89.495 78.539 36.01 90 96.515 90	89.221 78.653 36.026 90 96.573 90
Total reflections	30109 (2986)	78509 (7392)
Unique reflections	8819 (883)	22902 (2241)
Completeness (%)	99.62 (99.55)	99.65 (99.96)
Mean I/sigma(I)	11.18 (2.39)	13.41 (1.72)
Wilson B-factor	58.98	35.17
R-merge	0.08418 (0.4721)	0.05589 (0.6496)
R-meas	0.09974 (0.5645)	0.06618 (0.7767)
R-pim	0.05308 (0.3068)	0.03517 (0.4215)
Reflections used in refinement	8798 (883)	22833 (2241)
R-work	0.1879 (0.3236)	0.1812 (0.2858)
R-free	0.2458 (0.3968)	0.2176 (0.2884)
solvent	46	124
RMS(bonds)	0.013	0.012
RMS(angles)	1.86	1.63
Average B-factor	65.42	46.71

^aR_{sym} = $\sum h \sum j | \langle I(h) \rangle - I(h)j | / \sum h \sum j \langle I(h) \rangle$, where $\langle I(h) \rangle$ is the mean intensity of symmetry-related reflections. ^bR-value = $\sum | |F_{obs}| - |F_{calc}| | / \sum |F_{obs}|$. R_{free} for 5% of reflections excluded from refinement. Values in parentheses are for the highest resolution shell.

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List of abbreviations:

AA	LCMS method using ammonium acetate
Ac	acetate
ACN	acetonitrile
atm	atmosphere
aq	aqueous
9-BBN	9-borabicyclo(3.3.1)nonane
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
<i>t</i> Bu	<i>tert</i> -butyl
Bz	benzoyl
C	Celsius
DBAD	di- <i>tert</i> -butyl azodicarboxylate
DBU	1,8-diazabicyclo[5.4.0]undec-7-enedichloroacetic acid
DCE	dichloroethane
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
DMTr	4,4'-dimethoxytrityl
Et	ethyl
EtOH	ethanol
EtOAc	ethyl acetate
FA	LCMS method using formic acid
h	hours
HMPT	hexamethylphosphorous triamide

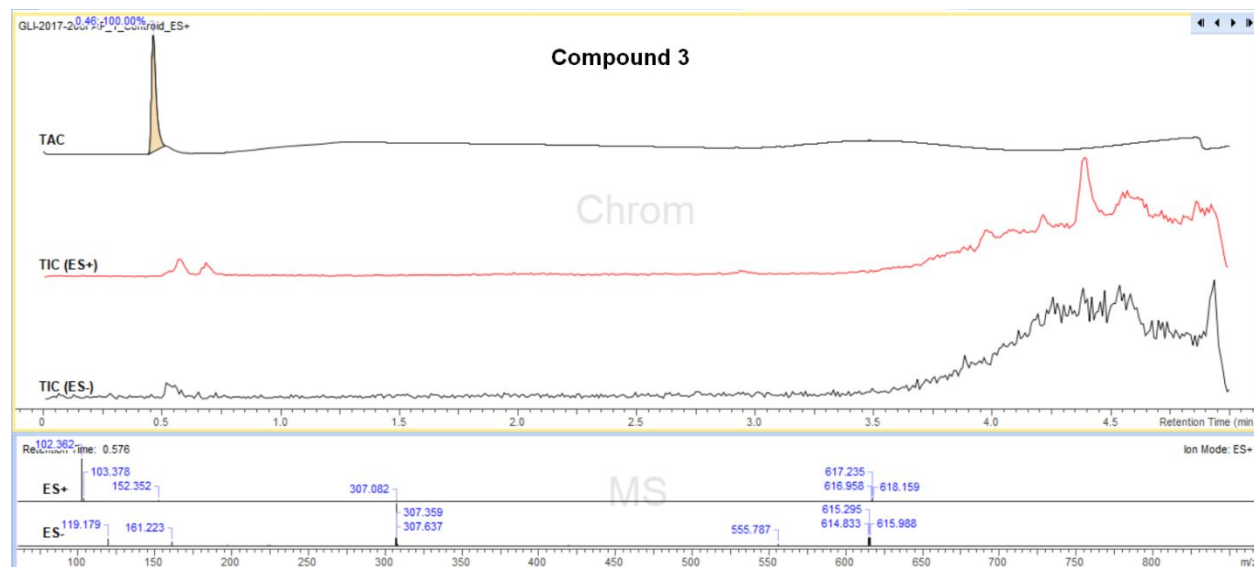
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectrometry
LCMS	liquid chromatography mass spectrometry
LDA	lithium diisopropylamide
<i>m/z</i>	mass to charge
MHz	mega hertz
Me	methyl
MeOH	methanol
min	minutes
mL	milliliters
MS	mass spectrum
NMR	nuclear magnetic resonance
PE	petroleum ether
Ph	phenyl
psi	pounds per square inch
pyr	pyridine
TBAF	tetrabutylammonium fluoride
TBS	<i>tert</i> -butyldimethylsilyl
TDA-1	Tris(3,6-dioxahexyl)amine
TEA	triethylamine
TFA	trifluoroacetic acid
TIDPSi	1,1,3,3-tetraisopropylidisiloxane
TIPS	triisopropylsilyl
THF	tetrahydrofuran
UPLC	ultra performance liquid chromatography

HPLC traces of Key Compounds

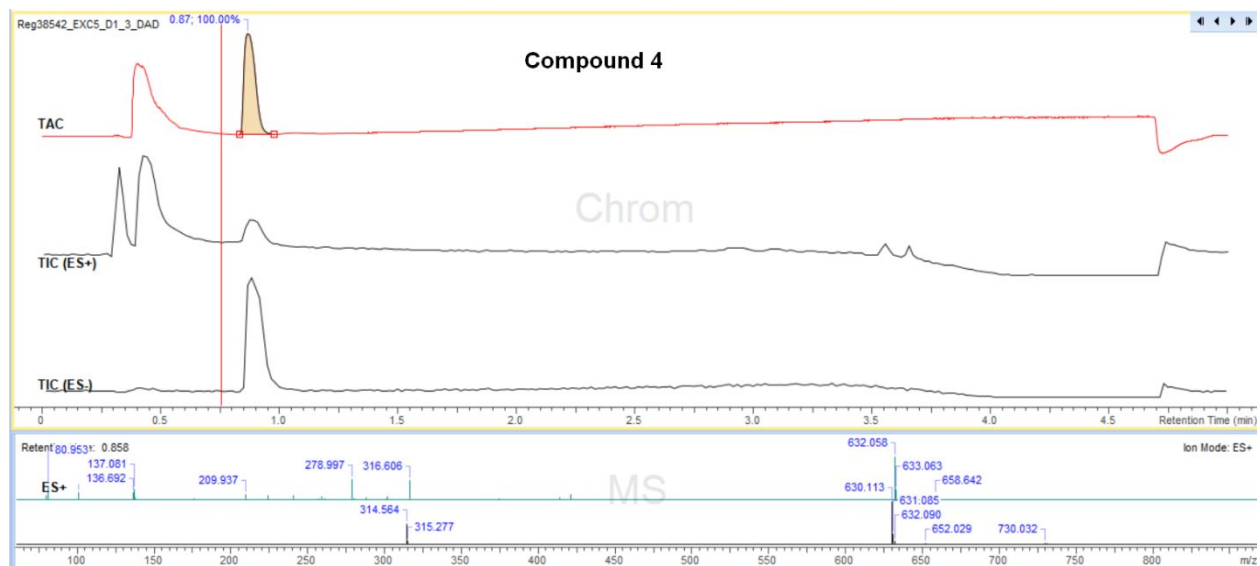
Compound purity was determined by analysis of the diode array UV trace of an LC-MS spectrum using the following procedure: compounds were dissolved in DMSO, methanol, or acetonitrile, and the solutions were analyzed using an Agilent 1290 Infinity UPLC system connected to an Agilent 6130 mass spectrometer, a Waters Acquity UPLC system connected to a Waters Acquity SQ mass spectrometer, or an Agilent 1100 Series HPLC system connected to a Waters Micromass ZQ mass spectrometer using reverse phase C18 columns. Various gradients and run times were selected in order to best characterize the compounds. Mobile phases were based on ACN/water or MeOH/water gradients and contained either 0.1% formic acid (methods indicated as **FA**) or 10 mM ammonium acetate (methods indicated as **AA**). One example of a solvent gradient that was used was 95% mobile phase A (mobile phase A = 99% water + 1% ACN + 0.1% formic acid) to 100% mobile phase B (mobile phase B = 95% ACN + 5% water + 0.1% formic acid) at a flow rate of 0.5 mL/min for a 5 min run.

UPLC Chromatograms

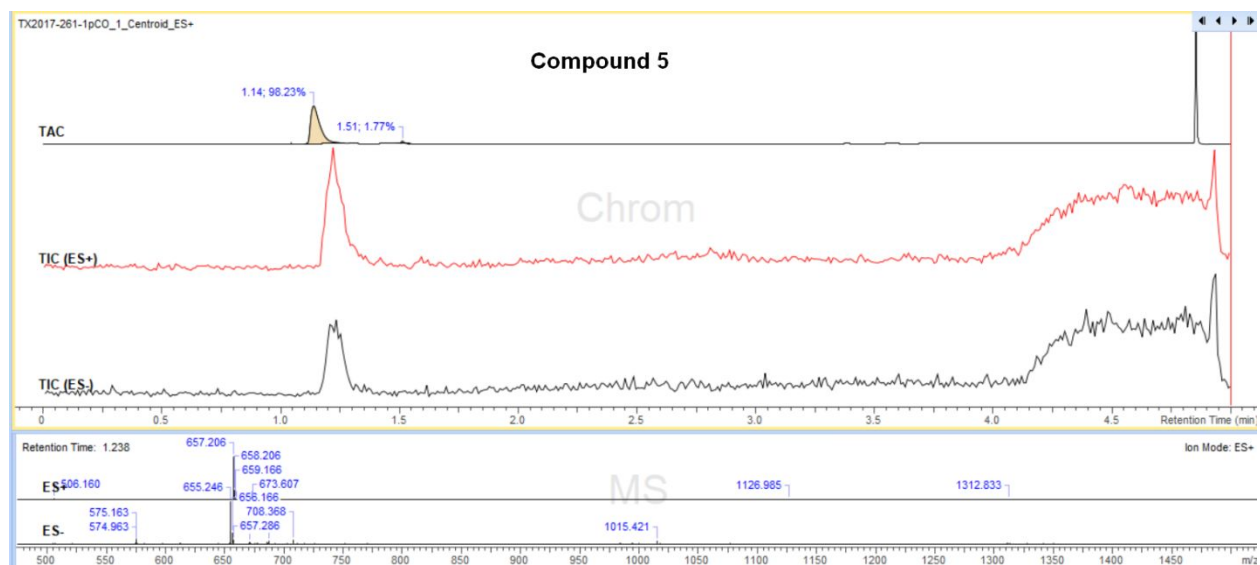
Compound **3**: Method (FA), Purity: 100%



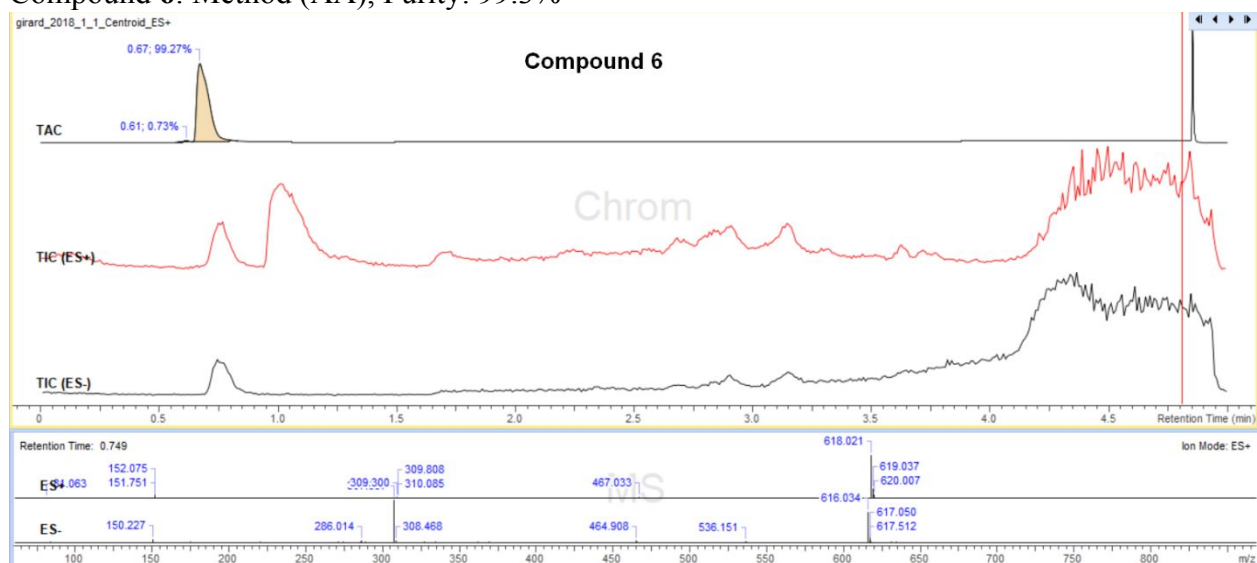
Compound 4: Method (FA), Purity: 100%



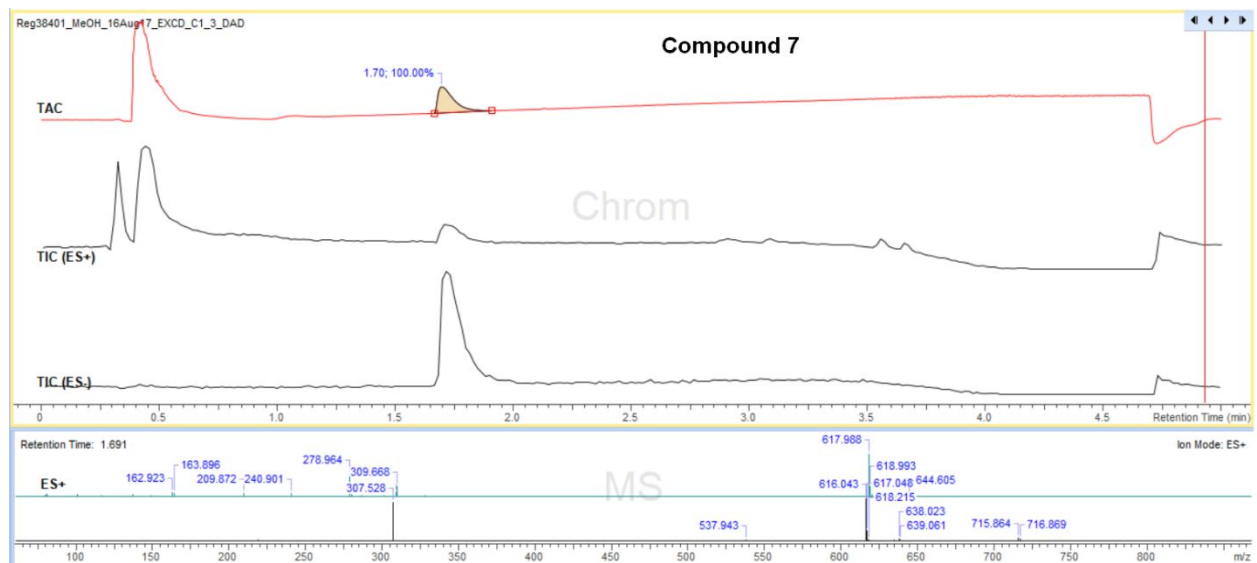
Compound 5: Method (AA), Purity: 98.2%



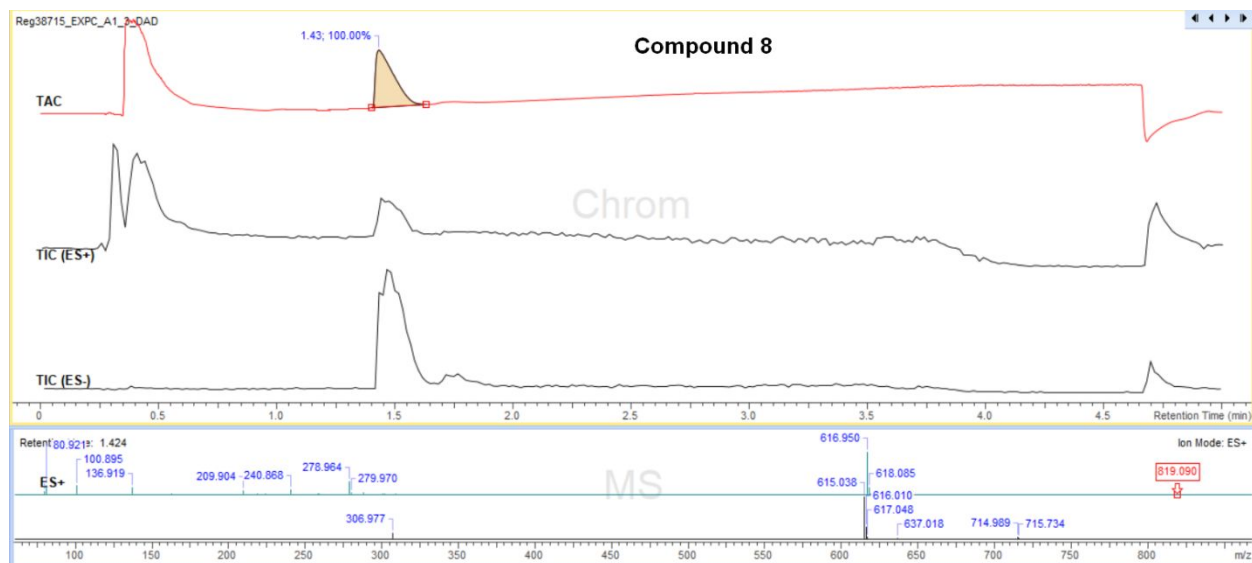
Compound 6: Method (AA), Purity: 99.3%



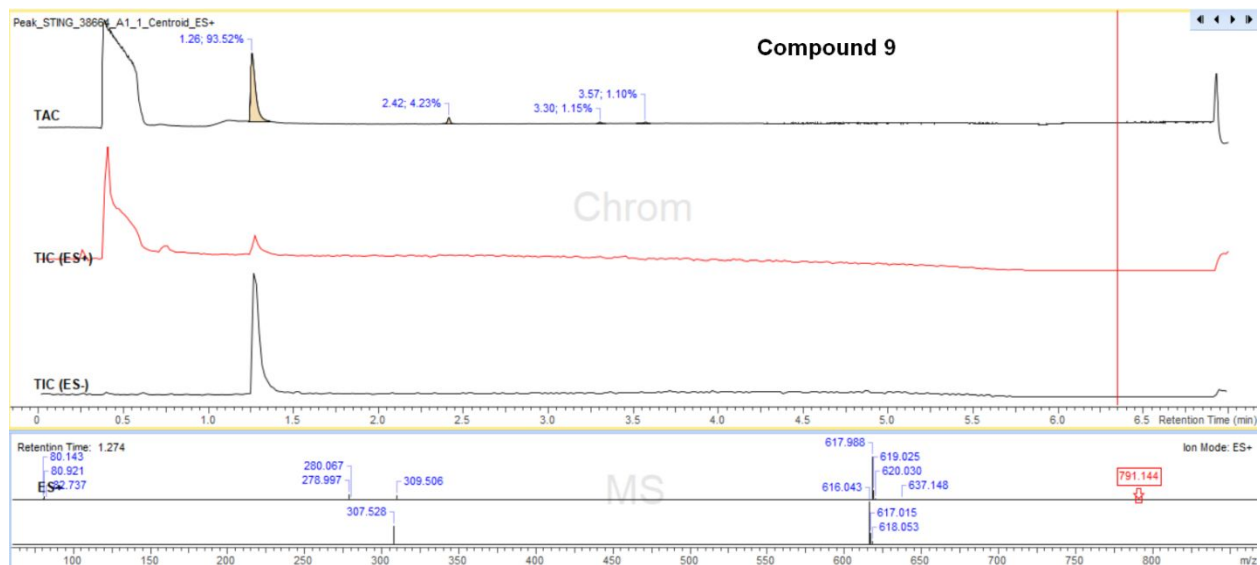
Compound 7: Method (FA), Purity: 100%



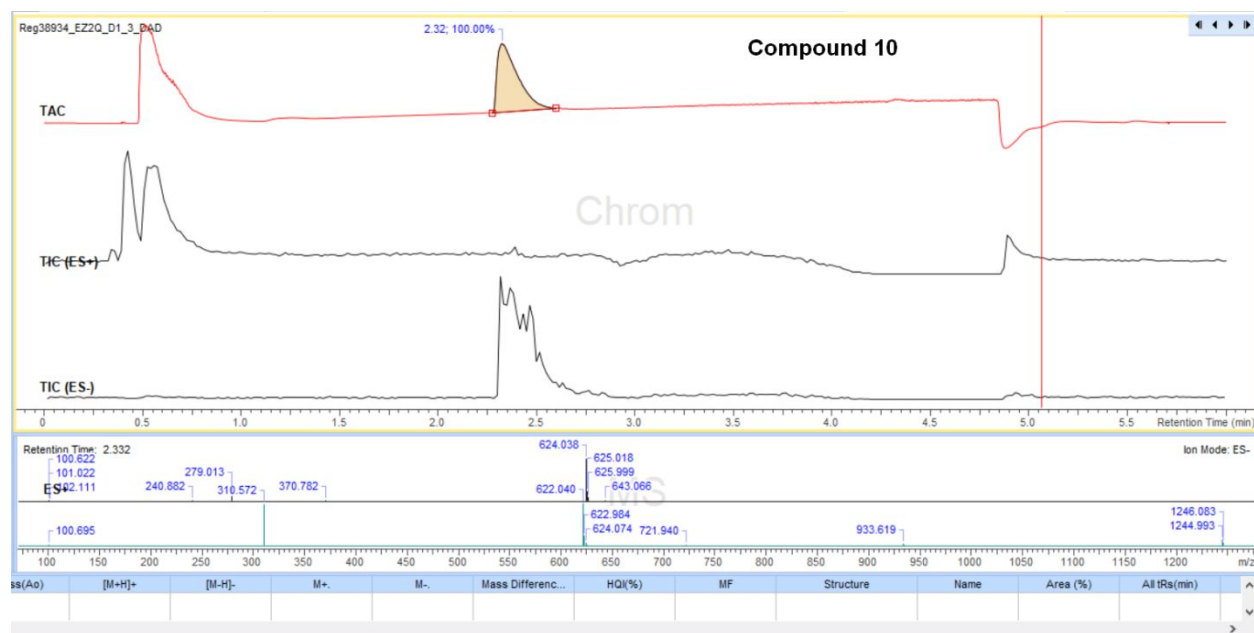
Compound **8**: Method (FA), Purity: 100%



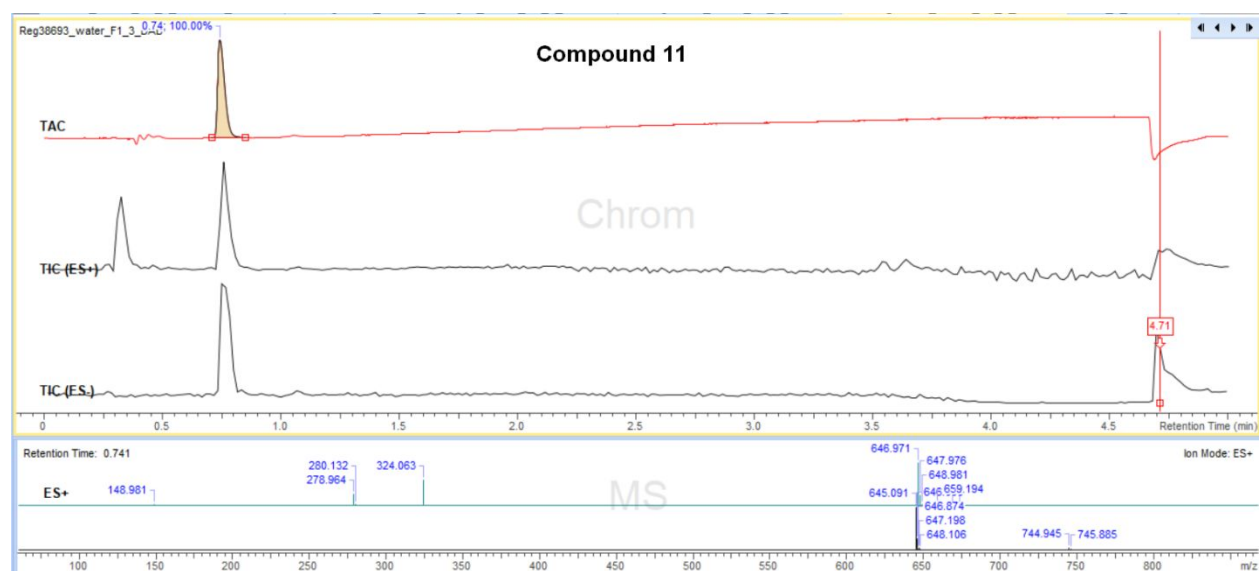
Compound **9**: Method (FA), Purity: 93.5%



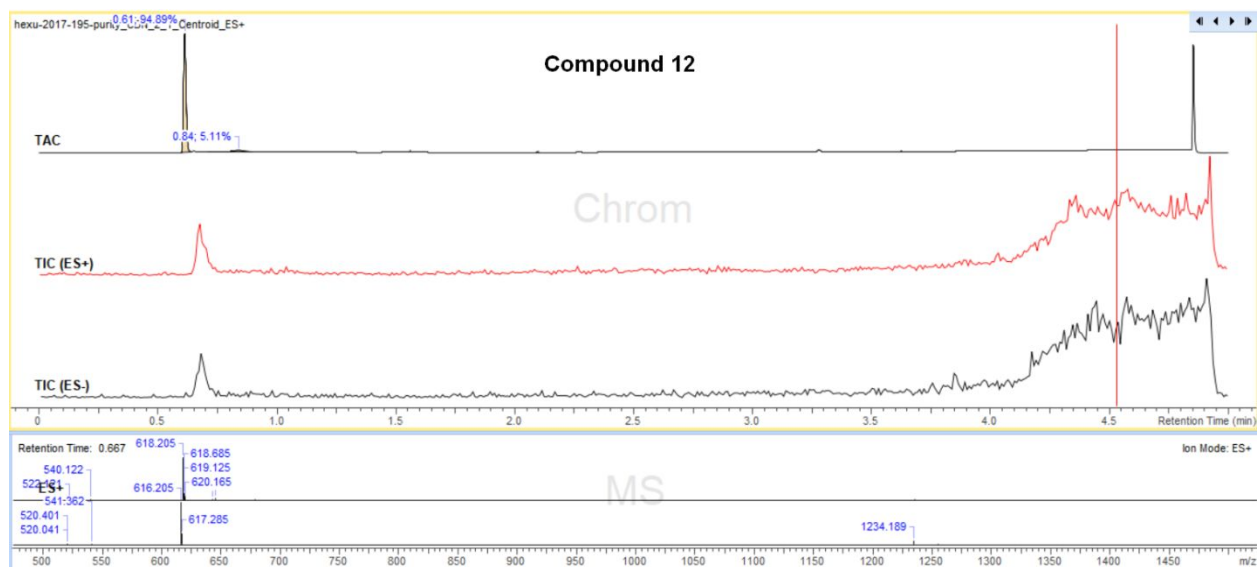
Compound **10**: Method (FA), Purity: 100%



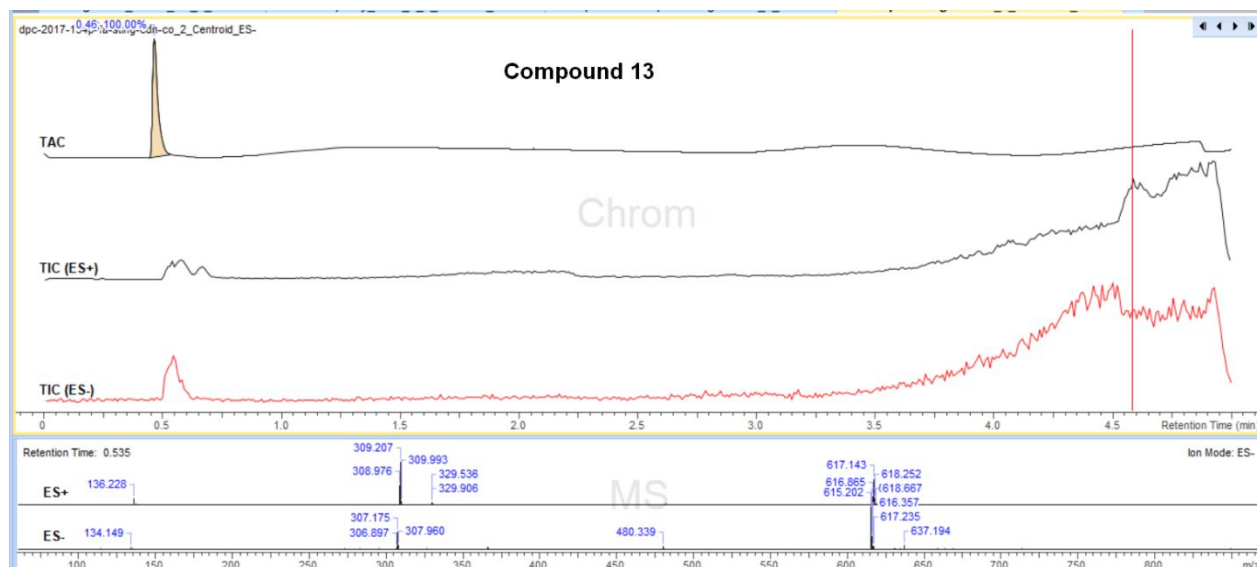
Compound **11**: Method (FA), Purity: 100%



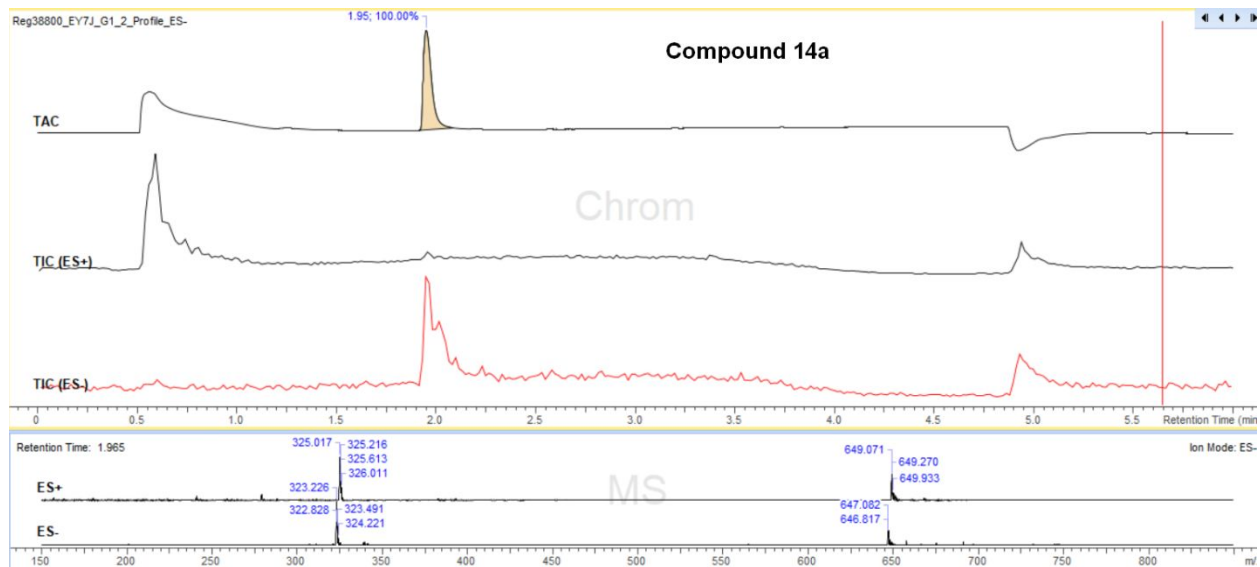
Compound **12**: Method (AA), Purity: 94.9%



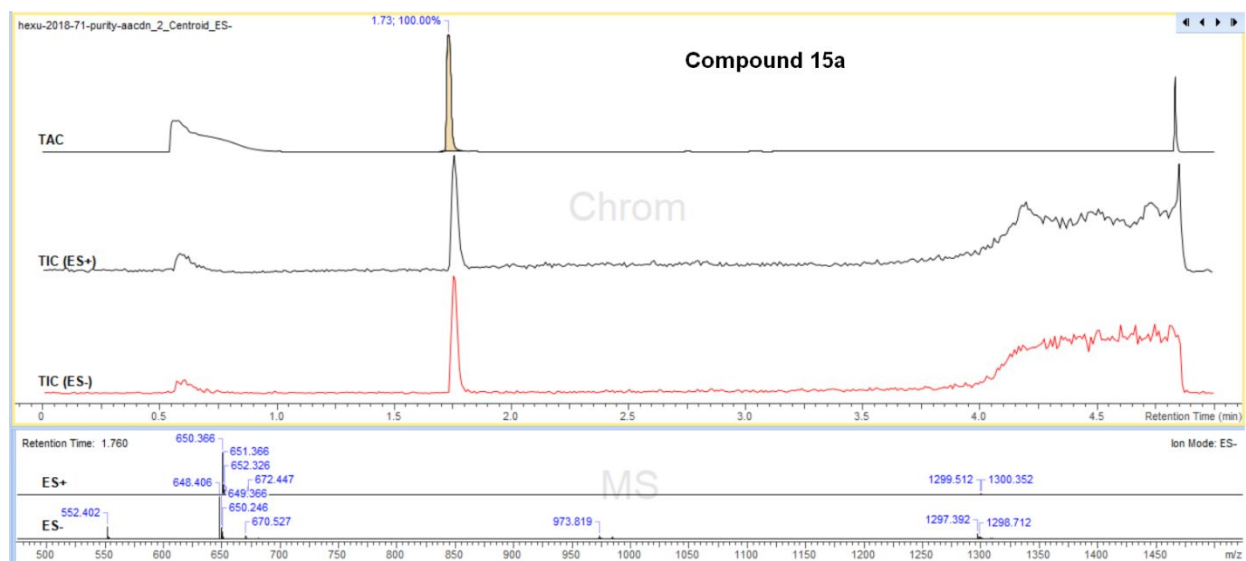
Compound **13**: Method (FA), Purity: 100%



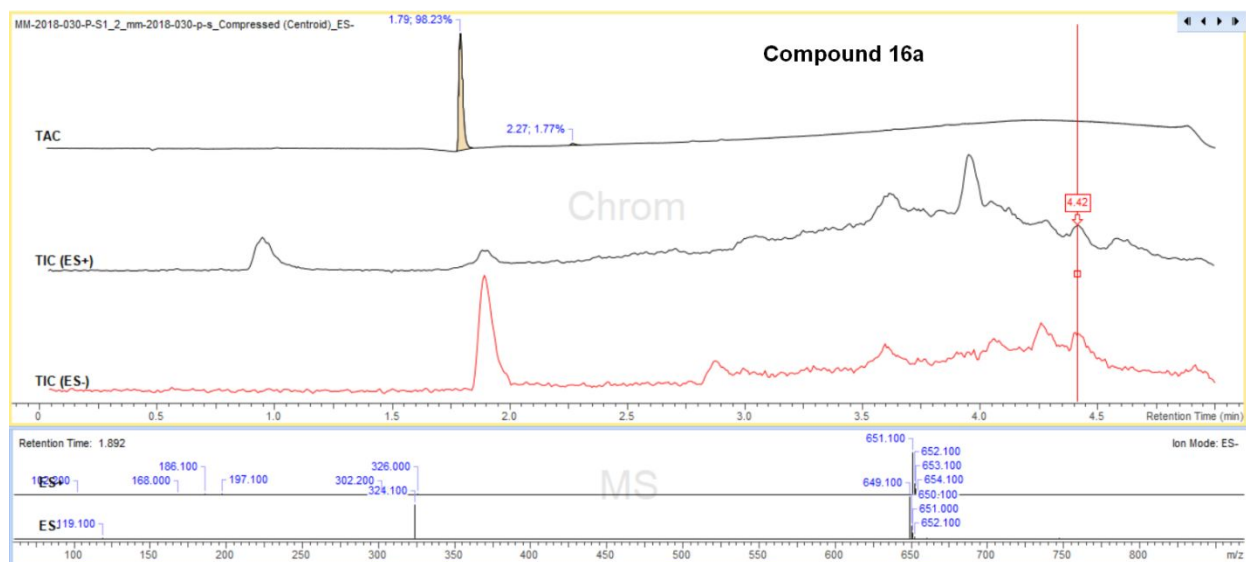
Compound **14a**: Method (FA), Purity: 100%



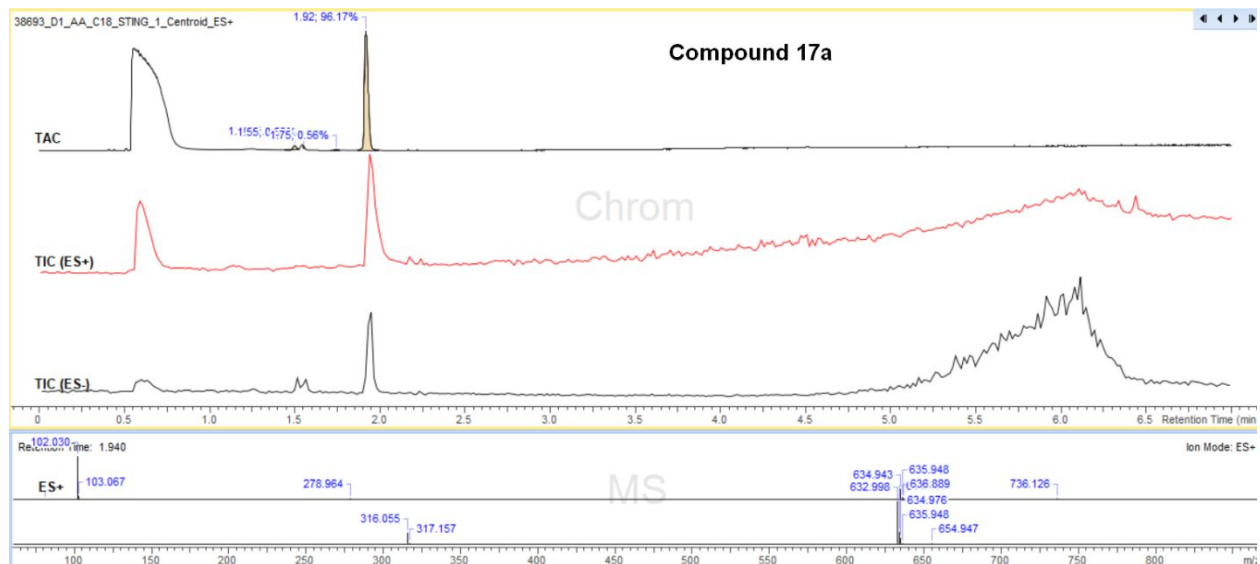
Compound **15a**: Method (AA), Purity: 100%



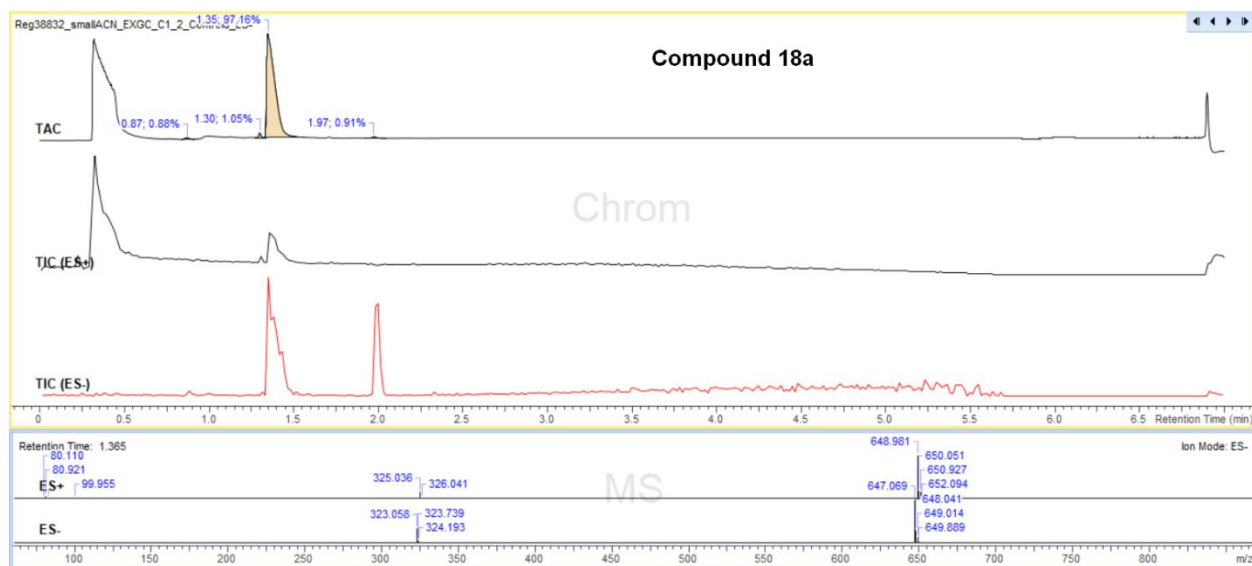
Compound **16a**: Method (AA), Purity: 98.2%



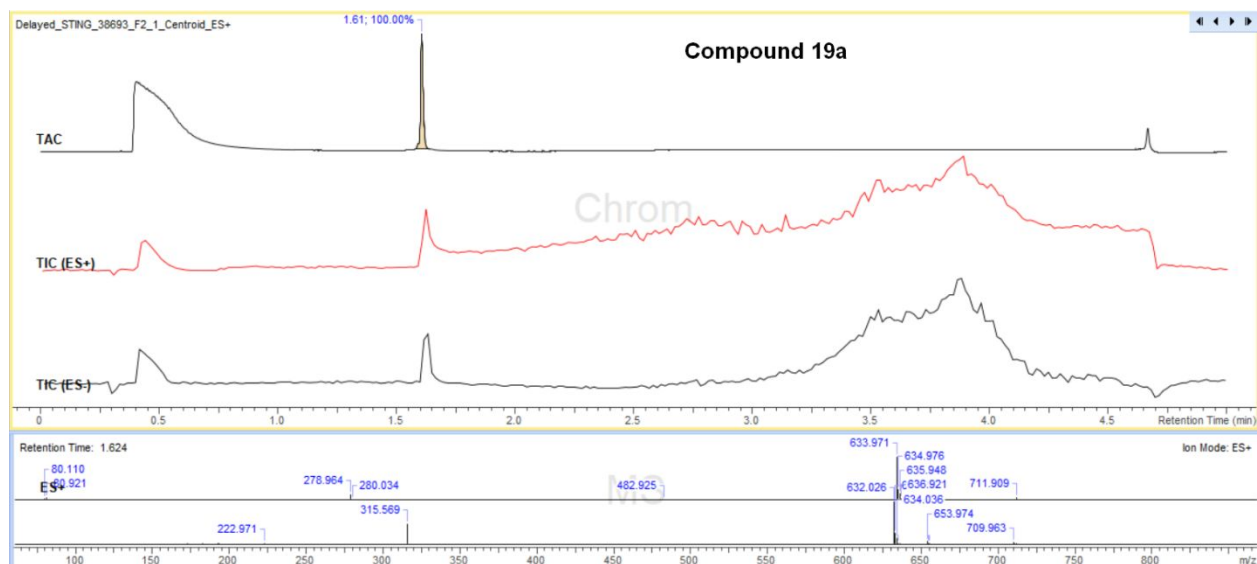
Compound **17a**: Method (FA), Purity: 96.2%



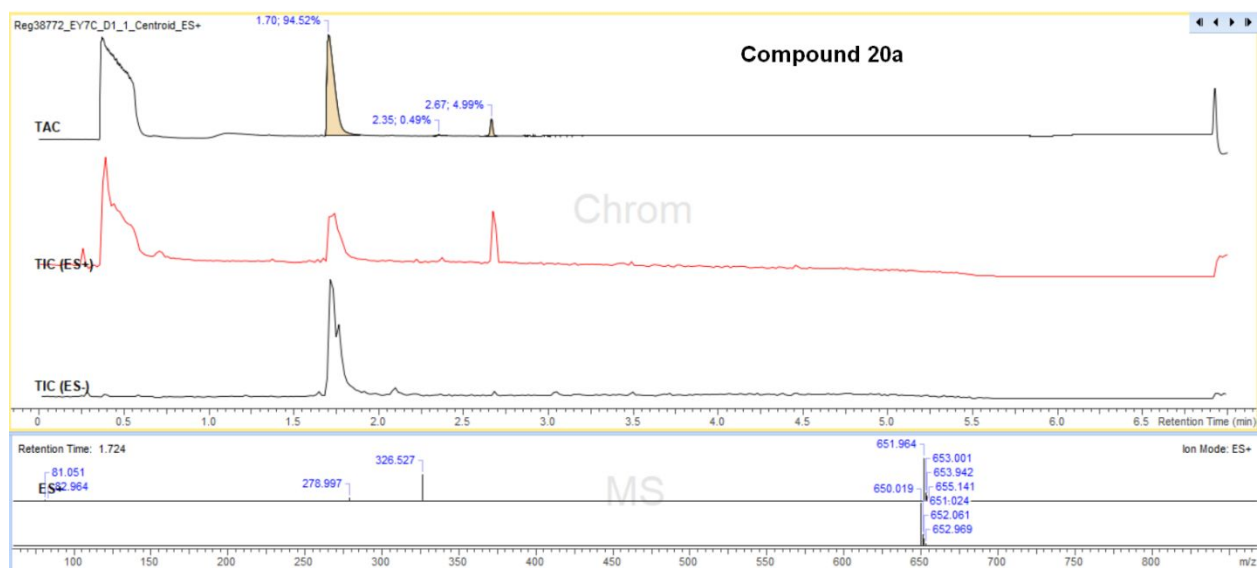
Compound **18a**: Method (FA), Purity: 97.2%



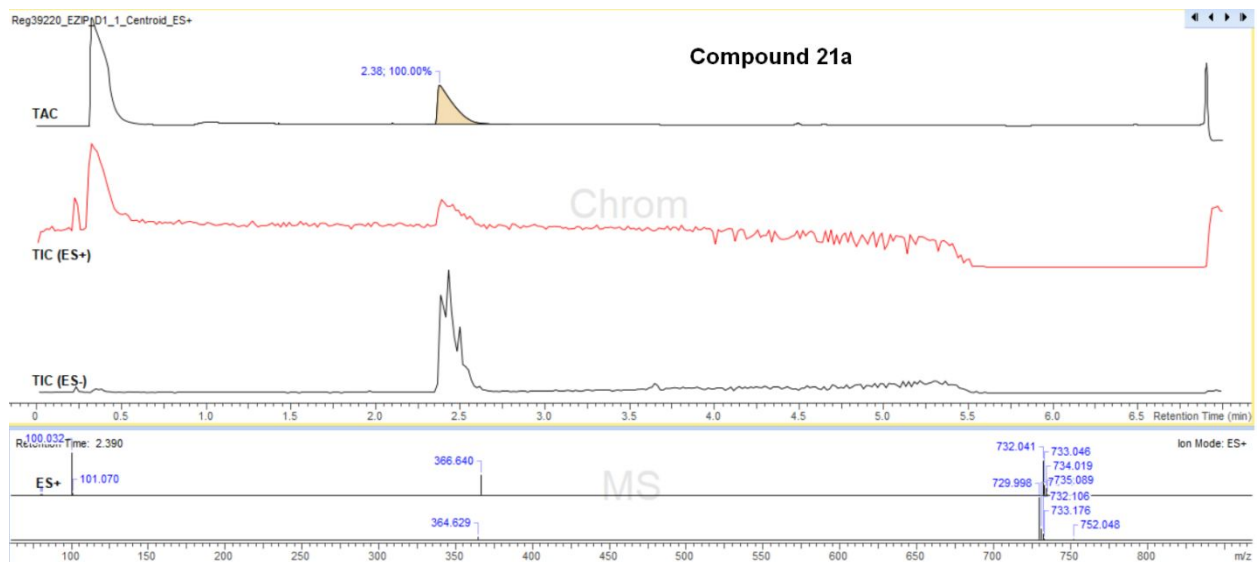
Compound **19a**: Method (FA), Purity: 100%



Compound **20a**: Method (FA), Purity: 94.5%



Compound **21a**: Method (FA), Purity: 100%



Compound **22a**: Method (AA), Purity: 98.4%

