Supplementary Material

Catechol-*O*-methyltransferase in Complex with Substituted 3'-Deoxyribose Bisubstrate Inhibitors

Manuel Ellermann,[a] Christian Lerner,[b] Guillaume Burgy,[b] Andreas Ehler,[b] Caterina Bissantz,[b] Roland Jakob-Roetne,[b] Ralph Paulini,[a] Oliver Allemann,[a] Heloïse Tissot,[a] Dan Grünstein,[a] Martine Stihle,[b] François Diederich,*[a] and Markus G. Rudolph*[b]

[a] *Laboratorium für Organische Chemie, ETH Zürich, Hönggerberg, HCI, CH-8093 Zürich, Switzerland. E-mail:* diederich@org.chem.ethz.ch; *Fax: (+41) 1-632-1109*

[b] *Pharma Division, Präklinische Forschung, F. Hoffmann-La Roche AG, CH-4070 Basel, Switzerland. E-mail:* markus.rudolph@roche.com

Table of Contents

Crystal Structure of the COMT/SAH semi-holo complex

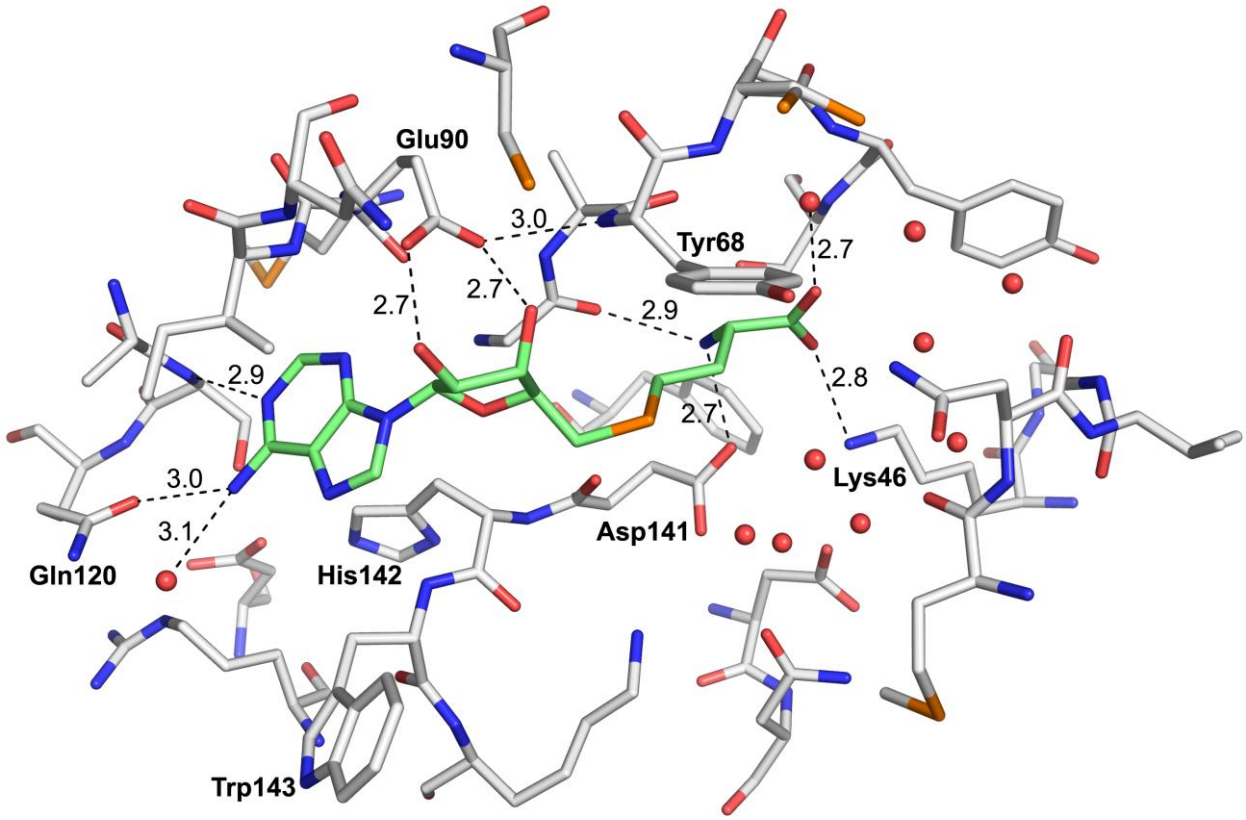
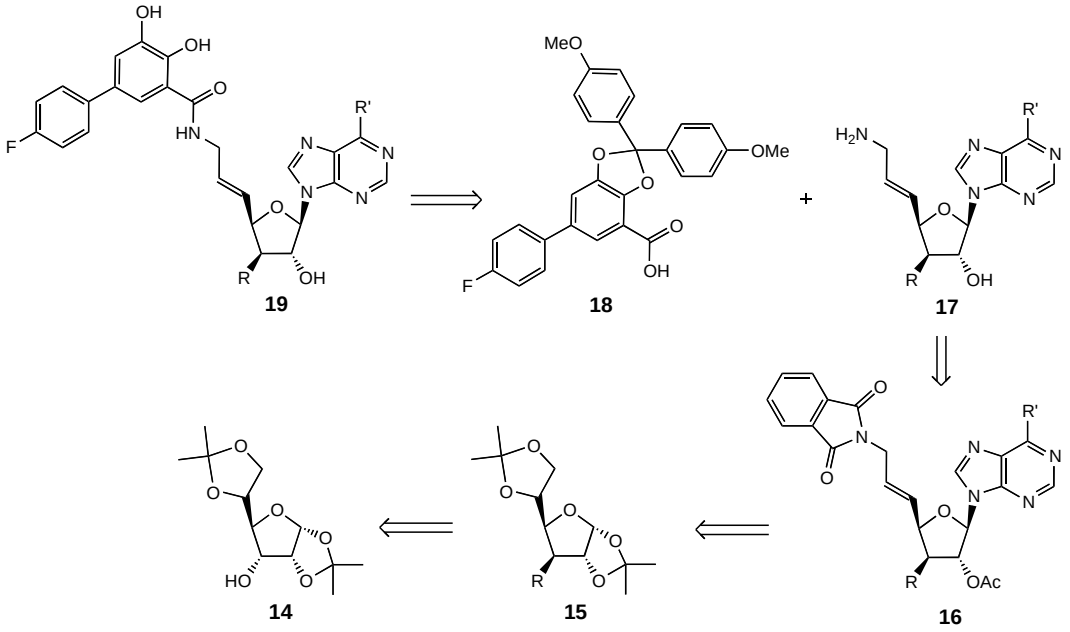


Figure S1 Binding site in the complex of SAH with COMT. Distances are given in Å (gray C_{COMT}, green C_{SAH}, PDB code: 3U81; red O, blue N, orange S). Selected water molecules are displayed as red spheres.

Experimental

Synthesis of bisubstrate inhibitors

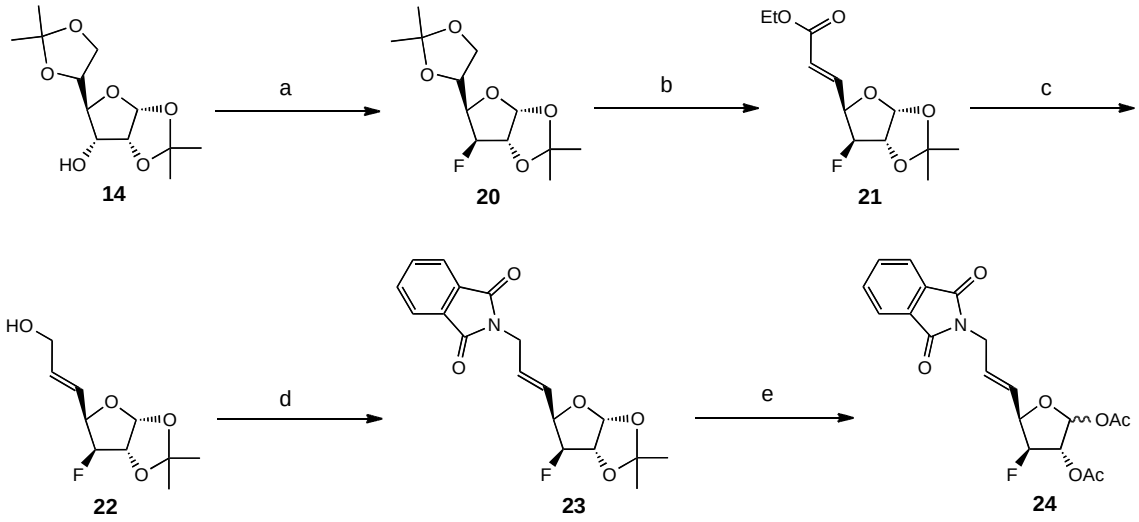
The retrosynthetic approach contains valuable steps from previous ligand developments,[1] the main difference being the novel *endo*-substituent (R = Me, F, Scheme 1) at C3' substituting for a protected *exo*-OH group (Scheme 1). Substitution of commercially available **14** should yield compound **15**.[2, 3] The nucleosides **16** can be obtained by the Vorbrüggen nucleosidation.[4] A following deprotection might yield allylic amines **17**. Amide coupling with catechol derivative **18**[1] might afford bisubstrate inhibitors **19** after deprotection.



Scheme 1 Retrosynthetic analysis of 3'-*endo*-substituted inhibitors starting from commercially available ribose **14**. R = Me, F. R' = Me, NHAlkyl.

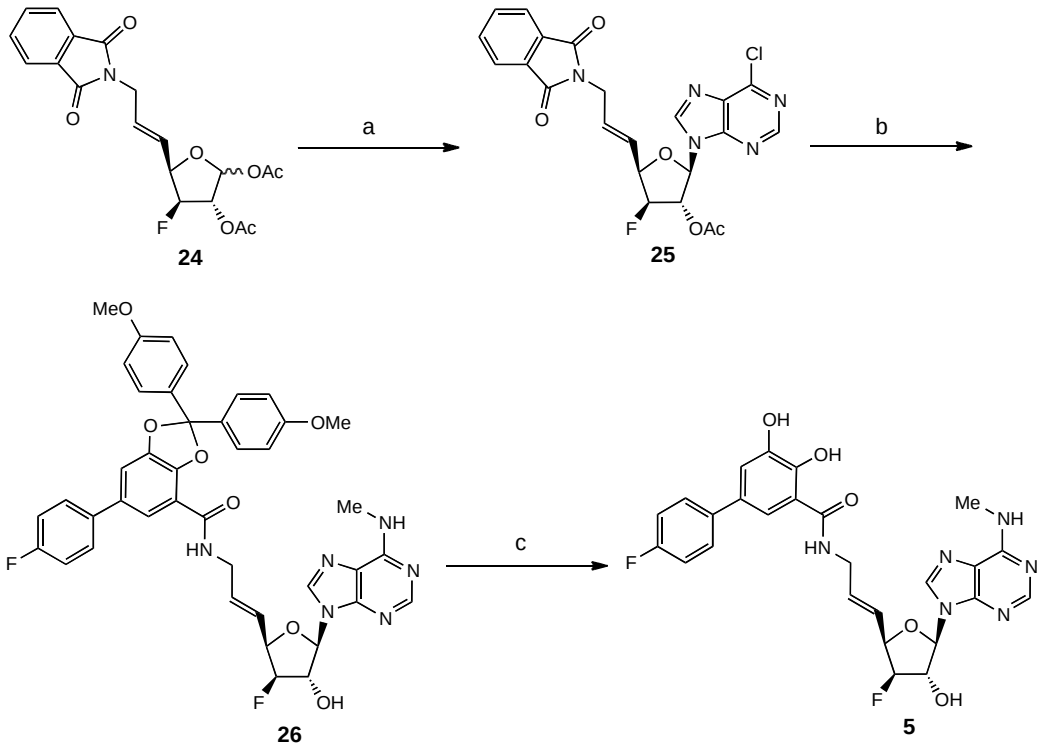
Synthesis of bisubstrate inhibitor 5

For the synthesis of 3'-fluorinated inhibitors, protected ribose **14** was fluorinated with DAST[5] to yield **20** according to the procedure of Mort *et al.* (Scheme 2).[3] After deprotection, oxidative diol cleavage and consecutive Wittig-Horner reaction, unsaturated *E*-ester **21** was obtained in 50% yield. Reduction of **21** with DIBAL-H led to allylic alcohol **22**, which was substituted with phthalimide in a Mitsunobu reaction to give ribose **23**. Acetonide **23** was deprotected under acidic conditions and directly converted into diacetyl 3'-deoxyribose **24** with Ac_2O.



Scheme 2 Synthesis of phthalimide **24** starting from ribose **14**. Reagents and conditions: a) DAST, pyridine, CH_2Cl_2, 20 °C, 20 h, 66%; b) i) H_5IO_6, $NaIO_4$, EtOAc, 0 to 20 °C, 7 h, ii) $Ph_3P{=}CHCO_2Et$, THF, 20 °C, 4 d, 50%; c) DIBAL-H, CH_2Cl_2, –78 °C, 4 h, 84%; d) PPh_3, DEAD, phthalimide, THF, 0 to 20 °C, 2d, 80%; e) i) HOAc (80%), 160 °C, 16 h, ii) Ac_2O, pyridine, DMAP, 20 °C, 2 h, 48%. DAST = Diethylaminosulfur trifluoride**,** DEAD = Diethyl azodicarboxylate, DMAP = 4-Dimethylaminopyridine.

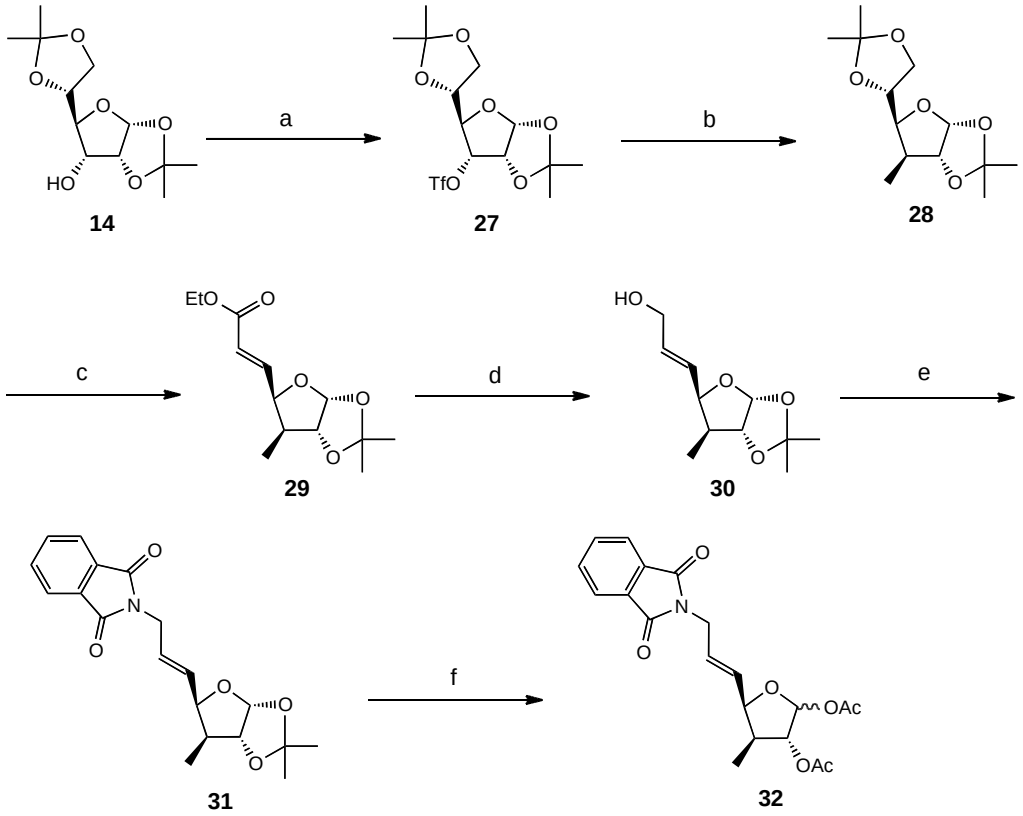
Vorbrüggen nucleosidation with 6-chloropurine afforded nucleoside **25** (Scheme 3). The 6-chloropurine undergoes nucleophilic *ipso*-substitution during the deprotection with methylamine to yield N(6)-methyladenine **25** after coupling with catechol **18**.[1, 6] Acidic deprotection yielded the targeted 3'-fluororinated inhibitor **5**.



Scheme 3 Synthesis of bisubstrate inhibitor **5** from acetonide **24**. a) 6-chloropurine, BSA, TMSOTf, DCE, 60 °C, 6 h, 52%; b) i) $MeNH_2$, EtOH, 20 °C, 15 h, then H_2NNH_2, MeOH, 40 °C, 15 h, ii) **18**, HBTU, *i*Pr_2NEt, HOBt, DMF, 20 °C, 18 h, 42%; c) TFA/H_2O/THF 1:1:1, 0 °C, 60 min, 53%. BSA = *N*,*O*-Bis(trimethylsilyl)acetamide. TMSOTf = Trimethylsyliltriflate. HBTU = (1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. TFA = Trifluoroacetic acid.

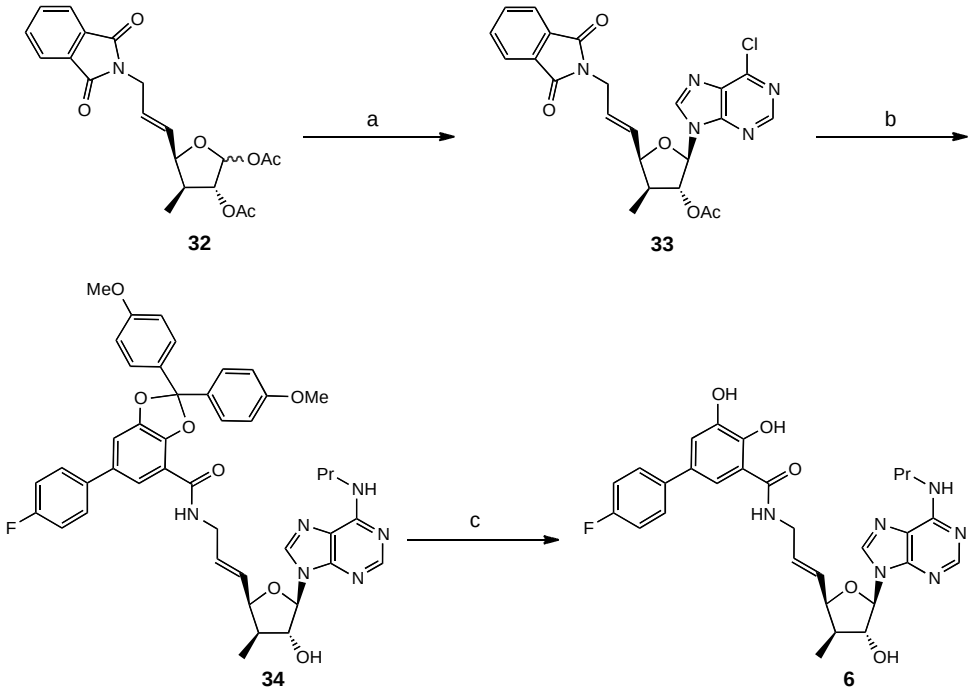
Synthesis of bisubstrate inhibitor 6

For the synthesis of the *endo*-3'-methylated bisubstrate inhibitor, ribose **14** was activated with a triflate leaving group to afford **27**. Nucleophilic attack of a methyl cuprate yielded **28** (Scheme 4), via a method described previously. Deprotection, oxidative diol cleavage, and Wittig-Horner reaction afforded ester **29**, which was reduced with DIBAL-H to give the allylic alcohol **30**. Mitsunobu reaction afforded phthalimide **31,** and deprotection/re-protection gave diacetyl 3'-deoxyribose **32**.



Scheme 4 Synthesis of diacetyl 3'-deoxyribose **32** from **14**. The key step is the introduction of a methyl substituent to triflate **27**. a) TfCl, DMAP, CH_2Cl_2, 0 °C, 1h, 76%; b) MeLi*LiBr, CuI, Et_2O, 0 °C, 3 h, then 20 °C, 12 h, 47%; c) i) H_5IO_6, $NaIO_4$, EtOAc, 20 °C, 3 h, ii) $(EtO)_2PO{=}CHCO_2Et$, LiCl, MeCN, 0 °C, 3 h, 46%; d) DIBAL-H, CH_2Cl_2, –78 °C, 4 h, 97%; e) PPh_3, DEAD, phthalimide, THF, 0 to 20 °C, 2 d, 99%; f) i) HOAc (80%), 120 °C, 16 h, ii) Ac_2O, pyridine, DMAP, 20 °C, 3 h, 65%.

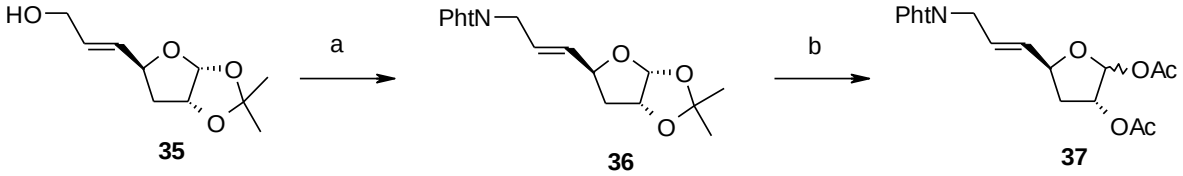
Vorbrüggen nucleosidation of 6-chloropurine with **32** led to **33** in low yield (18%) yield due to increased steric hindrance by the *endo*-3'-methyl substituent and problematic purification (Scheme 5). Reaction of crude **33** with propylamine, followed by coupling with **18** gave **34**, which was deprotected to yield the desired bisubstrate inhibitor **6**. Compounds **34** and **6** were contaminated (ca. 45%) with the isomeric nucleoside in which the ribose moiety is connected to N(7) of the adenine. All attempts to separate the two isomers by HPLC failed.



Scheme 5 Synthesis of 3'-methyl bisubstrate inhibitor **6**. a) 6-chloropurine, BSA, TMSOTf, DCE, 100 °C, 16 h, 18%; b) i) $PrNH_2$, EtOH, 20 °C, 72 h, ii) **18**, HBTU, *i*Pr_2NEt, HOBt, DMF, 0 to 20 °C, 18 h, 39%; c) TFA/H_2O/THF 1:1:1, 0 °C, 60 min, 33%. DCE = 1,2-Dichloroethane. HOBt = Hydroxybenzotriazole.

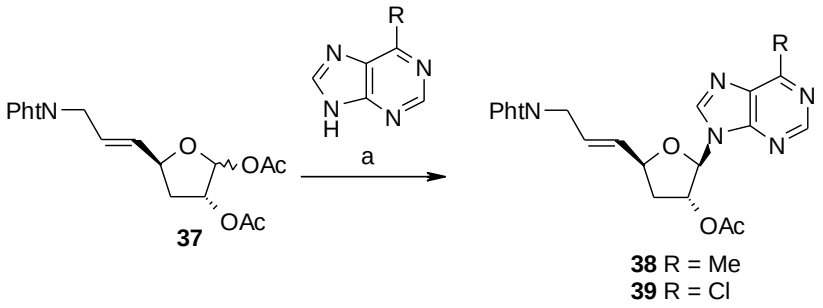
Synthesis of bisubstrate inhibitors 4 and 12

Alcohol **35**[7] was transformed to phthalimide **36** by a *Mitsunobu* reaction. In the following steps, the acetonide was cleaved with sulfuric acid and the resulting diol was acetylated to give product **37** (Scheme 6).



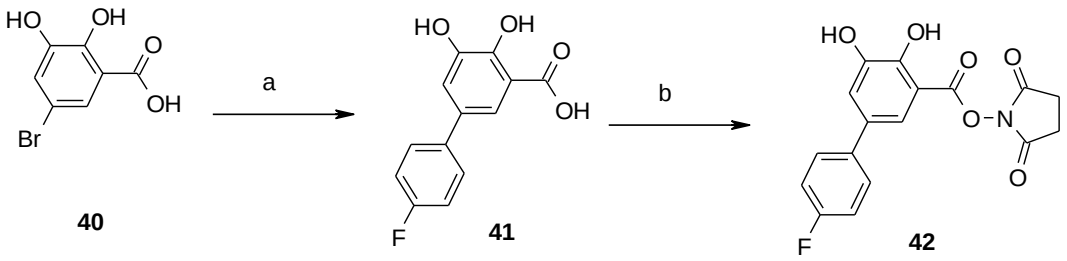
Scheme 6 Introduction of a phthalimide, hydrolysis of the acetonide, followed by acetylation. a) phthalimide, DIAD, PPh_3, 20 °C, 4 h, 80%; b) i) H_2SO_4, dioxane, 50 °C, 45 h, ii) Ac_2O, pyridine, DMAP, 20 °C, 20 h, 58%. DIAD = Diethyl azodicarboxylate.

Following nucleosidation reactions, according to a Vorbrüggen protocol, using bis(trimethylsilyl)acetamide (BSA) and TMSOTf in presence of the nucleobase methylpurine or chloropurine proceeded with a satisfying yield, to give the methyl (**38**) and the chloro (**39**) derivatives, respectively (Scheme 7).



Scheme 7 Nucleosidation reactions. a) BSA, TMSOTf, DCE, 60 °C, 5 h, **38**: 70%, **39**: 45%.

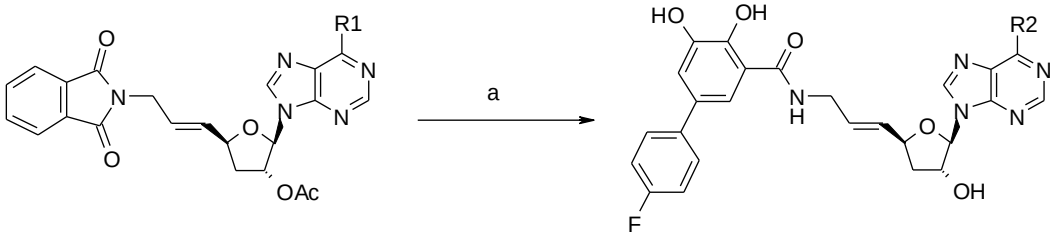
Suzuki reaction of 5-bromo-2,3-dihydroxy-benzoic acid (**40**) with 4-fluorophenylboronic acid gave acid **41**, which was converted to the hydroxysuccinimid ester **42** using *N*-hydroxy succinimide and a solid-supported DCC reagent (Scheme 8).



40 **41** **42**

Scheme 8 Synthesis of activated ester **42**. a) [Pd(dppf)Cl_2], 4-fluorophenylboronic acid, Na_2CO_3, dioxane, H_2O, 17 h, 80 °C, 97%; b) *N*-hydroxysuccinimide, DCC resin, THF, 20 h, 20 °C, 89%. DCC = *N*,*N*'-Dicyclohexyl-carbodiimide.

Deprotection of **38** and **39** with $MeNH_2$, followed by reaction of the released amines with succinimide ester **42** gave inhibitors **4** and **12** (Scheme 9).



38 R1 = Me
39 R1 = Cl

4 R2 = Me
12 R2 = NHMe

Scheme 9 Synthesis of target molecules **4** and **12**. a) i) $MeNH_2$, EtOH, 50 °C, 24 h, ii) **42**, DMF, $(i\text{-}Pr)_2NEt$, 20 °C, 4 h, **4:** 19%, **12:** 17%. DMF = *N*,*N*'-Dimethyl formamide.

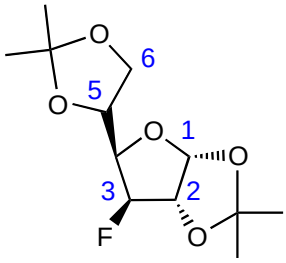
Materials and Methods

Reagents and solvents were purchased at reagent grade from Acros, Aldrich, and Fluka and used as received. *N*-cyclohexyl, *N'*-methyl carbodiimide polystyrene resin was purchased from Novabiochem. All reactions were carried out under an argon atmosphere unless otherwise stated. Column chromatography (CC) was carried out with SiO_2 60 (particle size 0.040–0.063 mm, 230–400 mesh; Fluka) and distilled technical solvents. Thin-layer chromatography (TLC) was conducted on aluminum sheets coated with SiO_2 60 F254 obtained from Macherey-Nagel; visualization with a UV lamp (254 nm). Analytical HPLC was performed on a Knauer ProntoSIL 120 C_{18} AQ column (250 x 4.6 mm, 5 μm); products were eluted with a linear gradient of CH_3CN in H_2O containing 0.1% formic acid over 50 min with a flow rate of 1 cm^3 min^{-1} with UV detection at l = 254 nm. Preparative HPLC was performed on a Knauer ProntoSIL 120-5 C18 AQ column (250 x 20 mm, 5 μm); products were eluted with a linear gradient of CH_3CN in H_2O containing 0.1% formic acid with a flow rate of 5–10 cm^3 min^{-1} with UV detection at l = 254 nm. Rotational angles ($[\alpha]_D^{20}$) were measured on a Perkin-Elmer 241 polarimeter. Measurements were performed at 549 nm (Na-D-line) with a 10 cm cuvette, concentrations are given in g/1000 cm^3. Melting points (m.p.): Büchi-B-540 capillary melting point apparatus; uncorrected. ^1H NMR and ^{13}C NMR spectra were measured with a Varian Gemini 300, a Varian Mercury 300, a Bruker ARX 300, Bruker AV 300, Bruker DRX 400, Bruker AV 400, Bruker DRX 500, or on a Bruker AVIII 600 spectrometer. Chemical shifts are reported in ppm relative to the signal of Me_4Si. Residual solvent signals in the ^1H and ^{13}C NMR spectra were used as an internal reference. Coupling constants (*J*) are given in Hz. The resonance multiplicity is described as s (singlet), d (doublet), t (triplet), q (quadruplet), quint. (quintet), hept. (heptet), m (multiplet), and br. (broad). Infrared spectra (IR) were recorded on a Varian 800 FT-IR spectrometer. The spectra were measured neat, selected absoption bands are reported in wavenumbers (cm^{-1}). ESI-MS were measured with a ESI-MS on Sciex API150 or

Agilent 6520 Accurate mass Q-TOF. EI-MS were measured with a Agilent 6890N. High-resolution (HR) ESI-MS and MALDI-MS spectra were measured with a Varian IonSpec FT-ICR-MS spectrometer using 3-hydroxypyridine-2-carboxylic acid (3-HPA) as matrix for MALDI-MS. Nomenclature follows the suggestions proposed by the software ACD Names of ACD/Labs.[8] Atom numbering is arbitrary and does not follow the numbering of the IUPAC nomenclature.

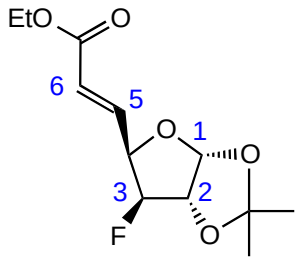
Syntheses and Characterization

3-Deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucopyranose (20).[2]



Compound **14** (11.52 g, 44.29 mmol) was dissolved in CH_2Cl_2 (220 cm^3), and pyridine (11.38 cm^3, 141.47 mmol) and DAST (9.35 cm^3, 70.75 mmol) were added at –30 °C. The mixture was stirred for 20 h at 20 °C, after which the reaction was quenched by addition of sat. $NaHCO_3$ (50 cm^3). The aqueous phase was extracted with CH_2Cl_2 (3 x 300 cm^3), the combined organic phases were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2; hexane/EtOAc 5:1) to give product **20** as a turbid oil (7.65 g, 66%). $[\alpha]_D^{20}$ –21 (c 1.7, $CHCl_3$) lit. [2]: $[\alpha]_D^{28}$ –37 (c 10, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2988*m*, 2938*w*, 2892*w*, 1736*w*, 1457*w*, 1373*s*, 1256*m*, 1212*s*, 1073*s*, 1018*s*, 954*w*, 788*m*, 637*w* cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.34 (s, 3 H), 1.38 (s, 3 H), 1.46 (s, 3 H), 1.52 (s, 3 H), 4.12 (m, 3 H), 4.30 (m, 1 H), 4.71 (dd, *J* 20.7, 3.7 Hz, 1 H), 5.02 (dd, *J* 49.8, 2.2 Hz, 1 H), 5.97 ppm (d, *J* 3.7 Hz, 1 H); δ_C (75 MHz, $CDCl_3$) 25.08, 26.10, 26.61, 26.77, 67.01, 71.72 (d, *J* 7.1 Hz), 80.46 (d, *J* 19.0 Hz), 82.34 (d, *J* 32.8 Hz), 93.60 (d, *J* 183.4 Hz), 104.95, 109.26, 112.13 ppm; δ_F (282 MHz, $CDCl_3$) –187.14 ppm (ddd, *J* 51.3, 24.5, 15.0 Hz); HR-EI-MS: *m*/*z* 43.0244 (100), 101.0569 (74), 247.0979 (65, calcd for $C_{11}H_{16}FO_5^+$ $[M\text{-}CH_3]^+$: 247.0976).

Ethyl (5*E*)-3,5,6-Trideoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-*xylo*-hept-5-enofuranuronate (21) and Ethyl (5*Z*)-3,5,6-Trideoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-*xylo*-hept-5-enofuranuronate (21b).



To a solution of **20** (7.59 g, 28.96 mmol) in EtOAc (150 cm^3), sodium periodate (6.19 g, 28.96 mmol) and *ortho*-periodic acid (7.59 g, 33.30 mmol) were added at 0 °C. The solution was stirred at 20 °C for 7 h. The resulting precipitate was filtered, washed with EtOAc, and dried *in vacuo*. THF (70 cm^3) and $Ph_3P{=}CHCO_2Et$ (12.61 g, 36.20 mmol) were added, and the resulting solution was stirred at 20 °C for 4 d. Afterwards, H_2O (100 cm^3) and EtOAc (200 cm^3) were added and the obtained phases separated. The aqueous phase was extracted with EtOAc (2 x 200 cm^3), the combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified twice by flash chromatography (SiO_2; hexane/EtOAc 95:5) to give **21** (3.73 g, 50%, three steps) and Z-isomer **21b** (2.06 g, 27%, three steps) as clear oils.

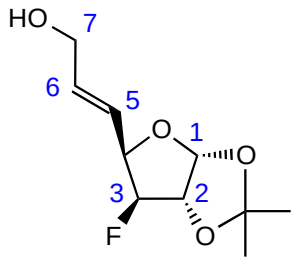
Z-isomer **21b** (1.92 g, 7.38 mmol) was dissolved in hexan (50 cm^3) and treated with $(PhS)_2$ (8.05 g, 36.91 g). The solution was stirred under UV light at 20 °C for 4 d and then concentrated *in vacuo*. The residue was purified twice by flash chromatography (SiO_2; hexane/EtOAc 95:5) to give **21** as a clear oil (0.59 g, 31%).

$[\alpha]_D^{20}$ –36 (c 0.90, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2994*m*, 2942*w*, 1719*s*, 1667*w*, 1653*m*, 1465*w*, 1376*m*, 1303*m* 1266*s*, 1232*m*, 1217*m*, 1181*s*, 1165*s*, 1076*s*, 1035*s*, 1019*s*, 977*m*, 915*w*, 887*w*, 858*w*, 735*m*, 636*s* cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.29 (t, *J* 7.1 Hz, 3 H), 1.34 (s, 3 H), 1.51 (s, 3 H), 4.20 (q, *J* 7.1 Hz, 2 H), 4.73 (dd, *J* 10.2, 3.9 Hz, 1 H), 4.82 (ddd, *J* 7.1, 6.1, 2.1 Hz, 1 H), 4.92 (dd, J 49.6, 2.4 Hz, 1 H), 6.03 (d, *J* 3.8 Hz, 1 H), 6.21 (dd, *J* 15.7, 1.7 Hz, 1 H), 6.90 ppm (dd, *J* 15.8, 5.1 Hz, 1 H); δ_C (75 MHz, $CDCl_3$) 14.16, 26.12, 26.64, 60.59, 78.77 (d, *J* 19.8 Hz), 82.58 (d, *J* 32.4 Hz), 94.41 (d, *J* 187.0 Hz), 104.77, 112.43, 124.65, 138.46 (d, *J* 6.7 Hz), 165.62 ppm; δ_F

(282 MHz, $CDCl_3$) –204.57 ppm (ddd, *J* 50.0, 29.3, 10.3 Hz); HR-EI-MS: *m*/*z* 43.0233 (100), 59.0497 (64), 245.0823 (46, calcd for $C_{11}H_{14}FO_5^+$ $[M\text{-}CH_3]^+$: 245.0820).

21b: $[\alpha]_D^{20}$ –19 (c 1.35, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2981*m*, 2938*w*, 1716*s*, 1654*s*, 1415*br*, 1385*m*, 1376*m*, 1195*s*, 1164*m*, 1080*s*, 1038*s*, 1017*s*, 828*m*, 635*s* cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.34 (t, *J* 7.2 Hz, 3 H), 1.38 (s, 3 H), 1.57 (s, 3 H), 4.23 (dq, *J* 7.2, 1.4 Hz, 2 H), 4.76 (dd, *J* 10.8, 3.9 Hz, 1 H), 5.21 (dd, *J* 49.8, 2.5 Hz, 1 H), 5.75 (m, 1 H), 6.02 (dd, *J* 11.7, 1.7 Hz, 1 H), 6.07 (d, *J* 3.9 Hz, 1 H), 6.28 ppm (dd, J 11.6, 6.6 Hz, 1 H); δ_C (75 MHz, $CDCl_3$) 14.16, 26.12, 26.64, 60.59, 78.77 (d, *J* 19.8 Hz), 82.58 (d, *J* 32.4 Hz), 94.41 (d, *J* 187.0 Hz), 104.77, 112.43, 124.65, 138.46 (d, *J* 6.7 Hz), 165.62 ppm; δ_F (282 MHz, $CDCl_3$) –204.04 ppm (ddd, J 49.9, 29.4, 10.4 Hz); HR-EI-MS: *m*/*z* 43.0232 (100), 245.0820 (56, calcd for $C_{11}H_{14}FO_5^+$ $[M\text{-}(CH_3)]^+$: 245.0820).

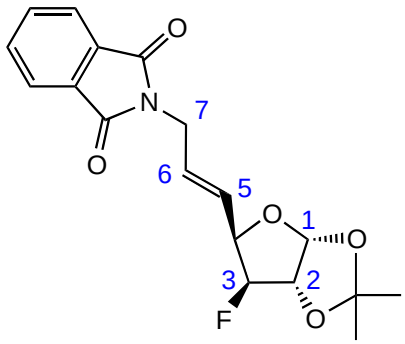
(5*E*)-3,5,6-Trideoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-*xylo*-hept-5-enofuranose (22).



Compound **21** (3.65 g, 14.03 mmol) was dissolved in CH_2Cl_2 (65 cm^3) and cooled to –78 °C. DIBAL-H (56.13 cm^3, 1M in CH_2Cl_2) was added slowly, and the solution was stirred for 4 h at –78 °C. A sat. aq. solution of NH_4Cl (2 cm^3) was added carefully at –78 °C, the solution was heated to 20 °C and a sat. aq. solution of ammonium tartrate (20 cm^3) was added. After stirring for 18 h at 20 °C, the aqueous phase was extracted with CH_2Cl_2 (3 x 100 cm^3), the combined organic phases were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2; hexane/EtOAc 95:5 → 4:1 → 2:1) to give product **22** as a clear oil (2.58 g, 84%).

$[\alpha]_D^{20}$ –27 (c 1.15, $CHCl_3$); IR (neat): $\tilde{\nu}$ 3422*br*, 2992*m*, 2942*w*, 2250*w*, 1385*m*, 1376*m*, 1215*m*, 1164*m*, 1075*s*, 1012*s*, 915*m*, 886*m*, 849*m*, 734*m*, 682*m*, 638*m*, 617*s* cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.28 (s, 3 H), 1.46 (s, 3 H), 1.57 (s, 1 H), 4.15 (d, *J* 4.8 Hz, 2 H), 4.64 (m, 2 H), 4.76 (dd, *J* 49.7, 2.4 Hz, 1 H), 5.76 (dd, *J* 15.7, 6.4 Hz, 1 H), 5.94 (d, *J* 3.7 Hz, 1 H), 6.04 ppm (td, *J* 15.6, 5.1 Hz, 1 H); δ_C (75 MHz, $CDCl_3$) 26.24, 26.71, 62.76, 79.96 (d, *J* 18.9 Hz), 82.72 (d, *J* 32.6 Hz), 95.00 (d, *J* 184.9 Hz), 104.52, 112.03, 122.41 (d, *J* 7.9 Hz), 135.37 ppm; δ_F (282 MHz, $CDCl_3$) –206.26 ppm (ddd, *J* 49.5, 29.1, 10.5 Hz); HR-EI-MS: *m*/*z* 43.0229 (100), 59.0484 (62), 203.0717 (14, calcd for $C_9H_{12}FO_4^+$ $[M-(CH_3)]^+$: 203.0714).

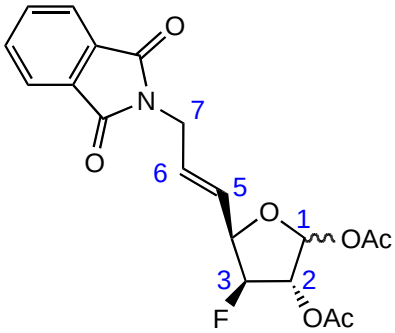
(5*E*)-3,5,6,7-Tetradeoxy-3-fluoro-1,2-*O*-isopropylidene-7-phthalimido-α-D-*xylo*-hept-5-enofuranose (23).



Triphenylphosphine (5.92 g, 22.56 mmol) was dissolved in THF (30 cm^3), and at 0 °C DEAD (4.1 cm^3, 22.50 mmol) was added. The solution was stirred for 20 h at 0 °C. Compound **22** (2.46 g, 11.28 mmol) was dissolved in THF (30 cm^3) and slowly added to the previous solution. Phthalimide (3.32 g, 22.56 mmol) was added, and the mixture was stirred for 20 h. Afterwards, H_2O (70 cm^3) and CH_2Cl_2 (30 cm^3) were added, and the obtained phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 30 cm^3), the combined organic phases were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified four times by flash chromatography (SiO_2; hexane/EtOAc 40:1) (CH_2Cl_2/MeOH 500:1) (CH_2Cl_2/MeOH 2000:1) (CH_2Cl_2/EtOAc 99:1) to give product **23** as a white solid (3.11 g, 80%).

Mp 95–103 °C (decomp.); $[\alpha]_D^{20}$ –33 (c 0.95, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2984*m*, 2937*w*, 2892*w*, 1740*s*, 1446*w*, 1373*m*, 1240*m*, 1047*s*, 735*w*, 633*w*, 609*w* cm^{-1}. δ_H (300 MHz, $CDCl_3$): 1.24 (s, 3 H), 1.40 (s, 3 H), 4.26 (d, *J* 6.0 Hz, 2 H), 4.58 (m, 2 H), 4.72 (dd, *J* 49.9, 2.2 Hz, 1 H), 5.74 (dd, *J* 15.6, 6.9 Hz, 1 H), 5.88 (d, *J* 3.9 Hz, 1 H), 5.95 (m, 1 H), 7.64 (dd, J 5.7, 3.2 Hz, 2 H), 7.77 ppm (dd, *J* 5.7, 3.0 Hz, 2 H); δ_C (75 MHz, $CDCl_3$) 26.26, 26.71, 38.98, 79.65 (*d*, *J* 19.0 Hz), 82.66 (d, *J* 32.5 Hz), 94.72 (d, *J* 180.5 Hz), 104.53, 112.07, 123.24, 125.52 (d, *J* 7.4 Hz), 129.32, 131.96, 133.88, 167.55 ppm; δ_F (282 MHz, $CDCl_3$) –206.12 ppm (ddd, *J* 49.9, 29.1, 10.5 Hz); HR-MALDI-MS (3-HPA): *m*/*z* 370.1059 (100, calcd for $C_{18}H_{18}FNNaO_5^+$ $[M+Na]^+$: 370.1061).

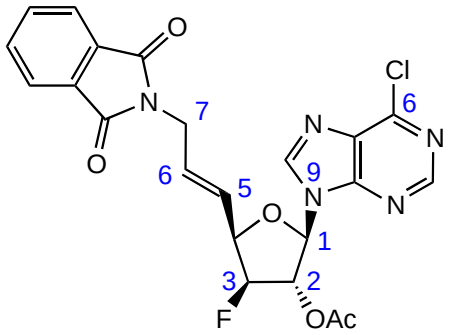
(5*E*)-1,2-Di-*O*-acetyl-3,5,6,7-tetradeoxy-3-fluoro-7-phthalimido-α/*β*-D-*xylo*-hept-5-enofuranose (24).



Compound **23** (1.79 g, 5.15 mmol) was treated with a solution of HOAc/H_2O (4:1, 40 cm^3) at 20 °C. The solution was stirred at 120 °C for 16 h, and the solvents were removed *in vacuo* with addition of toluene (2 x 50 cm^3) to give a residue, which was stirred with dry pyridine (10 cm^3), acetic acid (10 cm^3) and a catalytic amount of DMAP at 20 °C for 2 h. After evaporation, the oily residue was diluted with H_2O (200 cm^3) and EtOAc (200 cm^3) and the phases separated. The aqueous phase was extracted with EtOAc (2 x 200 cm^3); the combined organic phases were washed with a sat. aq. solution of NH_4Cl (200 cm^3), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2; pentane/ EtOAc 95:5) to give starting material **23** (0.22 g, 11%) and a *α*/*β*-mixture of **24** (*α*/*β* 1:2, 0.97 g, 53%, two steps, calculated from converted starting material) as clear oils.

IR (neat): $\tilde{\nu}$ 2980 *w*, 1769*m*, 1710*s*, 1496*w*, 1427*m*, 1381*m*, 1215*w*, 1167*w*, 1074*s*, 1037*s*, 991*m*, 943*m*, 855*s*, 787*s*, 725*s* cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.96 (s, 0.8 H), 2.01 (s, 0.8 H), 2.01 (s, 1.8 H), 2.02 (s, 1.8 H), 4.23-4.26 (m, 2 H), 4.47-4.56 (m, 0.3 H), 4.61-4.71 (m, 1.2 H), 4.96 (ddd, *J* 55.5, 6.0, 4.9 Hz, 0.3 H), 5.17-5.21 (m, 0.6 H), 5.27 (ddd, *J* 20.7, 6.0, 4.6 Hz, 0.3 H), 5.65-5.92 (m, 2 H), 6.10 (s, 0.6 H), 6.30 (d, *J* 4.6 Hz, 0.3 H), 7.64-7.68 (m, 2 H), 7.77-7.80 ppm (m, 2 H); δ_C (101 MHz, $CDCl_3$) 20.16, 20.47, 20.69, 20.91, 38.69, 76.82 (d, *J* 30.5 Hz), 79.23 (d, *J* 20.8 Hz), 79.33 (d, *J* 31.1 Hz), 83.14 (d, *J* 20.1 Hz), 92.75 (d, *J* 189.7 Hz), 93.11, 94.30 (d, *J* 189.1 Hz), 98.57, 123.24, 129.64, 129.83, 131.96, 134.00, 167.56, 168.95, 169.18, 169.36 ppm; δ_F (282 MHz, $CDCl_3$): –187.41-187.67 (m), –195.73 ppm (dt, *J* 55.0, 20.2 Hz); HR-ESI-MS: *m*/*z* 114.0866 (100), 414.0950 (78, calcd for $C_{19}H_{18}NFO_7Na^+$ $[M+Na]^+$: 414.0960).

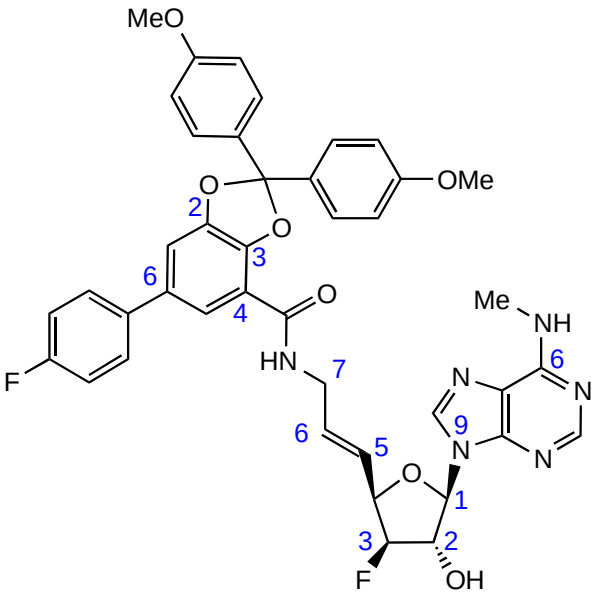
9-[(5*E*)-2-*O*-Acetyl-3,5,6,7-tetradeoxy-3-fluoro-7-phthalimido-*β*-D-*xylo*-hept-5-enofuranosyl]-6-chloropurine (25).



A dry N_2-flushed *Schlenk*-tube (20 cm^3), equipped with a magnetic stirrer and a septum, was charged with 6-chloropurine (519 mg, 3.66 mmol), 1,2-dichloroethane (12 cm^3), and *N*,*O*-bis(trimethylsilyl) acetamide (0.82 cm^3, 3.66 mmol). The mixture was heated to 60 °C. After 30 min, the mixture was cooled to 20 °C when **24** (439 mg, 1.12 mmol) and trimethylsilyl trifluoromethanesulfonate (0.61 cm^3, 3.66 mmol) were added slowly. The mixture was heated to 60 °C, stirred for 6 h, and then cooled to 20 °C. Afterwards, a sat. aq. solution of $NaHCO_3$ (200 cm^3) and CH_2Cl_2 (200 cm^3) were added and the obtained phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 200 cm^3), the combined organic phases were dried

($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified twice by flash chromatography (SiO_2; pentane/ EtOAc 1:1) to give **25** as a yellow oil (283 mg, 52%). $[\alpha]_D^{20}$ +6 (c 0.41, $CHCl_3$); IR (neat): $\tilde{\nu}$ 1754*m*, 1709*s*, 1561*w*, 1487*w*, 1425*m*, 1393*s*, 1336*m*, 1198*s*, 1233*m*, 1059*s*, 950*s*, 896*m*, 858*m*, 791*m*, 720*s*, 622*w* cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.21 (s, 3 H), 4.41 (d, *J* 5.7 Hz, 2 H), 4.81 (ddd, *J* 29.2, 6.9, 2.4 Hz, 1 H), 5.05 (ddd, *J* 49.5, 2.5, 0.7 Hz, 1 H), 5.57 (ddd, *J* 13.2, 1.2, 0.8 Hz, 1 H), 5.97 (ddd, *J* 15.6, 6.9, 0.5 Hz, 1 H), 6.11 (dtd, *J* 15.6, 5.8, 0.8 Hz, 1 H), 6.34 (d, *J* 1.3 Hz, 1 H), 7.77 (m, 2 H), 7.90 (m, 2 H), 8.77 (s, 1 H), 8.33 ppm (s, 1 H); δ_C ($CDCl_3$, 101 MHz) 20.51, 38.77, 79.82 (d, *J* 31.7 Hz), 82.74 (d, *J* 19.6 Hz), 87.29, 93.59 (d, *J* 186.8 Hz), 123.47, 124.05, 124.13, 131.66, 132.01, 134.15, 143.02, 143.11, 151.34, 152.33, 167.69, 168.78 ppm; δ_F (376 MHz, $CDCl_3$, decoup.): –198.63 ppm (s); HR-ESI-MS: *m*/*z* 217.1037 (100), 268.9978 (92), 508.0802 (44, calcd for $C_{22}H_{17}ClFN_5NaO_5^+$ $[M+Na]^+$: 508.0794).

9-{(5*E*)-3,5,6,7-Tetradeoxy-3-fluoro-7-[6-(4-fluorophenyl)-2,2-bis(4-methoxyphenyl)-1,3-benzodioxole-4-carboxamido]-*β*-D-*xylo*-hept-5-enofuranosyl}-N^6-methyladenine (26).

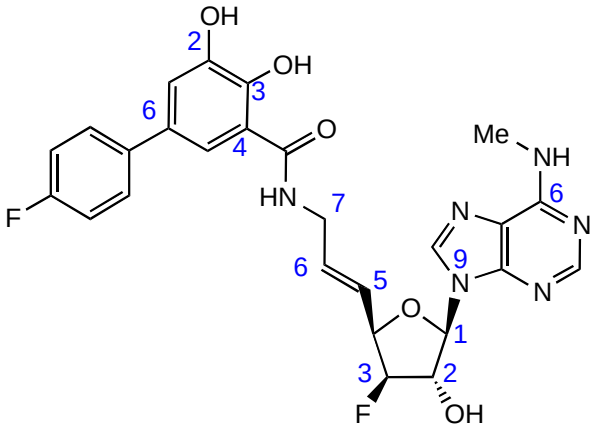


A dry N_2-flushed flask (25 cm^3), equipped with a magnetic stirrer and a septum, was charged with **25** (140 mg, 0.29 mmol), and a solution of methylamine (7 cm^3, 33% in ethanol) was added. The mixture was stirred for 11 d at 20 °C and evaporated. The residue was dissolved in

MeOH (10 cm^3) and treated with hydrazine hydrate (150 mg, 0.32 mmol) for 15 h at 40 °C. The solvent was removed. The residue was transferred into a *Schlenk*-tube (20 cm^3) and dried under high vacuum for 24 h to afford the primary amine. In a dry N_2-flushed flask (10 cm^3), equipped with a magnetic stirrer and a septum, **18**[1] (150 mg, 0.32 mmol) was dissolved in dry DMF (1 cm^3). *i*Pr_2NEt (0.05 cm^3, 0.58 mmol), HOBt (39 mg, 0.29 mmol), and HBTU (131 mg, 0.35 mmol) were added at 0 °C. The mixture was stirred for 30 min at 0 °C and then added slowly to a solution of the primary amine in DMF (2 cm^3) at 0 °C. The reaction was stopped by the addition of ice after 18 h. Two purifications with reverse-phase HPLC (H_2O/MeCN/HCOOH 90:10:0.01 → 20:80:0.01) and two-fold purification with flash chromatography (SiO_2; CH_2Cl_2/MeOH/NEt_3, 100:0:0.01 → 95:5:0.01) gave **26** as a white solid (93 mg, 42%, 2 steps).

Mp 110 °C (decomp.); $[\alpha]_D^{20}$ +4 (c 0.51, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.11 (s, 3 H), 3.69 (s, 3 H), 3.70 (s, 3 H), 4.15 (q, *J* 5.1 Hz, 2 H), 4.59 (dd, *J* 14.5, 1.3 Hz, 1 H), 5.83-4.05 (m, 2 H), 5.85-5.90 (m, 1 H), 6.02-6.09 (m, 2 H), 6.82 (m, 4 H), 7.00 (m, 2 H), 7.10 (d, *J* 1.9 Hz, 1 H), 7.22 (t, *J* 5.8 Hz, 1 H, CONH), 7.40 (m, 6 H), 7.68 (d, *J* 1.9 Hz, 1 H), 7.84 (s, 1 H), 8.25 ppm (s, 1 H); δ_C (101 MHz, $CDCl_3$) 34.46, 40.89, 53.00, 55.33, 79.67 (d, *J* 27.4 Hz), 82.62 (d, *J* 19.5 Hz), 90.36, 96.37 (d, *J* 185.6 Hz), 110.46, 113.76, 115.27, 115.61 (d, *J* 21.3 Hz), 119.49, 120.86, 123.32, 128.15, 128.48 (d, *J* 8.0 Hz), 130.98, 130.98, 133.16, 134.86, 136.29, 137.65, 137.72, 144.50, 152.89, 155.48, 160.64, 162.41 (d, *J* 246.4 Hz), 163.47 ppm; δ_F (282 MHz, $CDCl_3$): –115.27 (tt, *J* 9.0, 4.7 Hz), –196.89 ppm (ddd, *J* 50.3, 29.4, 13.5 Hz); HR-MALDI-MS (3-HPA): *m*/*z* 763.2691 (100, calcd for $C_{41}H_{37}F_2N_6O_7^+$ $[M+H]^+$: 763.2686).

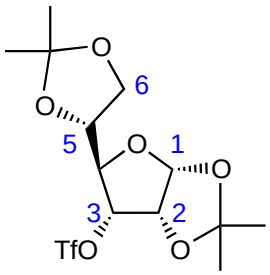
9-{(5*E*)-3,5,6,7-Tetradeoxy-3-fluoro-7-[5-(4-fluorophenyl)-2,3-dihydroxybenzamido]-*β*-D-*xylo*-hept-5-enofuranosyl}-N^6-methyladenine (5).



Nucleoside **26** (93 mg, 0.12 mmol) was treated with a mixture of THF (1 cm^3) and cold (0 °C) TFA/H_2O (1:1, 2 cm^3). The mixture was stirred at 0 °C for 60 min and evaporated. The residue was suspended MeCN. Two reverse-phase HPLC's (H_2O/MeCN/HCOOH 90:10:0.01 → 20:80:0.01) gave **5** as a white solid (35 mg, 53%).

Mp 150 °C (decomp.); $[\alpha]_D^{20}$ +20 (c 0.31, Me_2SO); IR (neat): $\tilde{\nu}$ 3389*br*., 2934*w*, 1605*s*, 1513*m*, 1469*s*, 1415*w*, 1371*w*, 1308*w*, 1249*w*, 1211*s*, 1173*s*, 1050*s*, 1023*s*, 952*s*, 829*s*, 790*w*, 643*m* cm^{-1}; δ_H (400 MHz, CD_3OD) 3.13-3.04 (s, 3 H), 4.09 (d, *J* 5.2 Hz, 2 H), 4.60 (d, *J* 12.7 Hz, 1 H), 4.58-4.98 (m, 2 H), 5.98-5.92 (m, 1 H), 6.07 (m, 2 H), 7.08 (m, 2 H), 7.15 (d, *J* 2.1 Hz, 1 H), 7.48 (d, *J* 2.1 Hz, 1 H), 7.54 (m, 2 H), 8.01 (s, 1 H), 8.23 ppm (s, 1 H); δ_C (CD_3OD, 101 MHz) 30.67, 41.74, 80.25 (d, *J* 27.3 Hz), 84.22 (d, *J* 18.7 Hz), 91.55, 97.88 (d, *J* 184.3 Hz), 116.40 (d, *J* 21.5 Hz), 116.93, 117.16, 118.13, 125.03, 125.12, 129.36 (d, *J* 8.0 Hz), 132.24, 133.76, 138.15, 139.48, 139.55, 147.81, 149.92, 154.05, 156.76, 163.56 (d, *J* 244.5 Hz), 171.25 ppm; δ_F (376 MHz, $(CD_3)_2SO$, decoup.): –118.10 ppm (s), –198.64 ppm (s); HR-MALDI-MS (3-HPA): *m*/*z* 539.1848 (100, calcd for $C_{26}H_{25}F_2N_6O_5^+$ $[M+H]^+$: 539.1849).

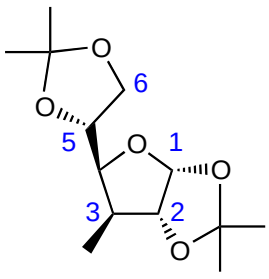
1,2:5,6-Di-*O*-isopropylidene-3-*O*-trifluoromethanesulfonyl-α-D-allopyranose (27).



A dry N_2-flushed flask, equipped with a magnetic stirrer and a septum, was charged with 4-(dimethylamino)pyridine (16.0 g, 130.7 mmol), ribose **14** (17.0 g, 65.4 mmol) and CH_2Cl_2 (250 cm^3). Trifluoromethanesulfonyl chloride (11.0 g, 65.4 mmol) was added dropwise at –20 °C. The mixture was stirred at 0 °C for 1 h. Then, the reaction was quenched with AcOH:H_2O (1:1). Afterwards, a sat. aq. solution of Na_2CO_3 (100 cm^3) was added and the obtained phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 x 80 cm^3), the combined organic phases were washed with a sat. aq. solution of $NaHCO_3$ (80 cm^3), dried ($MgSO_4$), filtered and concentrated in vacuo. The mixture was then purified by column chromatography (pentane/EtOAc 95:5) to give product **27** as a white oil (19.4 g, 76%).

$[\alpha]_D^{20}$ +68 (c 0.64, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2979*w*, 2918*w*, 1493*w*, 1462*w*, 1417*s*, 1380*m*, 1267*m*, 1247*m*, 1200*s*, 1146*s*, 1116*m*, 1078*w*, 994*s*, 932*m*, 872*s*, 845*s*, 803*w*, 703*m*, 652*m* cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.28 (s, 3 H), 1.31 (s, 3 H), 1.38 (s, 3 H), 1.51 (s, 3 H), 3.83 (dd, *J* 8.8, 4.7 Hz, 1H), 4.04 (dd, *J* 8.8, 6.4 Hz, 1H), 4.15-4.07 (m, 2H), 4.70 (dd, *J* 5.2, 3.9 Hz, 1H), 4.84 (dd, *J* 6.9, 5.3 Hz, 1H), 5.76 ppm (d, *J* 3.8 Hz, 1H); δ_C ($CDCl_3$, 101 MHz) 24.79, 26.23, 26.48, 26.85, 66.30, 75.23, 77.70, 77.90, 82.95, 104.19, 110.29, 114.40, 118.39 ppm (q, *J* 319.5 Hz); δ_F (282 MHz, $CDCl_3$): –74.81 ppm (t); HR-ESI-MS: *m*/*z* 172.0948 (100), 415.0644 (63, calcd for $C_{13}H_{19}F_3NaO_8S^+$ $[M+Na]^+$: 415.0645).

3-Deoxy-1,2:5,6-di-*O*-isopropylidene-3-methyl-α-D-glucopyranose (28).

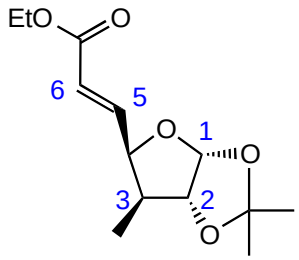


A dry N_2-flushed *Schlenk*-tube (50 cm^3), equipped with a magnetic stirrer and a septum, was charged with copper iodide (61.3 g, 28.57 mmol), and a solution of methyl lithium bromide in Et_2O (270 cm^3, 593 mmol) was added at –10 °C. The mixture was stirred at 0 °C. After 30 min,

a solution of **27** (19.4 g, 49.5 mmol) in THF was added dropwise and was stirred at 0 °C for 2 h. Then the mixture was heated to 20 °C and stirred for another 17 h. Afterwards, a sat. aq. solution of Na_2CO_3 (20 cm^3) was added and the obtained phases were separated. The aqueous phase was extracted with Et_2O (3 x 20 cm^3), the combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo. The mixture was then purified by column chromatography (pentane/EtOAc 4:1) to give starting material **27** (2.14 g, 2%) and product **28** (5.37 g, 47%, starting material substracted) as a yellow oils.

$[\alpha]_D^{20}$ +20 (c 0.51, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2985*m*, 2936*m*, 1456*w*, 1371*s*, 1253*w*, 1212*s*, 1167*m*, 1128*w*, 1061*s*, 1030*m*, 997*m*, 961*w*, 923*w*, 845*s*, 793*w* cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.89 (d, *J* 7.5 Hz, 3 H), 1.23 (s, 3 H), 1.28 (s, 3 H), 1.34 (s, 3 H), 1.45 (s, 3 H), 2.38 (dq, *J* 7.5, 4.2 Hz, 1 H), 3.86 (dt, *J* 8.2, 5.1 Hz, 1 H), 4.09-3.92 (m, 3 H), 4.29 (d, *J* 3.5 Hz, 1 H), 5.71 ppm (d, *J* 3.5 Hz, 1 H); δ_C ($CDCl_3$, 101 MHz) 11.05, 25.33, 26.08, 26.75, 26.81, 40.59, 68.42, 73.56, 80.74, 86.57, 104.79, 109.20, 111.23 ppm; HR-ESI-MS: *m*/*z* 281.1363 (100, calcd for $C_{13}H_{22}NaO_5^+$ $[M+Na]^+$: 281.1359).

Ethyl (5*E*/*Z*)-3,5,6-Trideoxy-1,2-*O*-isopropylidene-3-methyl-α-D-*xylo*-hept-5-enofuranuronate (29).

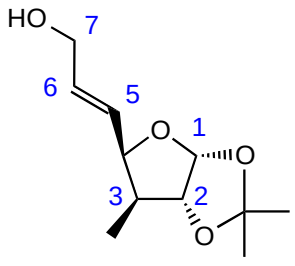


A dry N_2-flushed flask, equipped with a magnetic stirrer and a septum, was charged with ribose **28** (5.37 g, 22.1 mmol) in EtOAc (300 cm^3) and cooled to 0 °C. Sodium periodate (4.72 g, 22.1 mmol) and *ortho*periodic acid (5.03 g, 22.1 mmol) were added slowly. The mixture was stirred at 20 °C for 3 h, filtered, and the solvent was removed to give the aldehyde. A second dry N_2-flushed flask , equipped with a magnetic stirrer and a septum, was charged with LiCl (5.24 g,

123.7 mmol) in dry MeCN (100 cm^3). The suspension was cooled to 0 °C and the $(EtO)_2PO{=}CHCO_2Et$ (5.75 cm^3, 28.71 mmol) followed by iPr_2NEt (2.53 cm^3, 27.6 mmol) were added. The resulting mixture was stirred for 30 min at 20 °C and cooled back to 0 °C. The solution of the aldehyde in MeCN (200 cm^3) was slowly transferred into the mixture. The mixture was stirred for 3 h at 0 °C and was quenched with a sat. aq. solution of NH_4Cl. The aqueous phase was extracted with Et_2O (3 x 500 cm^3). The organic phase was washed with sat. aq. solution of $NaHCO_3$ and brine. The combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The mixture was then purified by column chromatography (pentane/EtOAc 4:1) to give product **29** as a yellow oil (2.61 g, 46%).

$[\alpha]_D^{20}$ –38 (c 0.16, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2981*m*, 1718*s*, 1662*w*, 1458*w*, 1373*m*, 1304*m*, 1259*m*, 1215*w*, 1168*s*, 1120*w*, 1061*s*, 1015*s*, 981*w*, 910*w*, 861*s*, 722*w*, 654*w*, 620*w* cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.73 (d, *J* 7.5 Hz, 3 H), 1.22 (t, *J* 7.2 Hz, 3 H), 1.25 (s, 3 H), 1.45 (s, 3 H), 2.35 (dq, *J* 7.5, 4.5 Hz, 1 H), 4.13 (q, *J* 7.1 Hz, 2 H), 4.34 (d, *J* 3.6 Hz, 1 H), 4.82 (dt, *J* 4.4, 1.84 Hz, 1 H), 5.78 (d, *J* 3.6 Hz, 1 H), 6.06 (dd, *J* 15.7, 1.9 Hz, 1 H), 6.79 ppm (dd, *J* 15.7, 4.4 Hz, 1 H); δ_C ($CDCl_3$, 101 MHz) 11.50, 14.32, 25.90, 26.86, 42.66, 60.39, 78.55, 86.34, 104.31, 111.34, 122.15, 143.23, 166.15 ppm; HR-ESI-MS: *m*/*z* 279.1203 (100, calcd for $C_{13}H_{20}NaO_5^+$ $[M+Na]^+$: 279.1205).

(5*E*)-3,5,6-Trideoxy-1,2-*O*-isopropylidene-3-methyl-α-D-*xylo*-hept-5-enofuranose (30).

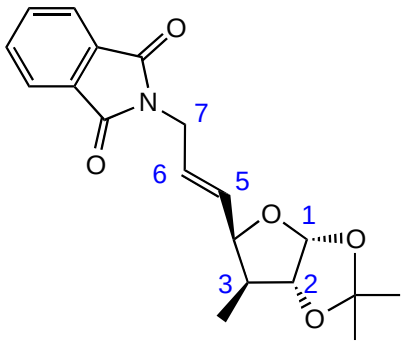


Compound **29** (2.61 g, 10.19 mmol) was dissolved in CH_2Cl_2 (45 cm^3) and cooled to –78 °C. DIBAL-H (27.51 cm^3, 1 M in cyclohexane, 27.51 mmol) was added slowly and the solution was stirred for 4 h at –78 °C. A sat. aq. solution of NH_4Cl (100 cm^3) was added carefully at –78 °C,

the solution was warmed to 20 °C and sat. aq. solution of ammonium tartrate (50 cm^3) was added. After stirring for 18 h, the aqueous phase was extracted with CH_2Cl_2 (3 x 200 cm^3), the combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2; pentane/EtOAc 3:1) to give product **30** as a clear oil (2.11 g, 97%).

$[\alpha]_D^{20}$ –41 (*c* 0.63, $CHCl_3$); IR (neat): $\tilde{\nu}$ 3433*br*, 2979*w*, 2936*w*, 2879*w*, 1667*w*, 1457*w*, 1374*s*, 1304*w*, 1256*w*, 1214*s*, 1168*m*, 1120*w*, 1097*w*, 1060*m*, 1168*s*, 1003*s*, 971*s*, 910*w*, 868*s*, 651*w*, 623*w* cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.84 (d, *J* 7.5 Hz, 3 H), 1.31 (s, 3 H), 1.52 (s, 3 H), 1.59 (br. s, 1 H), 2.29 (qd, *J* 7.5, 4.3 Hz, 1 H), 4.17 (d, *J* 5.2 Hz, 2 H), 4.39 (d, *J* 3.6 Hz, 1 H), 4.74 (t, *J* 5.4 Hz, 1 H), 5.69 (ddt, *J* 15.6, 6.6, 1.5 Hz, 1 H), 5.82 (d, *J* 3.6 Hz, 1 H), 5.98 ppm (dtd, *J* 15.6, 5.2, 1.1 Hz, 1 H); δ_C (75 MHz, $CDCl_3$) 11.30, 26.05, 26.65, 42.91, 62.96, 79.71, 86.51, 104.21, 110.98, 126.58, 132.68 ppm; HR-ESI-MS: *m*/*z* 172.09 (86), 237.1096 (100, calcd for $C_{11}H_{18}NaO_4^+$ $[M+Na]^+$: 237.1097).

(5*E*)-3,5,6,7-Tetradeoxy-1,2-*O*-isopropylidene-3-methyl-7-phthalimido-α-D-*xylo*-hept-5-enofuranose (31).

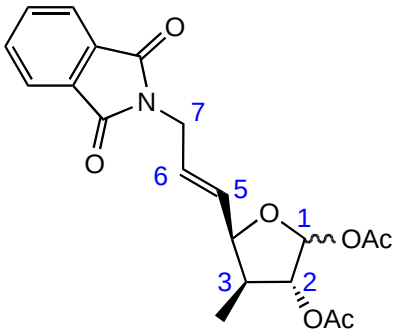


Triphenyl phosphine (5.14 g, 19.62 mmol) was dissolved in THF (70 cm^3), and at 0 °C DIAD (3.89 cm^3, 19.62 mmol) was added. The solution was stirred for 1 h. A solution of **30** (2.10 g, 9.81 mmol) in THF (40 cm^3) and slowly added. Phthalimide (2.88 g, 19.62 mmol) was added and the mixture was stirred for 20 h. Afterwards, H_2O (200 cm^3) and EtOAc (200 cm^3) were added and the obtained phases were separated. The aqueous phase was extracted with EtOAc (2

x 200 cm^3), the combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2; pentane/EtOAc 5:1) to give product **31** as a clear oil (3.33 g, 99%).

$[\alpha]_D^{20}$ –40 (*c* 1.79, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2980*w*, 2936*w*, 1771*w*, 1709*s*, 1467*w*, 1428*m*, 1393*s*, 1352*w*, 1321*w*, 1251*w*, 1214*m*, 1169*m*, 1110*m*, 1084*w*, 1061*s*, 1011*s*, 972*m*, 941*w*, 912*w*, 870*m*, 795*w*, 714*s*, 619*m* cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.79 (d, *J* 7.5 Hz, 3 H), 1.29 (s, 3 H), 1.48 (s, 3 H), 2.27 (qd, *J* 7.5, 4.4 Hz, 1 H), 4.30 (d, *J* 6.5 Hz, 2 H), 4.36 (d, *J* 3.6 Hz, 1 H), 4.71 (t, *J* 4.8 Hz, 1 H), 5.70 (ddt, *J* 15.5, 5.9, 1.2 Hz, 1 H), 5.78 (d, *J* 3.6 Hz, 1 H), 5.86 (dtd, *J* 15.5, 6.0, 1.3 Hz, 1 H), 7.71 (m, 2 H), 7.84 ppm (m, 2 H); δ_C (101 MHz, $CDCl_3$) 11.30, 26.09, 26.67, 39.09, 42.87, 79.27, 86.45, 111.03, 123.58, 126.03, 129.83, 132.15, 133.92, 134.28, 167.77 ppm; HR-ESI-MS: *m*/*z* 366.1318 (100, calcd for $C_{19}H_{21}NNaO_6^+$ $[M+Na]^+$: 366.1312).

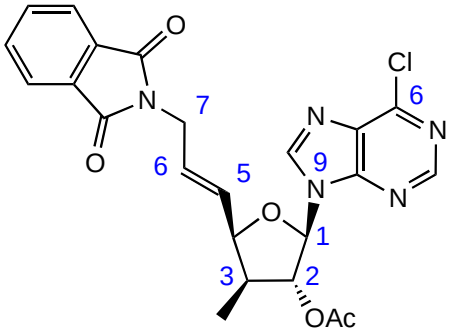
(5*E*)-1,2-Di-*O*-acetyl-3,5,6,7-tetradeoxy-3-methyl-7-phthalimido-α/*β*-D-*xylo*-hept-5-enofuranose (32).



Compound **31** (3.33 g, 9.69 mmol) was treated with a mixture of HOAc/H_2O 4:1 (80 cm^3) at 20 °C. The solution was stirred at 120 °C for 16 h, and the solvents were removed *in vacuo* with addition of toluene (2 x 50 cm^3) to give a residue, which was stirred with dry pyridine (20 cm^3), acetic acid anhydride (20 cm^3) and a catalytic amount of DMAP at 20 °C for 3 h. After evaporation the oily residue was diluted with H_2O (400 cm^3) and EtOAc (400 cm^3) and the phases separated. The aqueous phase was extracted with EtOAc (2 x 400 cm^3); the combined organic phases were washed with a sat. aq. solution of NH_4Cl (200 cm^3), dried ($MgSO_4$),

filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2; pentane/EtOAc 95:5 → 1:1) to give **32** (α/β 4:5, 2.43 g, 65%, two steps) as a clear oil. $[\alpha]_D^{20}$ +28 (*c* 1.52, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2974*w*, 2939*w*, 1743*m*, 1709*s*, 1467*w*, 1428*m*, 1393*m*, 1372*m*, 1238*s*, 1218*s*, 1010*w*, 1064*m*, 1046*m*, 1007*m*, 953*m*, 897*w*, 857*w*, 797*w*, 722*s* cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.98 (d, *J* 7.1 Hz, 3 H), 1.06 (d, *J* 7.6 Hz, 2.5 H), 2.05 (s, 3 H), 2.06 (s, 3 H), 2.06 (s, 3 H), 2.08 (s, 3 H), 2.37 (qd, *J* 7.2, 2.4 Hz, 0.8 H), 2.57 (qd, *J* 7.2, 1.0 Hz, 1 H,), 4.30 (m, 3.8 H), 4.75 (m, 1.9 H), 4.81 (dd, *J* 4.3, 8.6 Hz, 1 H,), 4.97 (d, *J* 2.4 Hz, 0.8 H), 5.64 (ddt, *J* 15.4, 6.7, 1.2 Hz, 1 H), 5.78 (m, 2.7 H), 6.06 (s, 0.8 H,), 6.34 (d, *J* 4.3 Hz, 1 H), 7.72 (m, 3.7 H), 7.86 ppm (m, 3.7 H); δ_C (101 MHz, $CDCl_3$) 12.38, 12.45, 20.53, 20.86, 20.99, 21.10, 38.00, 38.82, 41.52, 83.55, 83.64, 93.68, 100.05, 123.30, 130.33, 130.70, 132.11, 134.00, 167.73, 169.49, 169.67, 169.95, 170.15 ppm; HR-ESI-MS: *m*/*z* 410.1221 (100, calcd for $C_{20}H_{21}NO_7Na^+$ $[M+Na]^+$: 410.1210).

9-[(5*E*)-2-*O*-Acetyl-3,5,6,7-tetradeoxy-3-methyl-7-phthalimido-*β*-D-*xylo*-hept-5-enofuranosyl]-6-chloropurine (33).

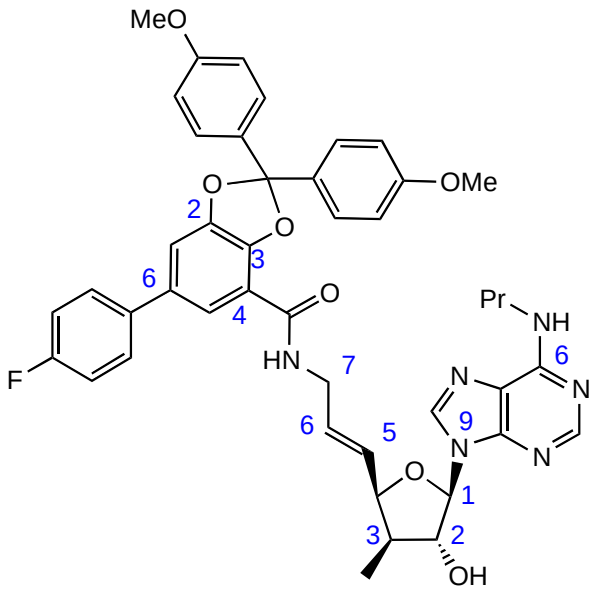


A dry N_2-flushed *Schlenk*-tube (250 cm^3), equipped with a magnetic stirrer and a septum, was charged with 6-chloropurine (1.44 g, 9.32 mmol), toluene (50 cm^3), and *N*,*O*-bis(trimethylsilyl) acetamide (1.89 cm^3, 7.77 mmol). The mixture was heated to 60 °C. After 30 min, the mixture was cooled to 20 °C when **32** (1.2 g, 3.11 mmol) and trimethylsilyl trifluoromethanesulfonate (3.37 cm^3, 18.65 mmol) were added slowly. The mixture was heated to 60 °C, stirred for 20 h and then cooled to 20 °C. Afterwards, a sat. aq. solution of $NaHCO_3$ (300 cm^3) and CH_2Cl_2 (300

cm^3) were added and the obtained phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 300 cm^3), the combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified twice by flash chromatography (SiO_2; pentane/EtOAc 9:1 → 1:9) (SiO_2; pentane/EtOAc 3:1 → 1:7) to give **33** as a yellow oil (270 mg, 18%) together with a crude fraction of **33**.

$[\alpha]_D^{20}$ +24 (c 0.41, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2971*w*, 2933*w*, 1747*m*, 1708*s*, 1592*m*, 1561*m*, 1489*w*, 1468*w*, 1394*s*, 1373*w*, 1338*m*, 1222*s*, 1205*s*, 1135*m*, 1112*m*, 1046*s*, 951*s*, 938*s*, 908*w*, 857*m*, 713*s*, 637*m*, 608*w* cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.11 (d, *J* 7.1 Hz, 3 H), 1.77 (s, 3 H), 2.75 (quint.d, *J* 6.9, 4.9 Hz, 1 H), 4.36 (d, *J* 6.0 Hz, 2 H), 5.11 (t, *J* 6.3 Hz, 1 H), 5.26 (t, *J* 4.8 Hz, 1 H), 5.76 (ddt, *J* 15.4, 6.3, 1.3 Hz, 1 H), 5.95 (dtd, *J* 15.4, 6.0, 1.2 Hz, 1 H), 6.64 (d, *J* 4.8 Hz, 1 H), 7.74 (m, 2 H), 7.87 (m, 2 H), 8.20 (s, 1 H), 8.72 ppm (s, 1 H); δ_C ($CDCl_3$, 101 MHz) 12.22, 20.33, 38.85, 41.61, 81.08, 81.81, 84.19, 123.39, 127.47, 129.24, 131.50, 132.04, 134.09, 144.00, 151.07, 151.25, 152.08, 167.75, 169.23 ppm; HR-ESI-MS: *m*/*z* 328.1183 (100), 482.1228 (49, calcd for $C_{23}H_{21}ClN_5O_5^+$ $[M+H]^+$: 482.1226).

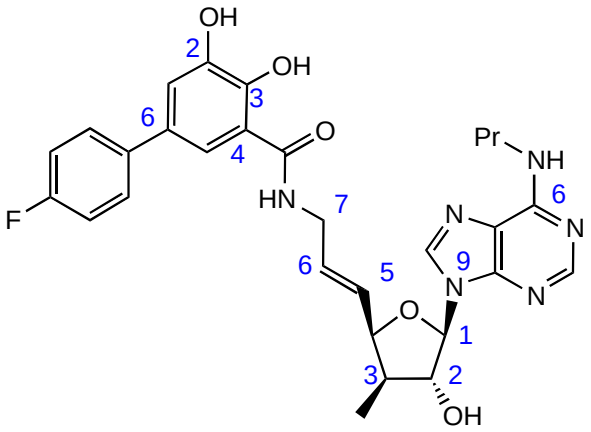
9-{(5*E*)-3,5,6,7-Tetradeoxy-7-[6-(4-fluorophenyl)-2,2-bis(4-methoxyphenyl)-1,3-benzodioxole-4-carboxamido]-3-methyl-*β*-D-*xylo*-hept-5-enofuranosyl}-*N*^{6}-propyladenine (34).



A dry N_2-flushed flask (25 cm^3), equipped with a magnetic stirrer and a septum, was charged with crude **33** (250 mg, 0.52 mmol, contained ca. 45% N7-isomer), EtOH (7 cm^3), and propylamine (0.75 cm^3) was added. The mixture was stirred for 5 d at 20 °C and evaporated. The residue was transferred into a *Schlenk*-tube (20 cm^3) and dried under high vacuum for 24 h to afford a mixture of the primary amine. In a dry N_2-flushed flask (10 cm^3), equipped with a magnetic stirrer and a septum, **18** (197 mg, 0.52 mmol) was dissolved in dry DMF (2 cm^3). *i*Pr_2NEt (0.07 cm^3, 0.78 mmol), HOBt (77 mg, 0.57 mmol), and HBTU (197 mg, 0.52 mmol) were added at 0 °C. The mixture was stirred for 60 min at 0 °C and then added slowly to a solution of the primary amine in DMF (2 cm^3) at 0 °C. The reaction was stopped after 18 h by the addition of ice. Two purifications with reverse-phase HPLC (H_2O/MeCN/HCOOH 90:10:0.01 → 20:80:0.01) gave **34** as a white solid (160 mg, 39%, 2 steps) with some impurities (N7 isomer and **18**).

$[\alpha]_D^{20}$ +2 (c 0.53, $CHCl_3$); IR (neat): $\tilde{\nu}$ 2934w, 1713*w*, 1666*w*, 1611*s*, 1513*s*, 1469*s*, 1416*w*, 1250*s*, 1209*s*, 1172*s*, 1115*w*, 1050*m*, 1026*m*, 1005*m*, 952*w*, 933*w*, 829*s*, 775*w*, 756*w*, 636*w*, 616*w* cm^{-1}; δ_H (400 MHz, $CDCl_3$, only peaks for **34** given) 0.88-0.95 (m, 6 H), 1.63-1.72 (m, 2 H), 2.54 (t, *J* 8.0 Hz, 1 H), 3.52 (br. m, 2 H), 3.71 (s, 6 H), 4.05-4.13 (m, 3 H), 4.79 (t, *J* 7.9 Hz, 1 H), 5.55-5.64 (m, 2 H), 5.78 (dt, *J* 15.4, 5.5 Hz, 1 H), 6.79-6.84 (m, 4 H), 6.95-7.02 (m, 2 H), 7.12 (d, *J* 1.9 Hz, 1 H), 7.31-7.37 (m, 2 H), 7.43-7.47 (m, 4 H), 7.55 (d, *J* 1.9 Hz, 1 H), 7.95 (s, 1 H), 8.25 ppm (s, 1 H); δ_C (101 MHz, $CDCl_3$, only peaks for **34** given) 11.61, 12.52, 21.20, 31.54, 40.96, 42.32, 55.45, 80.83, 84.22, 92.26, 113.74, 113.87, 115.77 (d, *J* 21.4 Hz), 119.24, 119.54, 121.04, 121.72, 128.31, 128.38, 128.53 (d, *J* 8.0 Hz), 128.64, 130.75, 131.02, 134.15, 135.09, 136.39, 144.55, 148.36, 149.30, 160.54, 160.85, 162.43 (d, *J* 246.5 Hz), 168.16 ppm; δ_F (376 MHz, $CDCl_3$, decoup.): –115.60 ppm (s); HR-ESI-MS: *m*/*z* 787.3257 (100, calcd for $C_{44}H_{44}FN_6O_7^+$ $[M+H]^+$: 787.3250).

9-{(5*E*)-3,5,6,7-Tetradeoxy-7-[5-(4-fluorophenyl)-2,3-dihydroxybenzamido]-3-methyl-*β*-D-*xylo*-hept-5-enofuranosyl}-N^6-propyladenine (6).



Crude nucleoside **34** (188 mg, 0.25 mmol) was treated with THF (1 cm^3) and cold (0 °C) TFA/H_2O (1:1, 1.5 cm^3). The mixture was stirred at 0 °C for 60 min. The solution was treated with Na_2CO_3 until pH 5 was reached. Two-fold purification by reverse-phase HPLC (H_2O/MeCN/HCOOH 90:10:0.01 → 20:80:0.01) gave crude **6** as a white solid (38 mg, 33%). Purification of bisubstrate inhibitor **6** proved to be very challenging. Multiple attempts to isolate the pure compound by reverse phase HPLC failed. **6** remained contaminated with ~45% of a regioisomer (N7 nucleoside). Mass spectrometry showed a single peak at 563.2410, underlining the fact that a regioisomer is present: HR-MALDI-MS (3-HPA): *m*/*z* (%): 563.2410 (100, calcd for $C_{29}H_{32}FN_6O_5^+$ $[M+H]^+$: 563.2413). However, even an attempted separation of these isomers by reverse phase HPLC and chiral reverse phase HPLC at Hoffmann-La Roche in Basel failed. Crude **6** was used for the biological assay. The reported IC_{50} and K_i values are the values obtained for crude **6**. The concentration was not corrected for by mathematical means and thus the real IC_{50} and K_i values for a pure **6** are expected to be lower by ~45%.

Mp 130–135 °C (decomp.); $[\alpha]_D^{20}$ +18 (c 0.40, Me_2SO); IR (neat): $\tilde{\nu}$ 3333*br*, 2967w, 2935w, 2878w, 1620*s*, 1605*s*, 1541*m*, 1475*s*, 1405*w*, 1306*s*, 1219*s*, 1160*w*, 1116*w*, 1099*w*, 1064*s*, 969*m*, 888*w*, 833*s*, 813*m*, 781*w*, 723*w*, 641*m*, 619*w* cm^{-1}; δ_H (400 MHz, CD_3OD, only peaks for **6** given) 0.99-1.08 (m, 6 H), 1.70 (dt, *J* 14.5, 7.3 Hz, 2 H), 2.52 (q, *J* 7.6 Hz, 1 H), 3.53 (br m, 2

H), 4.01-4.09 (m, 2 H), 4.66 (dd, *J* 8.4, 6.1 Hz, 1 H), 5.10 (t, *J* 6.5 Hz, 1 H), 5.75-5.87 (m, 2 H), 6.04 (dd, *J* 15.3, 7.8 Hz, 1 H), 7.10-7.15 (m, 2 H), 7.20 (d, *J* 2.1 Hz, 1 H), 7.51 (d, *J* 2.2 Hz, 1 H), 7.56-7.61 (m, 2 H), 8.12 (s, 1 H), 8.21 ppm (s, 1 H); δ_C (CD_3OD, 101 MHz, only peaks for **6** given) 11.63, 12.76, 23.76, 41.62, 44.29, 45.06, 79.46, 83.87, 91.01, 116.38 (d, *J* 21.2 Hz), 117.11, 118.18, 129.36 (d, *J* 7.9 Hz), 129.91, 130.20, 130.52, 130.82, 132.29, 138.12, 140.67, 141.49, 147.77, 149.82, 153.75, 156.07, 163.61 (d, *J* 244.6 Hz), 171.22 ppm; δ_F (376 MHz, CD_3OD, decoup.): –118.70 ppm (s); HR-MALDI-MS (3-HPA): *m/z* 563.2410 (100, calcd for $C_{29}H_{32}FN_6O_5^+$ $[M+H]^+$: 563.2413).

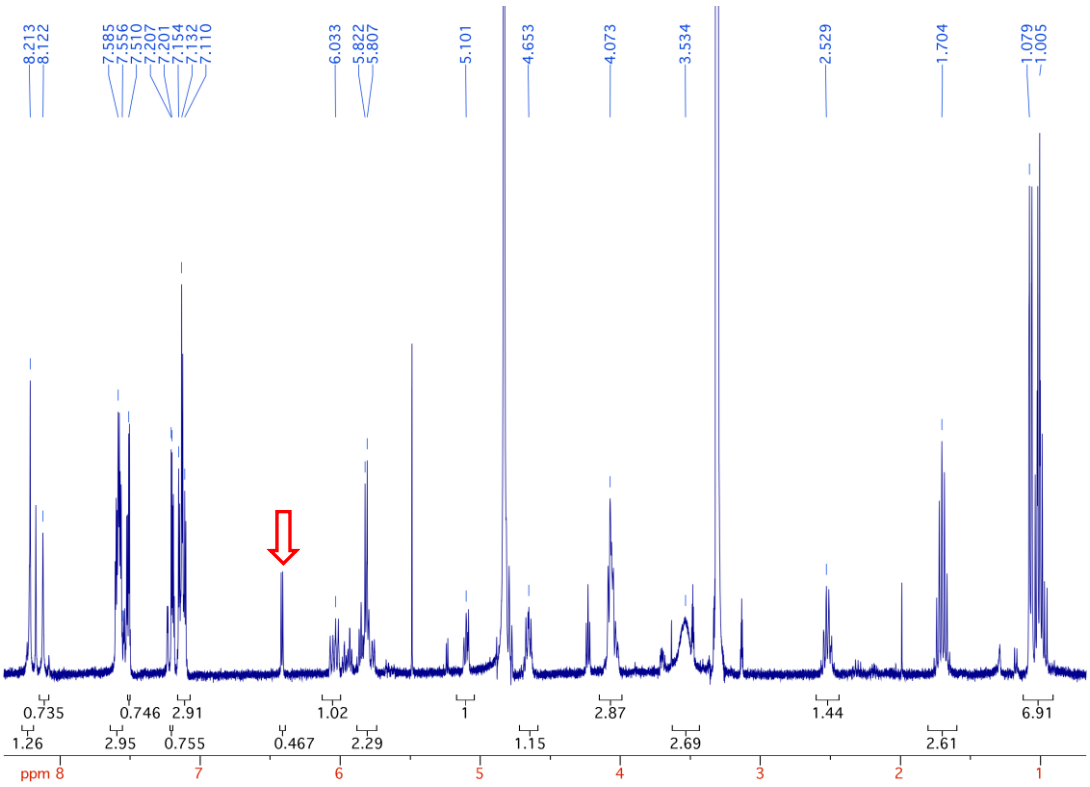
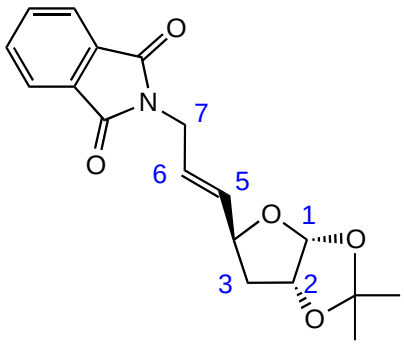


Fig. S2 NMR data for crude bisubstrate inhibitor **6**. Only peaks for **6** are labelled except peak highlighted in red which is from impurity to demonstrate the ratio: ~45%.

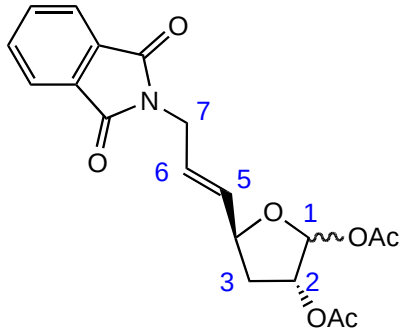
2-[(E)-3-((3aR,5S,6aR)-2,2-Dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-yl)-allyl]-isoindole-1,3-dione (36).



To a solution of phthalimide (1.6 g, 11 mmol, 1 eq) , triphenylphosphine (2.88 g, 11 mmol, 1 eq) and **35** (2.2 g, 11 mmol, 1 eq) in THF (53 mL) at 0 °C was added slowly a DEAD solution 40% in toluene (5.2 mL, 11.2 mmol, 1.02 eq), the solution was stirred for 4 hours at room temperature. The mixture was concentrated in vacuo. The residue was purified by flash chromatography to give the desired product **36** as a white solid (2.9 g, 80 %).

δ_H (600 MHz, $CDCl_3$) 1.30 (s, 3 H), 1.49 (s, 3 H), 1.55-1.61 (m, 1 H), 2.15 (dd, *J* 13.4, 4.2 Hz, 1 H), 4.28 (d, *J* 6.0 Hz, 2 H), 4.60-4.64 (m, 1 H), 4.71 (t, *J* 4.1 Hz, 1 H), 5.74 (dd, *J* 15.5, 6.5 Hz, 1 H), 5.80 (d, *J* 3.7 Hz, 1 H), 5.84-5.89 (m, 1 H), 7.71 (dd, *J* 5.4, 3.0 Hz, 2 H), 7.85 (dd, *J* 5.4, 3.0 Hz, 2 H) ppm; EI-MS: *m*/*z* 329, $M^{\cdot+}$.

Acetic acid (3R,5S)-3-acetoxy-5-[(E)-3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propenyl]-tetrahydro-furan-2-yl ester (37)

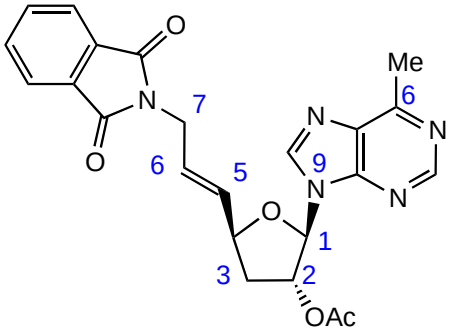


Aqueous H_2SO_4 (1 mL, 0.4 %) was added to a solution of **36** (100 mg, 0.3 mmol,1 eq) in dioxane (1 mL), and the solution was stirred during 48 h at rt. The mixture was neutralized with Na_2CO_3, filtered, and then concentrated to dryness. The residue was dissolved in pyridine (1 mL), and acetic anhydride (1 mL) and a catalytic amount of DMAP were added. The solution

was stirred overnight at rt. The solvent was evaporated and afterwards, aq. sat. NH_4Cl (2 mL) and EtOAc (2 mL) were added and the obtained phases were separated. The aqueous phase was extracted with EtOAc (2 x 5 mL), the combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash chromatography to give the desired product **37** (mixture of epimers) as a yellow oil (66 mg, 58%).

δ_H (600 MHz, $CDCl_3$) 2.00-2.10 (m, 6 H), 2.33-2.46 (m, 2 H), 3.82-3.85 (m, 1 H), 3.87-2.92 (m, 1 H), 5.05-5.09 (m, 1 H), 5.50-5.54 (m, 1 H), 5.57-5.62 (m, 1 H), 5.74-5.80 (m, 1 H), 6.82 (m, 6.81-6.82, 1 H), 7.72-7-74 (m, 2 H), 7.85-7.87 (m, 2 H) ppm; EI-MS: *m*/*z* 373, $M^{\cdot+}$.

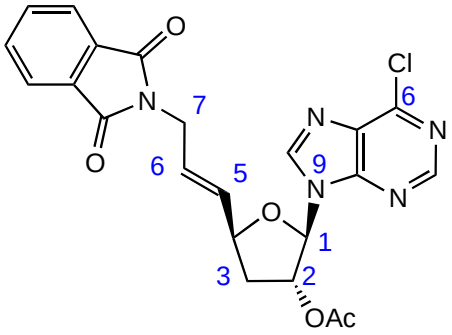
Acetic acid (2R,3R,5S)-5-[(E)-3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propenyl]-2-(6-methyl-purin-9-yl)-tetrahydro-furan-3-yl ester (38)



To a suspension of 6-methylpurine (100 mg, 0.75 mmol, 2 eq) in dichloroethane (3 mL), bis(trimethylsilyl)acetamide (154 mg, 0.56 mmol, 1.5 eq) and TMSOTf (250 mg, 1.125 mmol, 3 eq) were added. The solution was heated to 60 °C and after 5 min, **37** (140 mg, 0.375 mmol, 1 eq) in dichloroethane (3 mL) was added. The mixture was stirred 3 h at 60 °C. Saturated aq. $NaHCO_3$ (4 mL) and CH_2Cl_2 (4 mL) were added, and the obtained phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 5 mL), the combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash chromatography to give the desired product **38** as a clear oil (115 mg, 68%). δ_H (600 MHz, $CDCl_3$) 2.14 (s, 3 H), 2.29-2.33 (m, 1 H), 2.57-2.62 (m, 1 H), 2.85 (s, 3H), 4.31 (d, *J* 3.53 Hz, 2

H), 4.81-4.84 (m, 1 H), 5.70 (d, *J* 5.8 Hz, 1 H), 5.90-5.91 (m, 2 H), 6.07 (d, *J* 1.2 Hz, 1H), 7.72-7.75 (m, 2 H), 7.84-7.86 (m, 2 H), 8.07 (s, 1 H), 8.79 (s, 1 H) ppm; ESI-MS: *m/z* 448.1, $[M+H]^+$.

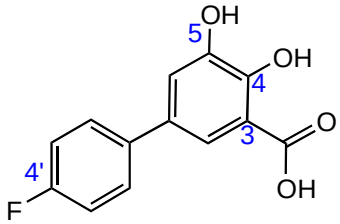
Acetic acid (2R,3R,5S)-2-(6-chloro-purin-9-yl)-5-[(E)-3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propenyl]-tetrahydro-furan-3-yl ester (39)



The procedure of product **38** was used but 6-methylpurine was replace by 6-chloropurine (120 mg, 0.75 mmol, 2 eq) to give the desired product **39** as a purple oil (85 mg, 45%).

δ_H (300 MHz, $CDCl_3$) 2.15 (s, 3 H), 2.33 (dd, *J* 14.1, 6.5 Hz, 1 H), 2.53-2.63 (m, 1 H), 4.32 (d, *J* 4.0 Hz, 2 H), 4.82-4.89 (m, 1 H), 5.68 (d, *J* 5.9 Hz, 1 H), 5.89-5.93 (m, 2 H), 6.08 (d, *J* 1.2 Hz, 1 H), 7.71-7.76 (m, 2 H), 7.83-7.88 (m, 2 H), 8.69 (s, 1 H), 8.70 (s, 1 H) ppm; ESI-MS: *m/z* 468.2, $[M+H]^+$.

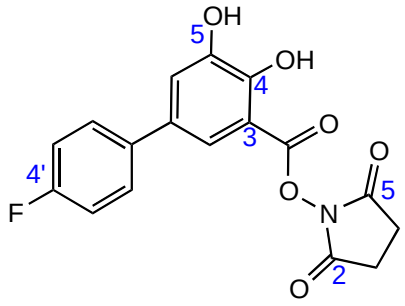
4'-Fluoro-4,5-dihydroxy-biphenyl-3-carboxylic acid (41)



Dichloro-1,1'-bis(diphenylphosphino)ferrocene palladium (II) dichloromethane adduct (2 g, 2 mmol, 0.03 eq), 4-fluorophenylboronic acid (17.43 g, 124 mmol, 1.5 eq), and a solution of sodium carbonate (26 g, 249 mmol, 3 eq) in water (78 ml) were added to 5-bromo-2,3-dihydroxybenzoic acid (19.35 g, 83 mmol, 1 eq) in dioxane (190 ml). The mixture was heated at 80 °C for 17 h. Dioxane was evaporated, water (500 ml) was added, and the mixture extracted with Et_2O (300 ml). The aqueous phase was acidified by addition of 25% aq HCl and extracted

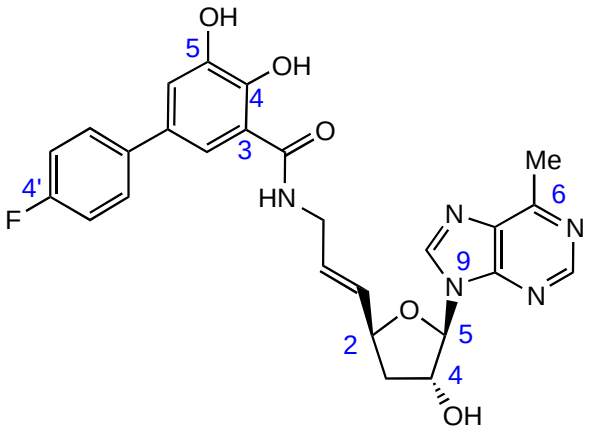
with EtOAc (3 x 300 ml). The combined organic layers were dried over Na_2SO_4 and concentrated to give **41** (20 g, 97 %) as a light brown solid. δ_H (300 MHz, CD_3OD) 5.61 (t, *J* 8.7 Hz, 2 H), 5.72 (d, *J* 2.1 Hz, 1 H), m (5.99-6.03, 3 H) ppm; ESI-MS: *m*/*z* 247.2, $[M-H]^-$.

4'-Fluoro-4,5-dihydroxy-biphenyl-3-carboxylic acid 2,5-dioxo-pyrrolidin-1-yl ester (42)



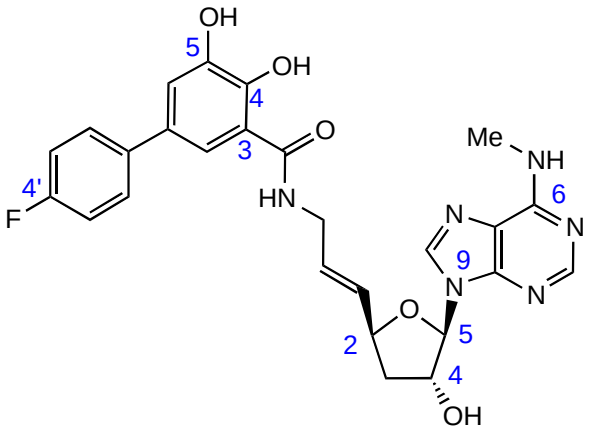
To **41** (300 mg, 1.21 mmol, 1 eq) in dry THF (10 mL) cooled to 0 °C, hydroxysuccinimide (139 mg, 1.21 mmol, 1 eq) and *N*-cyclohexyl, *N'*-methyl carbodiimide polystyrene resin (2.3 mmol/g, 1.05 g, 2.41 mmol, 2 eq) were added. The mixture was stirred overnight and allowed to slowly warm to rt. The mixture was filtered and concentrated *in vacuo* to give the desired product **42** (375 mg, 89%) as a brown solid, which was used without further purification in the next step. δ_H (300 MHz, $CDCl_3$) 2.95 (s, 4 H), 7.11 (t, *J* 8.7 Hz, 2 H), 7.41 (d, *J* 2.2 Hz, 1 H), 7.46-7.50 (m, 2 H), 7.69 (d, *J* 2.2 Hz, 1 H) ppm; ESI-MS: *m*/*z* 346.2, $[M+H]^+$.

4'-Fluoro-4,5-dihydroxy-biphenyl-3-carboxylic acid {(E)-3-[(2S,4R,5R)-4-hydroxy-5-(6-methyl-purin-9-yl)-tetrahydro-furan-2-yl]-allyl}-amide (4)



Compound **38** (150 mg, 0.335 mmol, 1 eq.) was dissolved with a solution of methylamine (7 mL, 33% in ethanol). The mixture was stirred for 48 h at 50 °C, the solvent was then removed in vacuo. The residue was dissolved in acetic acid (10 mL, 2%) and washed with CH_2Cl_2 (3x10 mL). The aqueous phase was evaporated to dryness. The residue was dissolved in DMF (4 mL), **42** (121 mg, 0.35 mmol, 1.05 eq) and *N*-*N*-diisopropyl ethyl amine (0.228 mL, 1.34 mmol, 4 eq) were added and stirred during two hours at rt. The mixture was purified by reversed phase HPLC to give the desired product **4** as a brown solid (31 mg, 19 %, 2 steps). δ_H (600 MHz, CD_3OD) 2.20-2.23 (m, 1 H), 2.30-2.35 (m, 1 H), 2.78 (s, 3 H), 4.06 (d, *J* 3.4 Hz, 2 H), 4.81-4.82 (m, 1 H), 4.95-4.98 (m, 1 H), 5.93-5.94 (m, 2 H), 6.01 (d, *J* 1.4 Hz, 1 H), 7.11-7.14 (m, 2 H), 7.19 (d, *J* 2.1 Hz, 1 H), 7.50 (d, *J* 2.2 Hz, 1 H), 7.56-7.59 (m, 2 H), 8.48 (s, 1 H), 8.73 (s, 1 H) ppm; ESI-MS: *m*/*z* 506.1, $[M+H]^+$.

4'-Fluoro-4,5-dihydroxy-biphenyl-3-carboxylic acid {(E)-3-[(2S,4R,5R)-4-hydroxy-5-(6-methylamino-purin-9-yl)-tetrahydro-furan-2-yl]-allyl}-amide (12)



The procedure of **4** was used but compound **38** was replaced by **39** (50 mg, 0.11 mmol, 1 eq) to give the desired product **12** as a brown solid (10 mg, 18%).

δ_H (300 MHz, CD_3OD) 2.21-2.24 (m, 2 H), 3.01 (s, 3 H), 4.08 (d, *J* 3.4 Hz, 2 H), 4.89-4.93 (m, 2 H), 5.93-5.97 (m, 2 H), 6.01-6.03 (m, 1 H), 7.12-7.17 (m, 2 H), 7.20-7.22 (m, 1 H), 7.51-7.55 (m, 1 H), 7.57-7.63 (m, 2 H), 8.0 (s, 1 H), 8.25 (s, 1 H) ppm; ESI-MS: *m*/*z* 519.2, $[M+H]^+$.

Binding Assay

The radiochemical assay was conducted as described previously:[6] "For the determination of IC_{50} values, 25 μL aqueous COMT solution (in the form of an aqueous rat liver homogenate) was mixed with 25 μL of various concentrations of the inhibitors in Me_2SO (0.12 nM to 120 μM), 10 μL $MgCl_2$ (0.1 mol L^{-1}), 170 μL potassium phosphate buffer (0.1 mol L^{-1}, pH 7.6), and 10 μL dithiothreitol (10 mg mL^{-1}). After preincubation for 15 min at 37 °C, 15 μL of the substrate benzene-1,2-diol (0.05 mol L^{-1}), and 10 μL of the cofactor [^{3}H]SAM (5.5 mmol L^{-1}, specific activity: 3.64 Ci mol^{-1}) were added. During the enzymatic reaction, which was stopped after 15 min by addition of 200 μL HOAc (5.7 %) containing guaiacol (0.1 g L^{-1}), the tritiated methyl group was transferred to the substrate. The resulting product was extracted into the organic phase by addition of 3 mL of scintillation fluid (5 g butyl-PBD, dissolved in 200 mL toluene and 800 mL n-hexane), whereas (^{3}H)-SAM remained in the aqueous phase. The product concentrations were measured by counting the decays per minute (dpm) of the organic phase in a Beckmann LS 6000 TA scintillation counter.

The K_i values were calculated by the following equation:

$$K_i = \frac{IC_{50}}{(S/K_m + 1)}$$

with K_m = 40 μM, S = 180 μM. S = [SAM].

The liver homogenate was prepared by homogenizing rat liver in ice cold water (1:10 *w*/*v*) containing 0.2 % Triton X-100, and centrifuging it at 12000 g for 20 min at 4 °C. Supernatants were further diluted (1:10) with water."

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