High-Affinity Alkynyl Bisubstrate Inhibitors of NicotinamideN-Methyltransferase (NNMT)

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ABSTRACT

Nicotinamide N-methyltransferase (NNMT) is a metabolic enzyme that methylates nicotinamide (NAM) using cofactor S-adenosylmethionine (SAM). NNMT overexpression has been linked to diabetes, obesity, and various cancers. In this work, structure-based rational design led to the development of potent and selective alkynyl bisubstrate inhibitors of NNMT. The reported nicotinamide-SAM conjugate (named NS1) features an alkyne as a key design element that closely mimics the linear, 180° transition state geometry found in the NNMT-catalyzed SAM → NAM methyl transfer reaction. NS1 was synthesized in 14 steps and found to be a high-affinity, subnanomolar NNMT inhibitor. An X-ray co-crystal structure and SAR study revealed the ability of an alkynyl linker to span the methyl transfer tunnel of NNMT with ideal shape complementarity. The compounds reported in this work represent the most potent and selective NNMT inhibitors reported to date. The

rational design principle described herein could potentially be extended to other methyltransferase enzymes.

INTRODUCTION

Nicotinamide N-methyltransferase (NNMT) is a metabolic enzyme responsible for the methylation of nicotinamide (NAM) using the cofactor S-adenosylmethionine (SAM), resulting in the production of 1-methylnicotinamide (MNAM) and S-adenosylhomocysteine (SAH).[1-2] While initially established as a vitamin B3 clearance enzyme, recent work has shown NNMT to be an important regulator of several intracellular pathways in fat, liver, and cancer cells.[2] NNMT can regulate methyl donor metabolism by direct interaction with proteins of the methionine cycle[3] and can regulate hepatic nutrient metabolism through Sirt1 stabilization[4]. By decreasing cellular SAM levels, NNMT can modulate the epigenetic landscape of cancer cells[5] and embryonic-stem cells[6].

Accordingly, NNMT has emerged as a disease-relevant enzyme and a possible point of therapeutic intervention. Abnormal NNMT expression is implicated in several diseases, including diabetes[7-8], obesity[7,9], and a variety of cancers[2,5,10]. Increased NNMT protein expression was observed in adipose and liver tissue of obese and diabetic mice[7], while increased NNMT mRNA expression was observed in humans[11]. NNMT is overexpressed in glioblastoma, leading to cellular SAM depletion, DNA hypomethylation, and accelerated tumor

growth.[12] Recently, NNMT was shown to be a master metabolic regulator of cancer-associated fibroblasts, with NNMT expression supporting ovarian cancer migration, proliferation, and metastasis.[13] The successful development of potent and selective NNMT inhibitors would aid efforts to elucidate the role of NNMT in disease, potentially enabling new strategies to treat a variety of metabolic disorders, cancers, and other pathologies.

Several NNMT inhibitors have been reported, including methylated quinolines[14], nicotinamide analogues[15-16], covalent inhibitors[17-18], and amino-adenosine derived bisubstrate inhibitors[19-21]. Bisubstrate inhibitors are compounds that bind both the substrate and cofactor binding pockets within an enzyme active site. The design of bisubstrate inhibitors relates closely to two well-established ligand design strategies: transition-state mimicry and fragment-based design.[22] Importantly, if two weak inhibitors that bind a protein at distinct sites are linked in an optimal manner, the resulting bisubstrate inhibitors can obtain several orders of magnitude increase in binding affinity.[23-24] As a result, linker identity is crucial to properly position the key interacting groups of the two component molecules and minimize unfavorable interactions between the linker and the protein.

In this work, we used a combination of structure-based design and molecular docking to develop high-affinity alkynyl bisubstrate inhibitors of NNMT. To begin, we examined the substrate-bound NNMT co-crystal structure (PDB ID 3ROD) and noted that the NAM nitrogen and SAH sulfur atoms are positioned ~ 4 Å apart and reside in a small tunnel that facilitates the S_N2-type methyl transfer reaction (Figure 1a,b).[25] We envisioned an alkyne as

the optimal linker between a NAM-like and a SAM-like fragment, as an alkyne linker resembles the linear, 180° transition state geometry found in the SAM → NAM methyl transfer reaction (Figure 1c,d).

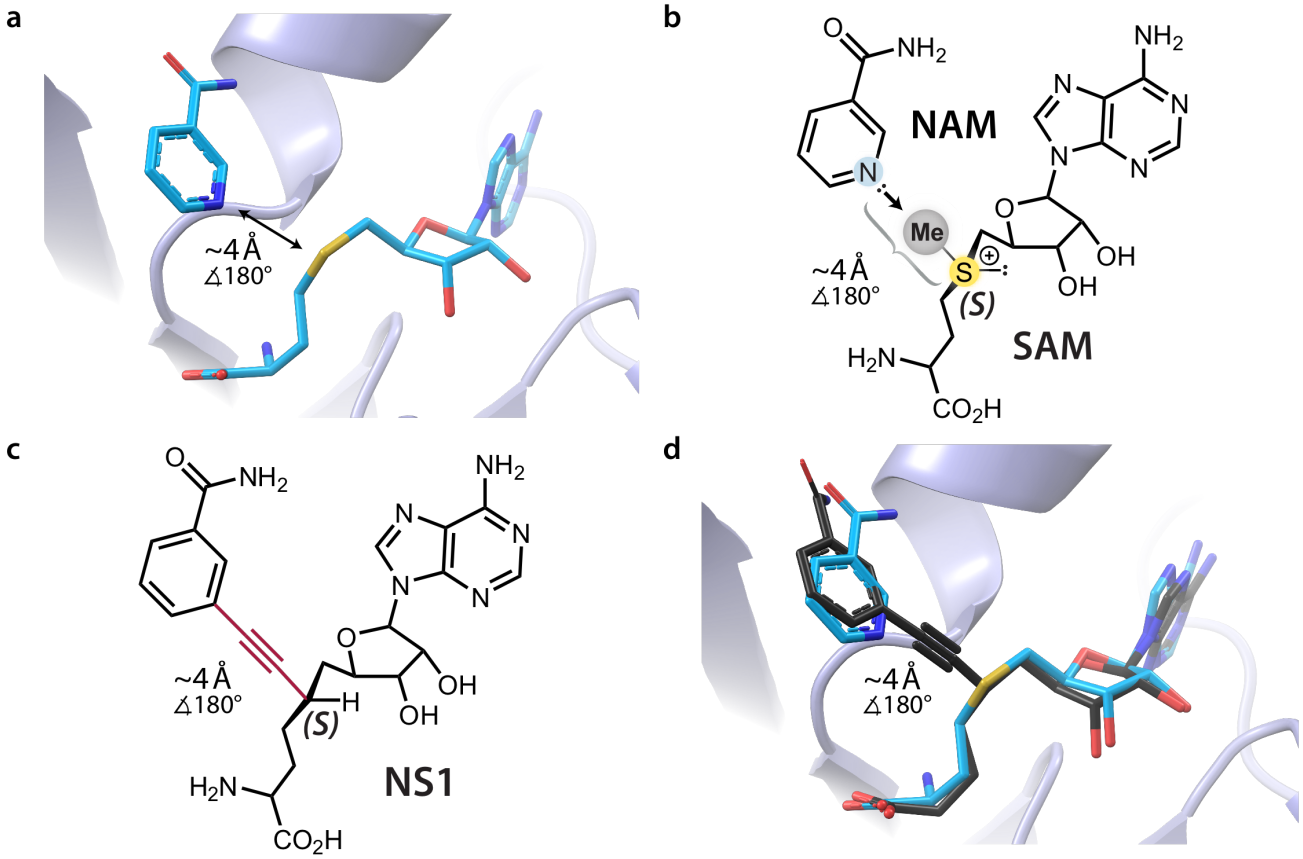


Figure 1. Overview of the alkynyl bisubstrate strategy. (a) Binding pose of ligands SAH and NAM (teal) rendered from previously published co-crystal structure (PDB ID 3ROD). (b) Graphical representation of the SAM → NAM methyl transfer reaction catalyzed by NNMT. (c) Graphical representation of alkynyl bisubstrate inhibitor NS1 posed to mimic the binding geometry of SAM/NAM. (d) Molecular docking output pose of alkynyl bisubstrate inhibitor NS1 (black) overlaid with SAH and NAM from structure 3ROD (teal).

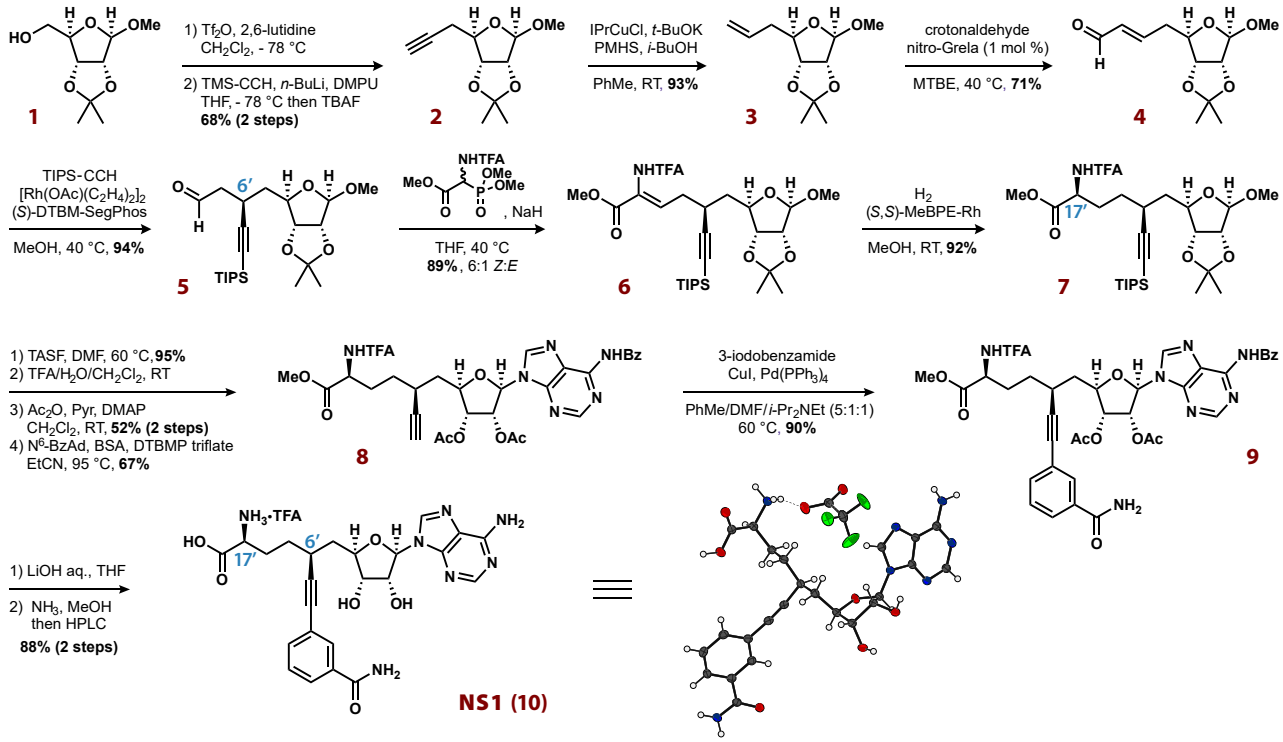
Thus, we rationally designed alkynyl nicotinamide-SAM conjugate 1 (named NS1, **10**). Molecular docking with Glide[26] predicted NS1 to be a better binder than SAM by > 3 kcal/mol (see *Supporting Information Part 1*). The docked pose of NS1 (Figure 1d) overlays well with NAM and SAH in the previously published substrate-bound crystal structure; the alkyne linker fits well into the methyl transfer tunnel. With molecular docking suggesting NS1 as a potential NNMT inhibitor, we aimed to synthesize and test NS1 in an NNMT inhibition assay.

6'-alkynyl nucleosides were first proposed and synthesized in 1990 as mixtures of alkynyl and amino acid diastereomers.[27] Unfortunately, the designed alkynyl nucleosides were poor inhibitors when tested against catechol O-methyltransferase (COMT).[28] In this work, we demonstrate that the alkynyl bisubstrate strategy toward SAM-dependent methyltransferase inhibition is a valuable approach. We synthesized the rationally designed alkynyl bisubstrate compound NS1 as a single enantiomer and diastereomer in 14 steps and showed it to be a high-affinity, selective NNMT inhibitor with a K_i of 500 pM.

RESULTS AND DISCUSSION

NS1 Synthesis. The synthesis of NS1 (**10**, Scheme 1) began with commercially available acetonide **1**. Alkylation of the corresponding triflate with lithium TMS-acetylide followed by silyl deprotection generated alkyne **2**. Copper-catalyzed semi-reduction[29] of **2** rendered terminal alkene **3**, which was converted to enal **4** by cross-metathesis with crotonaldehyde. Solvent and catalyst screening (Table S1, Figure S1) revealed methyl *tert*-butyl ether

(MTBE) and the commercially available Nitro-Grela olefin metathesis catalyst[30] as an optimal solvent/catalyst system to effect this transformation. The key C6' stereocenter was installed by a rhodium-catalyzed asymmetric alkynylation[31] to afford aldehyde **5** as a single diastereomer (see *Supporting Information Part 1, Section 3* for numbering scheme). A Horner-Wadsworth-Emmons olefination with a phosphonoglycine derivative yielded enamide **6**. The C17' stereocenter was set via rhodium-catalyzed asymmetric hydrogenation with a Burk-type 1,2-bis(phospholano)ethane (BPE) ligand,[32] providing (*S*)-trifluoroacetamide **7** as a single diastereomer. Subsequent acylation and Vorbrüggen glycosylation promoted by a 2,6-di-*tert*-butyl-4-methylpyridinium triflate salt[33] delivered nucleoside **8**. Introduction of the benzamide moiety by a Sonogashira cross-coupling and a global deprotection sequence completed the synthesis of NS1 (**10**) in a total of 14 steps with an overall yield of 9.1%. The small-molecule X-ray crystal structure of NS1•TFA was obtained, confirming the regiochemistry of the adenine nucleobase (N9-linkage) as well as the configuration of the C6' and C17' stereocenters.



Scheme 1. Synthesis and X-Ray Crystal Structure of NS1 (**10**)

Biochemical Evaluation of NS1 and Analogues. A fluorescence-based NNMT inhibition assay[34] revealed NS1 to be a highly potent, subnanomolar (500 pM) NNMT inhibitor. In the assay, NNMT catalyzes the SAM-dependent methylation of quinoline, forming 1-methyquinolinium (1MQ) and SAH. Quinoline as opposed to NAM as methyl-group acceptor has been previously employed by Loring and Thompson[35] who determined the detailed kinetic mechanism of NNMT (Rapid Equilibrium Random Bi Bi in Cleland's nomenclature[36-38]) and thus indirectly established that NAM and quinoline are mechanistically equivalent as mutually interchangeable methyl group acceptors.

More importantly for the purposes of our inhibition study, quinoline enables continuous real-time monitoring of the reaction progress, using fluorescence detection to monitor the

appearance of 1MQ. In contrast, previously described NNMT assays using NAM as native substrate were forced to rely on discontinuous (end-point) detection[39] and are thus unsuitable for real-time recording of continuous reaction progress, as was done in our study. Utilization of a quinoline-based continuous real-time assay was especially important because many enzymatic progress curves were prominently nonlinear, both in the presence and in the absence of inhibitors (Figure 2). In the specific case of NS1, the reaction progress curves were strongly nonlinear even within the first two minutes of the inhibition assay and therefore no "initial linear region" could be identified. Thus, as a matter of principle, the inhibitors reported in this study could not be studied by conventional initial rate kinetic methods.[40-41]

Therefore we analyzed the full reaction time-course using the software package DynaFit.[42-43] The mathematical models for each of several postulated kinetic mechanisms were systems of first-order Ordinary Differential Equations (ODEs) automatically derived by the DynaFit software. Inhibition constants were computed as the ratio $K_i = k_{off}/k_{on}$, where k_{off} is the microscopic rate constant for the dissociation of the enzyme-inhibitor complex and k_{on} is the corresponding microscopic association rate constant. (for details, see *Supporting Information Part 3, Section 2, pp. 13-15*). Because the K_i values reported in this work span six orders of magnitude, they are presented and discussed after logarithmic scaling, as pK_i (Figures 4, 5).

Note that the inhibition constants, K_i, reported in this study are true dissociation constants of the enzyme—inhibitor complex, as opposed to apparent inhibition constants, K_i^{app}, and therefore are entirely independent of the nature of the particular substrate (quinoline vs. NAM).[44] This interpretation is based on the assumption, supported by the X-Ray structural studies reported herein, that bisubstrate inhibitors in principle bind to the target enzyme in such a way that the binding of a bisubstrate inhibitor precludes any simultaneous binding of either of the two co-substrates.

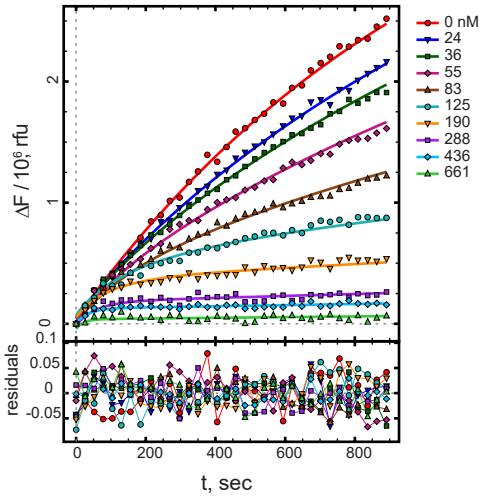


Figure 2. Kinetic studies of NNMT. A time course of the NNMT kinetic assay in the absence (red circles) or in the presence (remaining symbols) of NS1. Inhibitor concentrations are shown at top right. ΔF are scaled fluorescence changes in relative fluorescence units ("rfu"). The smooth curves represent the best least-squares fit to a system of first-order differential equations automatically generated by the DynaFit software package[42-43] according to the reaction mechanism shown in the *Supporting Information (Part 3: Enzyme Kinetics)*.

X-Ray Co-Crystal Structure of NS1 Bound to hNNMT. We determined the co-crystal structure of NS1-bound NNMT (Figure 3, PDB ID 6ORR, Table S2) and observed an NS1-shaped density in the NAM/SAM binding pocket in the electron density map (Figure 3a). Fitting NS1 into the density decreased the R_{free} value and improved the map quality, revealing that NS1 occupies both the NAM and SAM binding sites in the same orientation as the native substrates, with the alkynyl linker occupying the methyl transfer tunnel. The NS1 alkyne is an optimal surrogate for the SAM-dependent methyl transfer reaction of NNMT and effectively positions both structural components of NS1 properly in space, allowing NS1 to capture similar binding interactions as the native substrates (Figure 3b).

NS1 exploits the same amino acids that contact the native substrates to bind NNMT. To stabilize the adenosine portion of NS1, D142 forms a hydrogen bond with the primary amine on the adenine nucleobase, while the hydroxyl groups of the ribose hydrogen-bond extensively with the backbone nitrogen atom of S87 and the side chains of D85 and N90 (Figure 3c). The carboxylic acid of the NS1 amino acid accepts four hydrogen bonds from Y20, Y25, Y69, and T163, while the amino group hydrogen-bonds with the backbone carbonyl of G63 (Figure 3d). A hydrophobic clamp positions the aromatic ring of the benzamide moiety between Y204 and L164, with S201 and S213 stabilizing the amide group through hydrogen bonds (Figure 3e).

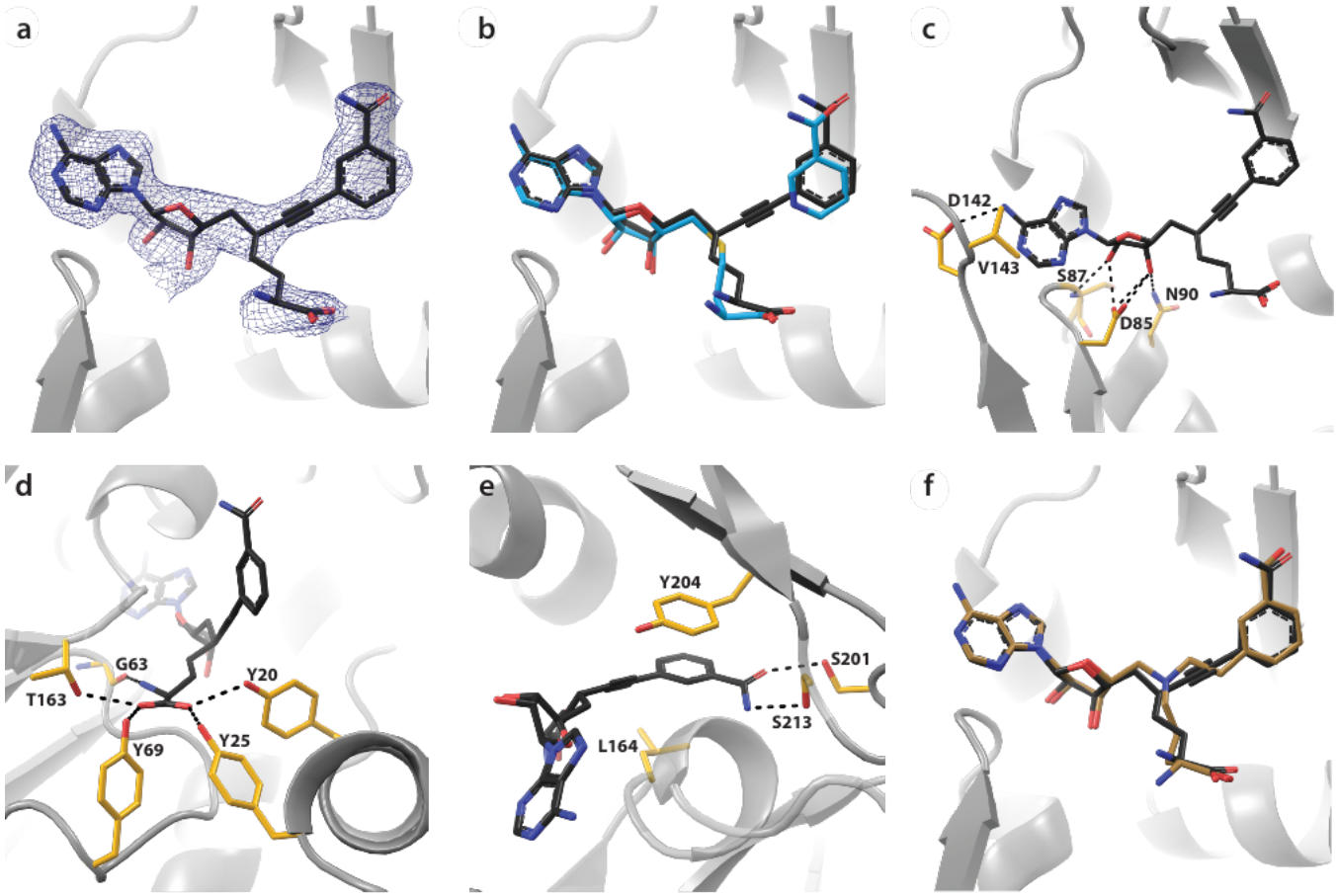
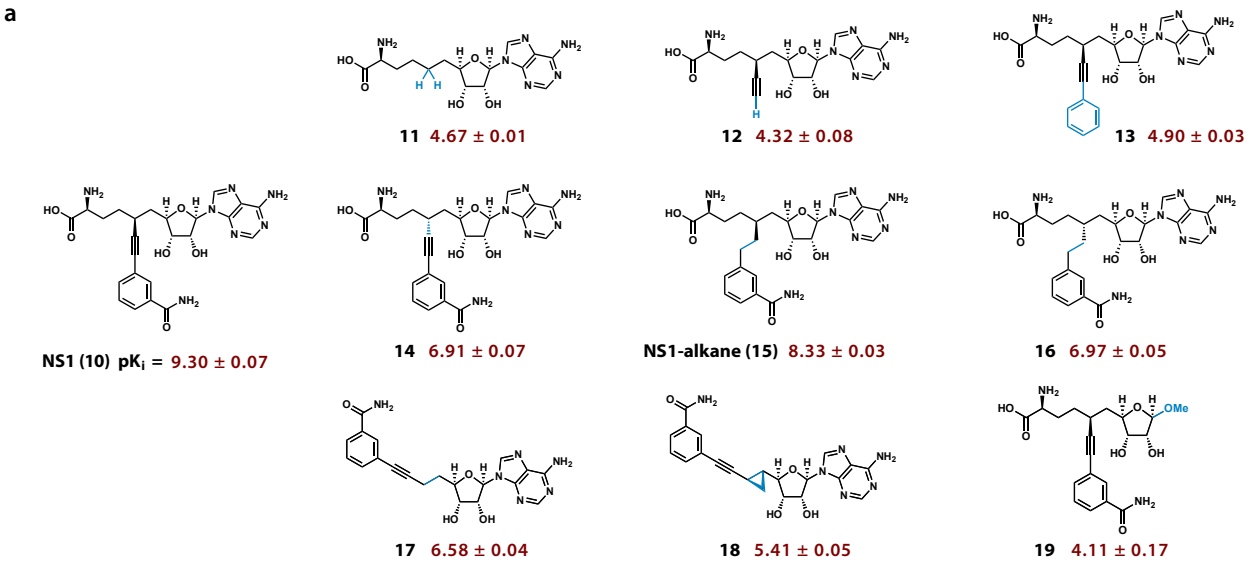


Figure 3. Co-crystal structure of NS1-bound NNMT (PDB ID 6ORR). (a) A $2F_o$-F_c omit map contoured at 1σ reveals NS1-shaped electron density in the binding pocket of NNMT. (b) NS1 (black) binds NNMT in an orientation that closely resembles previously published co-crystal structure containing ligands SAH and NAM (cyan; PDB ID 3ROD). (c-e) NS1 also exploits the same protein interactions that NNMT uses to bind its native substrates. Ligand-interacting residues from NNMT are shown in yellow; black dotted lines represent hydrogen bonds. (f) MS2756-bound NNMT (brown; PDB ID 6B1A) superimposed on NS1-bound NNMT.

Stucture-Activity Relationships (SAR). The synthesis of NS1 was designed to be highly modular to facilitate a systematic structure-activity analysis of our bisubstrate approach (Figures 4,5). SAR analysis began with the minimal motif desthia-SAH (**11**) and built up to the full NS1 structure incrementally (Figure 4a). Desthia-SAH, completely lacking an alkynyl-benzamide side chain, was a poor NNMT inhibitor. Addition of an acetylene (**12**) or a phenyl-acetylene (**13**) side chain yielded equally poor NNMT inhibitors. The potency of the parent compound NS1 (**10**) highlights the importance of the benzamide functionality in driving compound potency.

A fundamental aspect of our design strategy relied on the C6' stereocenter to properly direct the NS1 alkyne toward the NAM binding pocket. Analysis of the NNMT crystal structure (3ROD) suggested that the *S* stereochemistry would best capture the transition state geometry modeled in Figure 1. Accordingly, NS1 was a better inhibitor than the NS1-C6' epimer (**14**) by > 200-fold in terms of the inhibition constant K_i. The linear vector maintained by the alkynyl linker of NS1 proved optimal; NS1-alkane (**15**) was almost 10-fold less potent than NS1 itself (in terms of K_i). The NS1-alkane 6' epimer (**16**) and alkynyl



epimer **14** had similar pK_i values (6.97 vs. 6.91, respectively). These results may be explained by the shape of the NNMT binding pocket; the narrow width of the methyl transfer tunnel may accommodate linear alkynyl geometry better than a staggered aliphatic chain. Furthermore, the rigidity of the alkynyl linker could lower the entropic cost of binding relative to alkane containing analogues.

Figure 4. Structure-activity relationship (SAR) studies of bisubstrate NNMT inhibitors. pKi values ($-\log_{10}Ki$) are shown in red and are used to facilitate comparison between inhibitors of widely varying potency. (a) Exploration of the alkynyl bisubstrate approach to NNMT inhibition. (b) Amino acid modifications.

More drastic structural modifications to the NS1 scaffold included excision of the entire amino-acid side chain (**17**) or the entire adenine nucleobase (**19**). Compound **17**, a simplified bisubstrate inhibitor designed to target the adenosine and NAM binding pockets of NNMT, was a weaker inhibitor than NS1 by ~ 500-fold in terms of K_i. Attempts to improve the inhibitory activity of **17** by conformational restriction (via the installation of a cyclopropyl group; **18**) proved detrimental. Removal of the adenine nucleobase (**19**) completely abrogated NNMT inhibition.

SAR: Amino Acid Modification. Alteration of the NS1 amino acid (Figure 4b) revealed the C17' amino group of NS1 to be critical for inhibition, as compound **20** (lacking the

C17' amine) was a poor NNMT inhibitor. Removal of the carboxylic acid was better tolerated, as compound **21** (which retains the C17' amine) had a pK_i of 7.24 ± 0.02. Amide- (**22**) and methyl ester-containing (**23**) analogues were poor NNMT inhibitors (5.98 ± 0.01 and 6.36 ± 0.08, respectively), while amino-amide- (**24**) or urea-containing (**25**) analogues were slightly better (7.77 ± 0.02 and 7.12 ± 0.05, respectively). These results are consistent with the structural features of **24** and **25**, as both contain the same number of potential-hydrogen bonding groups as NS1 and native substrate SAH (although differing slightly in their donor/acceptor capacities and positions). Compound **22** has one fewer hydrogen-bonding atom than NS1. In **23**, the methyl ester likely adds too much bulk to fit in the amino acid pocket while also reducing the hydrogen bonding ability of the amino acid carboxylate.

Only one side chain modification yielded a more potent compound than NS1: the addition of a single methylene unit in the NS1 backbone (**26**, homo-NS1). Homo-NS1 had a pK_i of 9.62 ± 0.05 (compared to NS1 with pK_i = 9.30 ± 0.07), indicating a ~ 2-fold increase in inhibitory activity in terms of K_i. This finding is consistent with prior reports showing that the addition of a methylene unit in amino-bisubstrate NNMT inhibitors can improve potency.[20] We also synthesized and tested previously reported compounds MS2734 and MS2756 (Figure 5b). In our hands, MS2734—a one-carbon chain-extended homologue of MS2756—was a 10-fold better inhibitor than MS2756 in terms of K_i (pK_i of 7.05 ± 0.01 vs. 6.04 ± 0.06). The benefit of an extra methylene group in bisubstrate inhibitors could

arise because an added methylene better mimics the longer C—S bonds of the native substrates. The trimethylene linker of homo-NS1 would also be more flexible than the dimethylene linker of NS1, which may allow the molecule more room to optimally satisfy hydrogen bonds between the amino acid, benzamide, ribose and adenine moieties to the NNMT binding pocket.

SAR: Aryl Modification. After examining the impact of side chain modifications on inhibitor potency, we tested the effects of aryl group modification. We purposely designed the NS1 synthesis to accommodate a late-stage Sonogashira cross-coupling reaction to allow for easy introduction of a desired aryl moiety late in our synthesis. Leveraging this capability, we prepared gram quantities of intermediate **8** (Scheme 1) and synthesized a variety of aryl substituted NS1 analogues (Figure 5a).

These studies revealed that proper positioning of an amide group on NS1 is essential for NNMT binding, as *para*-benzamide (**27**), *ortho*-benzamide (**28**), and sulfonamide (**29**) substituted NS1 analogues were much less potent than NS1. We designed fluorine (**30**), methyl (**31**), trifluoromethyl (**32**), and chlorine (**33**) containing analogues to 1) bind a small hydrophobic pocket in the nicotinamide binding site, 2) modulate the pK_a of the NS1 benzamide group through inductive effects, and 3) potentially alter the rotation of the NS1 amide relative to the NS1 benzamide phenyl ring. While **30**, **31**, and **32** were potent NNMT inhibitors ($pK_i > 8.0$), only chloro-substitution (**33**) yielded a more potent NNMT inhibitor than NS1 ($pK_i = 9.72 \pm 0.15$). As shown in Figure 6a, there is a small hydrophobic region

in the NAM binding pocket *para* to the alkyne of the NS1 scaffold. The small, hydrophobic chlorine atom of **33** likely makes Van der Waals contacts with this region, providing additional binding affinity.

After interrogating ring substitution at the *para*-position, we attempted to restrict rotation of the NS1 amide by converting the benzamide moiety to a benzolactam (Figure 5a, **34** and **35**). Benzolactams **34** and **35** were moderate NNMT inhibitors, with a six-membered ring (**34**) better tolerated than a five-membered ring (**35**). We also examined C13'/C14' substitution with methylenedioxy analogue **36** ($pK_i = 7.49 \pm 0.05$) or introduced a heterocyclic nitrogen atom (to better mimic NAM, **37**, **38**, **39**, **40**). Compounds **36**-**40** all had pK_i values below that of NS1, demonstrating that C13'/C14' substitution or inclusion of a nitrogen atom are not beneficial in these cases. Lastly, we designed and synthesized amino naphthalene derivative **41** (a bisubstrate analogue of the previously reported NNMT inhibitor 5-amino-1-methylquinolinium[45]), but **41** was also a poor inhibitor ($pK_i = 5.43 \pm 0.07$).

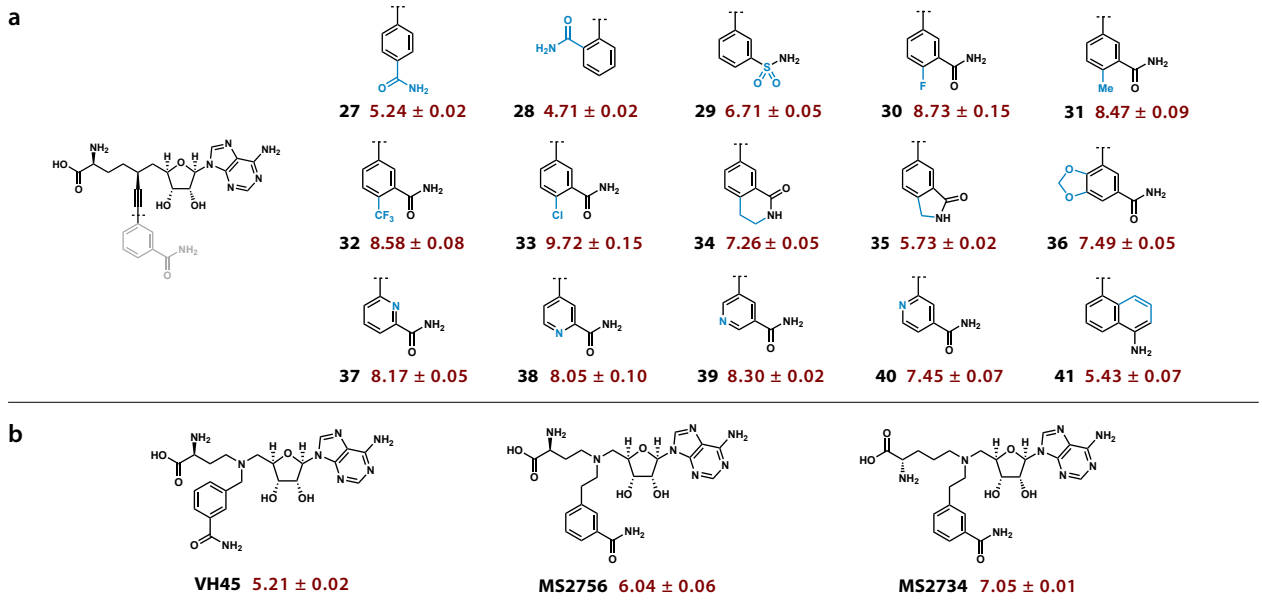


Figure 5. SAR studies of bisubstrate inhibitors. pKi values ($-\log_{10}K_i$) are shown in red and are used to facilitate comparison between inhibitors of widely varying potency. (a) Modification of the benzamide moiety of NS1. (b) Previously published amino-bisubstrate NNMT inhibitors were synthesized and tested alongside NS1 and analogues.

NS1-Alkane (15) vs. Amino-Bisubstrate Inhibitor MS2756. In the final stage of our SAR analysis we compared our inhibitors with previously reported amino bisubstrate NNMT inhibitors (Figure 5b). Previously reported compounds VH45[19] , MS2756[20], and MS2734[20] use a tertiary amine core and an alkyl tether to link a SAM-like fragment to a NAM-like fragment. An alignment of NS1-bound NNMT with MS2756-bound NNMT[20] shows the similar binding modes of the two compounds (Figure 3f). We synthesized and tested all three of these compounds alongside NS1-alkane (**15**, Figure 4a) using the same

assay protocols and kinetic analyses to determine K_i values. We compared MS2756 and NS1-alkane *directly* because they are close structural analogues in which the tertiary amine core of MS2756 has been replaced with an sp^3 hybridized carbon stereocenter. These studies revealed that NS1-alkane was approximately 200-fold more potent than MS2756 in terms of K_i.

The contrast between the observed activity of MS2756 and NS1-alkane is noteworthy; changing a *single* non-hydrogen atom (tertiary nitrogen to sp^3 carbon stereocenter) substantially alters compound potency. We hypothesize that a variety of factors could be responsible for this observation. It is evident that the stereochemical definition at the C6' stereocenter of NS1-alkane is beneficial as evidenced by the ~ 20-fold difference in K_i between NS1-alkane and its C6' epimer (**16**). A much larger difference (~ 250-fold) in K_i between NS1 and its C6' epimer (**14**) was also observed, demonstrating that as linker rigidity increases, C6' stereodefinition becomes more important.

While NS1-alkane and NS1 both show large differences in K_i values between their C6' stereoisomers, the tertiary nitrogen atom in MS2756 maintains sp^3 tetrahedral geometry but is free to invert rapidly at room temperature. In solution, we imagine that NS1-alkane and NS1 assume the optimal tetrahedral geometry at C6' necessary for binding, whereas MS2756 is free to occupy conformations that are unproductive for binding because of nitrogen inversion. It is also likely that the hydrophobic, carbon-based scaffolds of NS1-alkane and NS1 are preferred over the polar, tertiary amine core of MS2756. NS1-alkane

and NS1 likely form favorable Van der Waals contacts with the NNMT active site, as the closest residue to the NS1-alkane/NS1 C6' carbon stereocenter is F15 (~ 4 Å away). This phenylalanine residue presents a large hydrophobic[46] surface in the NNMT ligand binding pocket (Figure 6a), near the NS1-alkane/NS1 C6' stereocenter.

Finally, the transition state structure of PNMT (phenylethanolamine N-methyltransferase[47]), the second closest homologue of NNMT, involves even distribution of positive charge along the *entire* axis of methyl transfer.[48] If NNMT has a transition state structure similar to that of PNMT, analogues like MS2756, which localize positive charge on a single nitrogen atom, do not accurately capture the transient electrostatics of the methyl transfer transition state. While not positively charged, carbon-based linkers avoid the localized positive charge of MS2756 and other amino-bisubstrate inhibitors.

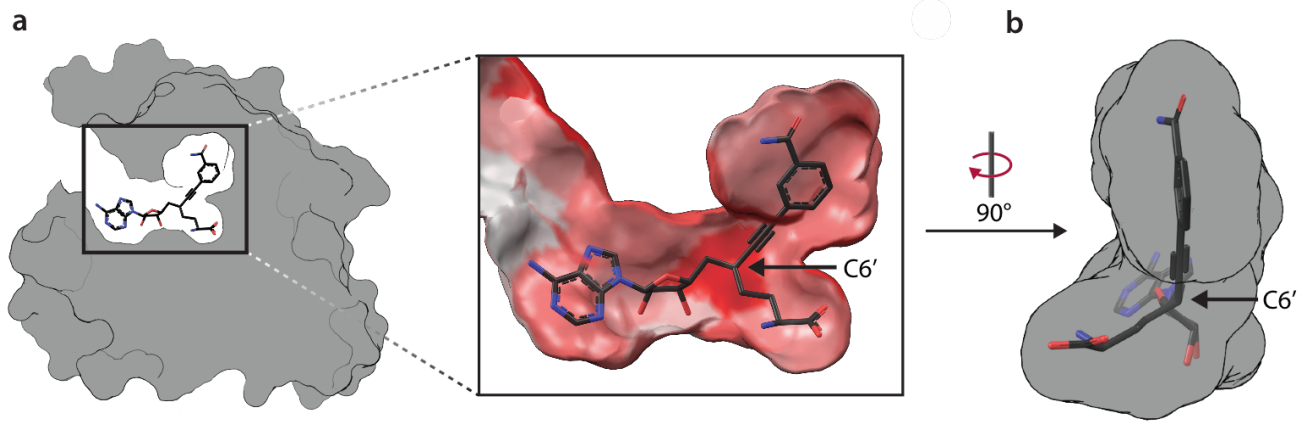


Figure 6. The chemical properties and geometry of NS1 closely match the hydrophobic profile and shape of the NNMT binding pocket. (a) The binding pocket is buried in the hydrophobic core of the protein (gray). (Inset) A solvent-accessible surface representation

of the binding pocket colored by the Eisenberg hydrophobicity scale[46] reveals a hydrophobic patch (dark red) that contacts the C6' carbon atom of NS1. (b) A side-on view shows that the binding pocket has shape complementarity specific to the *S* diastereomer of NS1 at the C6' carbon.

Selectivity Evaluation. While several previously reported NNMT inhibitors were described as selective for NNMT, none of them have been tested against the closest homologues[25] of NNMT or other small-molecule methyltransferases[49-50]. We aimed to critically evaluate the selectivity profile of NS1 by testing it against the methyltransferases most like NNMT (Table 1). Sequence similarity analysis and generation of a sequence similarity network[51-52] (Figure S2, Table S3) showed that NNMT, INMT (indolethylamine N-methyltransferase[53]), and PNMT cluster tightly, with INMT and PNMT having 53% and 39% sequence identity to NNMT, respectively. A complementary approach based on structural homology analysis via the DALI[54] server demonstrated that INMT and PNMT are also the closest structural homologues of NNMT (Figure S3, S4, Table S4).[25] In addition to the selectivity of NS1 amongst small-molecule methyltransferases, we tested NS1 against a few representative DNA, histone, and protein-arginine methyltransferases (Table 1, Table S5). These studies revealed thiopurine S-methyltransferase (TPMT) to be the closest off-target of NS1, with an IC_{50} of ~ 1 μM. This result might be explained by the flexibility of the TPMT active site[55] and the ability of TPMT to bind a variety of small molecule substrates

and inhibitors[56]. INMT was the second closest off-target of NS1, with an IC_{50} of 3.4 μM (Figure S5).

While in this case NS1 proved selective for NNMT, the introduction of different aryl groups on the NS1 scaffold could likely be used to target other methyltransferases selectively. In this work, cross coupling of compound **8** (Scheme 1) with 3-iodobenzamide generated NS1; cross coupling of **8** with indole or thiopurine fragments could provide inhibitors of INMT and TPMT, respectively. In cases where off-target binding or inhibition is observed, aryl groups could be modified until a desired selectivity profile is achieved.

Table 1. Methyltransferase Selectivity Profile of NS1

small-molecule methyltransferase	inhibition at 10 μM (%)	inhibition at 1 μM (%)
TPMT[a]	76	51
INMT[b,*]	53	14
COMT[c]	43	0
PNMT[d]	26	0
GAMT[e]	26	0
GNMT[f]	23	4
HNMT[g]	2	6
protein or DNA methyltransferase	**IC_{50} (μM)**	
hDNMT3a[h]	7.6	
PRMT1[i]	>10	
ASH1L[j]	>10	
DOT1L[k]	>10	
EHMT1[l]	>10	
G9a[m]	>10	
SETDB1[n]	>10	

[a]thiopurine S-methyltransferase. [b]indolethylamine N-methyltransferase; *values interpolated from the full IC_{50} curve shown in Figure S5. [c]catechol O-methyltransferase. [d]phenylethanolamine N-methyltransferase. [e]guanidinoacetate N-methyltransferase. [f]glycine N-methyltransferase. [g]histamine N-methyltransferase. [h]DNA (cytosine-5)-methyltransferase 3A. [i]protein arginine N-methyltransferase 1. [j]histone-lysine N-methyltransferase ASH1L. [k]histone-lysine N-methyltransferase, H3 lysine-79 specific. [l]histone-lysine N-methyltransferase EHMT1. [m]histone-lysine N-methyltransferase EHMT2. [n]histone-lysine N-methyltransferase SETDB1.

Thermal Stabilization and Cell-Based Evaluation. Along with biochemical profiling of NS1 and analogues, we found that NS1 binds and stabilizes NNMT in lysate of K562 cells (a chronic myelogenous leukemia line known to express NNMT[17]). The cellular thermal shift assay[57] revealed that NS1 and **25** shift the melting curve of NNMT by 8 and 5 °C, respectively (Figures S6, S8) and in a dose-dependent manner (Figures S7, S9). We also evaluated several compounds for cytotoxicity via the CellTiter-Glo luminescent cell viability assay in U2OS cells (a human bone osteosarcoma line known to express NNMT[15]). None of the compounds tested (NS1, **21**, **23**, **24**, **25)** were toxic to U2OS cells after 48 h at a 100 μM dose (Table S7).

We tested the ability of NS1, **21**, **23**, **24**, and **25** to decrease MNAM levels in U2OS cells, but the effects were modest (Table S8). NS1-methyl ester (**23**), designed to be more cell permeable than NS1, performed best in this assay, decreasing MNAM levels by 30% at a 31.6 μM dose. A-B permeability testing (Caco-2, pH 6.5/7.4) showed that NS1 and analogues have limited permeability (most permeable out of those tested was NS1-amine **21**, 1.3 x 10^{-6} cm/s, Table S9). Ongoing work is aimed at developing more cell-penetrant alkynyl NNMT inhibitors that would be useful probes in cell-based studies.

CONLUSIONS

In this work, we designed and synthesized the most potent and selective NNMT inhibitors reported to date. A modular, scalable synthesis of alkynyl nucleosides enabled us to make and test a variety of alkynyl bisubstrate inhibitors, leading to the discovery of NS1 as a

selective, picomolar NNMT inhibitor. Alkynyl bisubstrate inhibition could be a generalizable strategy; covalent tethering of alkynyl nucleosides to substrate mimics of other small molecule methyltransferases could provide a method for selective inhibition of these enzymes. The stereodefined alkyne present in the NS1 scaffold could be used for click chemistry or other functionalization, enabling the development of bisubstrate probes or inhibitors of protein arginine/lysine methyltransferases.[58-59]

Finally, this work demonstrates the value of alkynes in small-molecule ligand design in the context of methyltransferase inhibition. Alkynes provide unique rigidity, geometry, and spatial occupancy not captured by common fragment linkers. Current state-of-the-art bisubstrate inhibitor designs often rely upon reductive amination, alkylation, and amide-bond forming reactions to link fragments together. These reactions are generally fast and easy to implement, but often produce highly flexible structures characterized by relatively weak overall binding affinity. This report demonstrates that C6' alkynyl nucleosides—while more synthetically complex—can be used to generate potent bisubstrate methyltransferase inhibitors.

EXPERIMENTAL SECTION

General Chemistry Procedures

All reactions were performed under an atmosphere of nitrogen in flame- or oven-dried glassware unless otherwise noted. Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 covered glass backed plates (0.25 mm, F_{254}). TLC plates

were visualized under UV light and/or exposure to an acidic solution of *p*-anisaldehyde, an aqueous solution of ceric ammonium molybdate (CAM), or an aqueous solution of potassium permanganate, followed by heating. Reactions were also monitored by analytical LCMS using an Agilent 6120 Quadrupole MS and an Agilent 1260 Infinity II LC system equipped with (1) a Phenomenex Kinetex F5 column (2.6 μm, 100 Å, 100 × 3.0 mm), (2) a Phenomenex Kinetex Biphenyl column (2.6 μm, 100 Å, 100 × 3.0 mm) or (3) a Thermo Scientific Accucore aQ C18 column (2.6 μm, 100 × 2.1 mm). For analytical LCMS, solvent A consisted of 0.1% (v/v) FA/water and solvent B consisted of 0.1% (v/v) FA/CH_3CN. Two methods were used: *method A* for columns 1, 2, and 3: 5% B for 1 min, 5% → 95% B over 8 min, 95% B for 2 min, and reequilibration for 2 min; flow rate: 0.55 mL/min (1), 0.95 mL/min (2), 0.70 mL/min (3), column temperature: 50 °C; UV_{254}, and *method B* for columns 2 and 3: 0% B for 1 min, 0% → 40% B over 8 min, 40% → 95% B over 2 min, 95% B for 2 min, and reequilibration for 2 min; flow rate: 0.95 mL/min (2), 0.70 mL/min (3), column temperature: 50 °C; UV_{254}. Column 2 (*method B*) was used for the final analysis of very polar compounds, including NS1 (**10**) and analogues. Flash column chromatography was performed on an Interchim PuriFlash 215 system with prepacked silica gel cartridges (Büchi FlashPure (35 to 45 μm), Büchi Reveleris HP (16 to 24 μm), Teledyne Isco RediSep Rf (35 to 70 μm) and Teledyne Isco RediSep Rf Gold (20 to 40 μm). Preparative HPLC was performed on an Agilent 1260 Infinity II Preparative LC system equipped with a Kromasil C18 column (10 μm, 100 Å, 21.2 × 250 mm) using solvents A (0.1% (v/v)

FA/water) and B (0.1% (v/v) FA/CH_3CN) or A' (0.1% (v/v) TFA/water) and B' (0.1% (v/v) TFA/CH_3CN) at flow rate 10 mL/min delivering gradients specified in individual experimental protocols. All compounds reported in this manuscript and *Supporting Information* have a purity of ≥ 95% as determined by analytical LCMS and NMR (^1H and ^{13}C) unless otherwise noted.

Instrumentation

^1H NMR spectra were recorded on Varian INOVA-600 or Varian INOVA-500 spectrometers at ambient temperature. Chemical shifts are reported in ppm (δ scale) relative to residual undeuterated solvent ($CDCl_3$: 7.26 ($CHCl_3$), CD_3OD: 3.31 (CD_2HOD), CD_3CN: 1.94 (CD_2HCN), D_2O: 4.79 (HOD)). Data are reported as follows: chemical shift (δ ppm) (integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent, or a combination of these), coupling constant(s) J (Hz)). When a mixture of deuterated solvents is employed, the residual undeuterated solvent used for reference is indicated in bold. ^{13}C NMR spectra were recorded on Varian INOVA-500, Varian MERCURY-400 and JEOL J400 spectrometers at ambient temperature. Data are reported as follows: chemical shifts (δ scale, ppm) relative to the carbon resonances of the solvent ($CDCl_3$: 77.16, CD_3OD: 49.00, CD_3CN: 1.32). When a mixture of deuterated solvents is employed, the solvent used for reference is indicated in bold. ^{19}F NMR spectra were rec-

orded on Varian INOVA-500 and Varian MERCURY-400 spectrometers at ambient temperature. Data are reported as follows: chemical shifts (δ scale, ppm) relative to $CFCl_3$ and referenced to benzotrifluoride (BTF), hexafluorobenzene (HFB), or trifluoroacetic acid (TFA) as internal standards (BTF IStd: δ –63.7, HFB IStd: δ –164.9, TFA IStd: δ –76.6). ^{31}P NMR spectra were recorded on a Varian MERCURY-400 spectrometer at ambient temperature. Data are reported as follows: chemical shifts (δ scale, ppm), calibrated to an external standard of triphenyl phosphate in $CDCl_3$ (0.0485 M, δ –17.6 relative to H_3PO_4 at δ 0). Infrared (FTIR) spectra were recorded on a Bruker Alpha FT-IR spectrophotometer. Data is reported in frequency of absorption (cm^{-1}). The IR spectrum of each compound (solid or liquid) was acquired directly neat or on a thin layer at ambient temperature. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II or an Agilent 6220 LC-TOF equipped with an electrospray ionization source (ESI+).

Materials

Commercial reagents and solvents were used as received unless otherwise noted. All solvents were of commercially available anhydrous grade (DriSolv® or equivalent) unless otherwise noted. The molarity of *n*-butyllithium solutions was determined by titration using N-benzylbenzamide[60] or N-pivaloyl-*o*-toluidine[61] with similar results (average of three determinations). $[Rh(OAc)(C_2H_4)_2]_2$ was prepared according to a known procedure[62] using $[RhCl(C_2H_4)_2]_2$ (CAS: 12081-16-2) obtained from Strem Chemicals, Inc. (catalog #: 45-

0270). The following catalysts were all obtained from Strem Chemicals, Inc.: nitro-Grela catalyst ([1,3-bis(2,4,6-trimethylphenylimidazolidin-2-ylidene)]-(2-*i*-propoxy-5-nitrobenzylidene)ruthenium(II) dichloride), CAS: 502964-52-5, catalog #: 44-0758; (*S*,*S*)-MeBPE-Rh catalyst[63] ((-)-1,2-bis((2*S*,5*S*)-2,5-dimethylphospholano)ethane(1,5-cyclooctadiene)rhodium(I) tetrafluoroborate), CAS: 213343-65-8, catalog #: 45-0169; (*S*)-DTBM-SegPhos ((*S*)-(+)-5,5'-bis[di(3,5-di-*t*-butyl-4-methoxyphenyl)phosphino]-4,4'-bi-1,3-benzodioxole), CAS: 210169-40-7, catalog #: 15-0067; $[Rh(nbd)_2]BF_4$, CAS: 36620-11-8, catalog #: 45-0230; (*S*,*S*)-MeDUPHOS ((+)-1,2-Bis((2*S*,5*S*)-2,5-dimethylphospholano)benzene), CAS: 136735-95-0, catalog #: 15-0092. (±)-Z-α-phosphonoglycine trimethyl ester, CAS: 88568-95-0, was obtained from Chem-Impex International, (Catalog #: 14125).

Synthesis of NS1 (10)

(3a*R*,4*R*,6*R*,6a*R*)-4-methoxy-2,2-dimethyl-6-(prop-2-yn-1-yl)tetrahydrofuro[3,4-*d*][1,3]dioxole (2). The following is an adaption of a published procedure.[64] To a stirred solution of 2,6-lutidine (9.85 mL, 84.6 mmol, 1.1 equiv.) in CH_2Cl_2 (130 mL) at –78 °C was added trifluoromethanesulfonic anhydride (13.7 mL, 81.5 mmol, 1.06 equiv.) over 5 min (*flask A*, 1 L rbf), followed by the addition of a solution of alcohol **1** (15.7 g, 76.9 mmol) in CH_2Cl_2 (33 mL) over 10 min. The mixture was stirred at –78 °C for 2 h and warmed to 0 °C. Meanwhile, to a separate flask (*flask B*, 500 mL rbf) containing a solution of TMS-acetylene (32.0 mL, 231 mmol, 3.00 equiv.) in dry THF (130 mL) at –78 °C was

added *n*-butyllithium (2.56 M in hexanes, 93.7 mL, 240 mmol, 3.12 equiv.) at a rate of 5 mL/min *via* syringe. The resulting mixture was stirred at –78 °C for 1.5 h and warmed to 0 °C. While the contents of flask B remained at 0 °C, flask A was removed from the ice/water bath, volatiles were removed *in vacuo* (bath temperature at 20 °C) and a brief subjection to high vacuum yielded a red-orange paste. The crude triflate in flask A was resuspended in dry THF (65 mL) and DMPU (35 mL) was added. The contents were stirred vigorously for 10 min until complete solubilization was noted and cooled to –78 °C. The contents of flask B were cooled to –78 °C and transferred to flask A *via* cannula. The resulting mixture was stirred at –78 °C for 1 h and at –12 °C for 3 h (NaCl/ice bath), before the slow addition of TBAF (**caution, gas evolution!**, 1.0 M in THF, 250 mL, 250 mmol, 3.25 equiv.) *via* cannula. The mixture was allowed to warm to RT and was stirred for another 30 min before volatiles were removed *in vacuo*. The reddish-brown residue was resuspended in MTBE (350 mL) and a sat. aq. NH_4Cl solution (300 mL) was added. The layers were roughly separated and the organic phase, largely emulsified, was filtered through a pad of $CaCO_3$/celite to give two clear layers in the filtrate. The layers were separated and the combined aqueous phases were extracted with MTBE (2 × 200 mL) and EtOAc (200 mL). The combined organic extracts were washed with a 5% aq. LiCl solution (800 mL) and then with brine (800 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by dry column vacuum chromatography[65] (cyclohexane/EtOAc, 0 → 30%) to yield pure **2** as well as semi-pure **2** which was repurified by silica gel chromatography

(hexanes/EtOAc, 0 → 30%). Compound **2** (11.1 g, 52.3 mmol, 68%) was obtained as a light-yellow oil. R_f = 0.60 (hexanes/EtOAc, 67:33); FTIR (neat), ν_{max} (cm^{-1}): 3286, 2989, 2938, 2835, 1438, 1209, 1090, 1053, 1041; ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.95 (s, 1H), 4.69 (d, J = 5.9 Hz, 1H), 4.60 (d, J = 5.9 Hz, 1H), 4.30 (dd, J = 9.3, 6.6 Hz, 1H), 3.32 (s, 3H), 2.52 (ddd, J = 16.7, 6.6, 2.7 Hz, 1H), 2.42 (ddd, J = 16.6, 9.4, 2.7 Hz, 1H), 2.04 (t, J = 2.7 Hz, 1H), 1.46 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 112.3, 109.6, 85.2, 85.1, 83.3, 80.2, 70.4, 54.7, 26.4, 24.9, 24.6; HRMS (ESI+): $[C_{11}H_{16}O_4Na]^+$ calc. 235.0941, meas. 235.0938, Δ 1.0 ppm.

(3a*R*,4*R*,6*R*,6a*R*)-4-allyl-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole (3). Alkyne **2** (10.6 g, 49.9 mmol) was reduced according to protocols adapted from Cox et al.[66] Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 30%) to afford alkene **3** (9.97 g, 46.5 mmol, 93%) as a clear, viscous oil. The flash column chromatography removed *ca*. 98% of PHMS, yielding material that was suitable for use in subsequent reactions. An analytically pure sample was prepared by subjecting 2.0 g of the post-column material to short path distillation (Kugelrohr) at 130 °C and 200 mTorr. R_f = 0.50 (hexanes/EtOAc, 80:20); FTIR (neat), ν_{max} (cm^{-1}): 3078, 2987, 2938, 2833, 1643, 1381, 1372, 1209, 1193, 1105, 1090, 1059, 1031; ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.82 (dddd, J = 16.7, 10.3, 7.4, 6.2 Hz, 1H), 5.15–5.09 (m, 2H), 4.96 (s, 1H), 4.61 (d, J = 5.9 Hz, 1H), 4.57 (dd, J = 6.0, 1.0 Hz, 1H), 4.23 (td, J = 7.8, 1.0 Hz, 1H), 3.34 (s, 3H), 2.46–2.38 (m, 1H), 2.32–2.24 (m, 1H), 1.48 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 134.5, 117.7,

112.4, 109.6, 86.6, 85.7, 83.6, 55.0, 39.6, 26.6, 25.2; HRMS (ESI+): $[C_{11}H_{18}O_4Na]^+$ calc. 237.1095, meas. 237.1097, Δ 1.0 ppm.

(*E*)-4-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)but-2-enal (4). To a solution of alkene **3** (2.0 g, 9.3 mmol) in MTBE (degassed prior to addition by sparging with nitrogen for 15 min, 28 mL) at RT, were added *E*-crotonaldehyde (3.9 mL, 47 mmol, 5.0 equiv.) and nitro-Grela metathesis catalyst (63 mg, 93 μmol, 1 mol %) sequentially. The flask headspace was purged with nitrogen for 10 min and the mixture was stirred at 40 °C for 48 h, at which point TLC analysis indicated *ca*. 50% conversion. More nitro-Grela catalyst (63 mg, 93 μmol, 1 mol %) and *E*-crotonaldehyde (3.9 mL, 47 mmol, 5.0 equiv.) were added and the reaction mixture was stirred at 40 °C for another 24 h, at which point complete conversion was indicated by TLC analysis. Volatiles were removed *in vacuo* and the crude material was purified by silica gel chromatography (hexanes/EtOAc, 2 → 50%) to afford enal **4** (1.60 g, 6.60 mmol, 71%) as a yellow oil. R_f = 0.41 (hexanes/EtOAc, 50:50); FTIR (neat), ν_{max} (cm^{-1}): 2990, 2936, 2832, 2735, 1688, 1641, 1453, 1382, 1377, 1298, 1269, 1263, 1242, 1210, 1194, 1161, 1140, 1104, 1089, 1058, 1026; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.54 (d, J = 7.8 Hz, 1H), 6.84 (dt, J = 15.7, 6.8 Hz, 1H), 6.21 (ddt, J =15.7, 7.8, 1.5 Hz, 1H), 4.98 (s, 1H), 4.64 (d, J = 5.9 Hz, 1H), 4.56 (dd, J = 6.0, 1.1 Hz, 1H), 4.35 (ddd, J= 8.8, 6.4, 1.0 Hz, 1H), 3.34 (s, 3H), 2.68 (dddd, J = 15.4, 8.5, 6.6, 1.6 Hz, 1H), 2.58 (dtd, J = 15.2, 6.7,1.5 Hz, 1H), 1.48 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 193.6, 153.5, 134.8, 112.6, 109.8, 85.4, 85.3, 83.7,

54.7, 38.4, 26.5, 25.0; HRMS (ESI+): calcd. for $[C_{12}H_{18}O_5Na]^+$ 265.1046, meas. 265.1062, Δ 6.0 ppm.

(*R*)-3-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-5-(triisopropylsilyl)pent-4-ynal (5). Aldehyde **5** was prepared using protocols adapted from Nishimura et al.[31] A 250 mL flask was charged with $[Rh(OAc)(C_2H_4)_2]_2$ catalyst (126 mg, 0.289 mmol, 2.5 mol %), (*S*)-DTBM-SegPhos (818 mg, 0.693 mmol, 6.0 mol%), and a large stir bar. The flask headspace was evacuated and backfilled with nitrogen (3 ×) and methanol was added (22 mL). The resulting orange suspension was stirred vigorously at RT until a red-orange solution was obtained (30 min). At this time, a solution of enal **4** (2.80 g, 11.6 mmol) in MeOH (10 mL) and TIPS-acetylene (5.19 mL, 23.1 mmol, 2.0 equiv.) were added sequentially to the reaction mixture. The resulting red mixture was stirred at 40 °C for 15 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 50%) to afford aldehyde **5** (4.60 g, 10.8 mmol, 94%) as a single diastereomer, as a light yellow oil. R_f = 0.42 (cyclohexane/EtOAc, 75:25); FTIR (neat), ν_{max} (cm^{-1}): 2941, 2865, 2722, 2167, 1728, 1463, 1381, 1371, 1271, 1241, 1210, 1193, 1160, 1093, 1058, 1018; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.82 (t, *J* = 2.1 Hz, 1H), 4.95 (s, 1H), 4.61 (dd, *J* = 5.9, 1.2 Hz, 1H), 4.58 (d, *J* = 5.9 Hz, 1H), 4.49 (ddd, *J* = 10.6, 4.2, 1.2 Hz, 1H), 3.35 (s, 3H), 3.16 (dtd, *J* = 11.2, 6.9, 3.9 Hz, 1H), 2.60 (ddd, *J* = 16.5, 7.5, 2.2 Hz, 1H), 2.55 (ddd, *J* = 16.5, 6.4, 2.1 Hz, 1H), 1.74 (ddd, *J* = 13.3, 10.6, 4.0 Hz, 1H), 1.64 (ddd, *J* = 13.2, 11.1, 4.2 Hz, 1H), 1.47 (s, 3H), 1.31 (s,

3H), 1.11–0.97 (m, 21H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 200.7, 112.6, 109.9, 108.6, 85.5, 85.3, 84.5, 84.2, 55.4, 49.0, 40.7, 26.7, 25.4, 25.1, 18.7, 11.3; HRMS (ESI+): calcd. for $[C_{23}H_{40}O_5Si_1Na]^+$ 447.2537, meas. 447.2527, Δ 2.3 ppm.

Methyl (*S*,*Z*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)-7-(triisopropylsilyl)hept-2-en-6-ynoate (6). A solution of methyl 2-(dimethoxyphosphoryl)-2-(2,2,2-trifluoroacetamido)acetate (**S2**, 3.11 g, 10.6 mmol, 1.5 equiv.) in THF (21 mL) was added dropwise over 20 min to a stirred suspension of NaH (95%, 254 mg, 10.6 mmol, 1.5 equiv.) in THF (10 mL) at 0 °C. The resulting cloudy mixture was stirred at 0 °C for 30 min and a solution of aldehyde **5** (3.00 g, 7.06 mmol) in THF (7.1 mL) was added over 5 min. The resulting mixture was allowed to warm gradually to RT and was stirred for 2 h, at which point the reaction media was heated to 40 °C and stirred for another 6 h. The mixture was allowed to cool to RT and volatiles were removed *in vacuo*. The residue was partitioned between MTBE (25 mL) and 1× PBS (15 mL). The mixture was stirred vigorously for 10 min and the layers were separated. The aqueous phase was extracted with MTBE (3 × 25 mL). The combined organic extracts were washed with brine (80 mL), dried over $MgSO_4$, filtered, and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 30%) to yield enamide **6** (3.74 g, 6.32 mmol, 89%) as a 6:1 mixture of *Z* and *E* isomers as a light yellow oil. *Note: *Z* and *E* isomers are not separated for the following step, as they both react to form the desired product. R_f = 0.48 (hexanes/EtOAc,

67:33); FTIR (neat), ν_{max} (cm^{-1}): 3297, 2943, 2866, 1720, 1664, 1528, 1463, 1439, 1382, 1373, 1335, 1283, 1210, 1160, 1106, 1092, 1062, 1043, 1016; ^{1}H NMR (600 MHz, $CDCl_3$): *δ (Z-isomer)* 7.73 (s, 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 4.94 (s, 1H), 4.61 (dd, *J* = 6.0, 1.1 Hz, 1H), 4.58 (d, *J* = 5.9 Hz, 1H), 4.44 (ddd, *J* = 10.4, 4.4, 1.1 Hz, 1H), 3.81 (s, 3H), 3.32 (s, 3H), 2.87 (ddd, *J* = 8.3, 5.5, 3.7 Hz, 1H), 2.40 (ddd, *J* = 15.8, 7.2, 5.1 Hz, 1H), 2.26 (ddd, *J* = 15.8, 8.7, 7.2 Hz, 1H), 1.71 (ddd, *J* = 13.3, 10.4, 4.2 Hz, 1H), 1.65 (ddd, *J* = 13.4, 10.8, 4.5 Hz, 1H), 1.46 (s, 3H), 1.30 (s, 3H), 1.06–1.02 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): *δ (Z-isomer)* 163.3, 155.1 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 138.3, 133.3, 123.8, 115.6 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 112.0, 109.4, 109.1, 85.2, 84.2, 83.7, 54.5, 52.2, 40.6, 34.4, 29.2, 26.2, 24.8, 18.3, 11.0; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): *δ (Z-isomer)* –76.4; HRMS (ESI+): calcd. for $[C_{28}H_{44}F_3N_1O_7Si_1K]^+$ 630.2471, meas. 630.2462, Δ 1.4 ppm.

Methyl (2*S*,5*S*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)-7-(triisopropylsilyl)hept-6-ynoate (7). To a flask charged with olefin **6** (300 mg, 0.507 mmol), as a 6:1 mixture of *Z* and *E* isomers, was added MeOH (20 mL) at RT under a nitrogen atmosphere. The contents were stirred until full dissolution was noted. Separately, (*S*,*S*)-MeBPE-Rh (28 mg, 0.051 mmol, 10 mol %) was weighed out into a vial in a glove box. The vial was removed from the glove box, placed under a nitrogen atmosphere, and MeOH (10 mL) was added. The methanolic solution of (*S*,*S*)-MeBPE-Rh was transferred *via* syringe to the methanolic solution of olefin **6**. The flask headspace was purged with hydrogen (balloon), the outlet

needle was removed, and the balloon was refilled with hydrogen. The contents were stirred vigorously at RT under a hydrogen atmosphere (balloon) for 4 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 50%) to afford protected amino acid **7** (276 mg, 0.465 mmol, 92%) as a single diastereomer, as a clear viscous oil. R_f = 0.45 (hexanes/EtOAc, 67:33); FTIR (neat), ν_{max} (cm^{-1}): 3321, 2942, 2865, 2165, 1750, 1719, 1549, 1462, 1440, 1382, 1372, 1208, 1160, 1095, 1060, 1017; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.84 (d, J = 7.8 Hz, 1H), 4.93 (s, 1H), 4.66 (td, J = 7.7, 4.6 Hz, 1H), 4.61 (dd, J = 5.9, 1.2 Hz, 1H), 4.57 (d, J = 5.9 Hz, 1H), 4.43 (ddd, J = 10.1, 4.8, 1.2 Hz, 1H), 3.79 (s, 3H), 3.30 (s, 3H), 2.70–2.62 (m, 1H), 2.14 (dddd, J = 14.0, 10.8, 5.8, 4.6 Hz, 1H), 2.08–1.99 (m, 1H), 1.68–1.57 (m, 2H), 1.54 (dddd, J = 13.1, 10.7, 5.9, 4.5 Hz, 1H), 1.46 (s, 3H), 1.36 (dddd, J = 13.1, 11.2, 9.7, 4.4 Hz, 1H), (s, 3H), 1.08–1.03 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4, 156.9 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 112.5, 109.8, 109.5, 85.6, 85.5, 84.5, 83.7, 55.1, 53.0, 52.3, 41.1, 31.1, 30.1, 29.8, 26.7, 25.4, 18.7, 11.3; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{28}H_{46}F_3N_1O_7Si_1Na]^+$ 616.2888, meas. 616.2890, Δ 0.4 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-ethynyl-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (8). To a suspension of N^6-benzoyladenine (742 mg, 3.10 mmol, 2.15 equiv.) in propionitrile (dried over 4 Å molecular sieves, 10 mL) at RT, was added *N,O*-bis(trimethylsilyl)acetamide (0.97 mL, 4.0 mmol, 2.75 equiv.). The resulting mixture was stirred at 80 °C for 10 min,

upon which complete solubilization of N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S4** (dried by azeotropic distillation with benzene (4 ×), 735 mg, 1.44 mmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (51 mg, 0.14 mmol, 10 mol %) was added propionitrile (10 mL). The resulting mixture was stirred at RT for 5 min until a clear solution was obtained and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 3 h, cooled to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (PhMe/MeCN, 0 → 50%) to afford nucleoside **8** (663 mg, 0.963 mmol, 67%) as a white amorphous solid. R_f = 0.48 (PhMe/MeCN, 50:50); FTIR (thin-film), ν_{max} (cm^{-1}): 3273, 3086, 2928, 1748, 1720, 1611, 1582, 1511, 1487, 1456, 1374, 1329, 1243, 1217, 1182, 1160, 1099, 1074, 1047, 1029; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.09 (s, 1H), 8.77 (s, 1H), 8.09 (s, 1H), 8.03–7.99 (m, 2H), 7.62–7.57 (m, 1H), 7.53–7.48 (m, 2H), 7.06 (d, *J* = 7.9 Hz, 1H), 6.12 (d, *J* = 5.4 Hz, 1H), 6.07 (t, *J* = 5.4 Hz, 1H), 5.55 (dd, *J* = 5.5, 4.5 Hz, 1H), 4.61 (td, *J* = 7.8, 5.0 Hz, 1H), 4.48 (ddd, *J* = 10.9, 4.5, 2.9 Hz, 1H), 3.77 (s, 3H), 2.59 (ddtd, *J* = 11.4, 9.1, 4.4, 2.5 Hz, 1H), 2.16 (d, *J* = 2.4 Hz, 1H), 2.15 (s, 3H), 2.11–2.00 (m, 2H), 2.06 (s, 3H), 1.95 (dddd, *J* = 13.9, 10.9, 7.7, 4.6 Hz, 1H), 1.86 (ddd, *J* = 13.8, 11.2, 2.9 Hz, 1H), 1.61–1.54 (m, 1H), 1.45 (dddd, *J* = 13.6, 10.9, 9.3, 4.6 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.2, 169.8, 169.6, 165.2, 157.0 (q, $^2J_{C\text{-}F}$ = 37.6 Hz), 152.6, 151.6, 149.9, 142.4, 133.5, 132.8, 128.8, 128.1, 123.9, 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 87.0, 84.7, 80.7, 73.5, 72.9,

71.7, 53.0, 52.3, 38.2, 30.8, 29.6, 27.9, 20.6, 20.4; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.7; HRMS (ESI+): calcd. for $[C_{31}H_{31}F_3N_6O_9Na]^+$ 711.1988, meas. 711.1997, Δ 1.2 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((3-carbamoylphenyl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (9). To a vial charged with alkyne **8** (79 mg, 0.11 mmol) were added 3-iodobenzamide (57 mg, 0.23 mmol, 2.0 equiv.), CuI (4 mg, 0.02 mmol, 20 mol %), and $Pd(PPh_3)_4$ (7 mg, 6 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 3.5 mL) was added at RT. The resulting mixture was stirred at 70 °C for 3 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (PhMe/MeCN, 0 → 100%) to afford protected NS1 **9** (83 mg, 0.10 mmol, 90%) as a white amorphous solid. R_f = 0.17 (EtOAc/*i*-PrOH, 50:50); FTIR (neat), ν_{max} (cm^{-1}): 3255, 3064, 2929, 1744, 1717, 1666, 1610, 1578, 1511, 1486, 1455, 1374, 1329, 1240, 1212, 1180, 1158, 1096, 1073, 1046, 1029; ^{1}H NMR (600 MHz, CD_2Cl_2): δ 9.03 (br s, 1H), 8.75 (s, 1H), 8.14 (s, 1H), 8.00 (t, *J* = 1.3 Hz, 1H), 7.99 (d, *J* = 1.5 Hz, 1H), 7.87 (td, *J* = 1.8, 0.6 Hz, 1H), 7.77 (ddd, *J* = 7.8, 1.9, 1.1 Hz, 1H), 7.64 (ddt, *J* = 8.0, 7.0, 1.3 Hz, 1H), 7.57–7.54 (m, 2H), 7.53 (dd, *J* = 1.6, 1.0 Hz, 1H), 7.40 (td, *J* = 7.8, 0.6 Hz, 1H), 7.15 (d, *J* = 7.7 Hz, 1H), 6.53 (br s, 1H), 6.18 (d, *J* = 5.8 Hz, 1H), 6.15 (t, *J* = 5.5 Hz, 1H), 5.70 (br s, 1H), 5.65 (dd, *J* = 5.3, 4.0 Hz, 1H), 4.65 (td, *J* = 7.5, 5.5 Hz, 1H), 4.54 (dt, *J* = 10.1, 3.9 Hz, 1H), 3.77 (s, 3H), 2.83 (ddt, *J* = 11.0, 9.0, 4.4 Hz,

1H), 2.19 (ddd, J = 13.8, 10.1, 4.0 Hz, 1H), 2.15 (s, 3H), 2.11 (dt, J = 10.5, 5.1 Hz, 1H), 2.11–1.99 (m, 2H), 2.04 (s, 3H), 1.68 (ddt, J = 13.0, 10.7, 5.6 Hz, 1H), 1.57 (dddd, J = 13.5, 10.8, 9.3, 4.8 Hz, 1H); ^{13}C NMR (101 MHz, CD_2Cl_2): δ 171.6, 170.4, 170.1, 168.8, 165.4, 157.4 (q, $^{2}J_{C\text{-}F}$ = 37 Hz), 152.8, 152.1, 150.3, 142.9, 134.8, 134.1, 134.0, 133.1, 131.1, 129.1, 128.9, 128.3, 127.4, 124.6, 123.9, 116.1 (q, $^{1}J_{C\text{-}F}$ = 287 Hz), 91.9, 87.3, 83.1, 81.5, 74.1, 73.0, 53.3, 53.0, 38.8, 31.4, 29.8, 29.1, 20.8, 20.6; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.7; HRMS (ESI+): calcd. for $[C_{38}H_{37}F_3N_7O_{10}]^+$ 808.2549, meas. 808.2538, Δ 1.3 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoylphenyl)hept-6-ynoic acid (NS1, 10). To a solution of alkyne **9** (158 mg, 196 μmol) in THF (2.0 mL) at RT, was added a 0.5 M aq. LiOH solution (2.0 mL). The resulting mixture was stirred for 2.5 h, cooled to 0 °C, and a 10% aq. AcOH solution was added dropwise until pH 7 was reached. Volatiles were removed *in vacuo* and the residue was dissolved in a solution of ammonia in methanol (7 N, 8 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (gradient elution: 5% → 55% B over 45 min) to afford NS1 **10** (107 mg, 172 μmol, 88% over 2 steps) as the TFA salt. Single crystals suitable for X-ray diffraction studies were grown from a 4:1:1 volumetric mixture of 10 mM aq. NS1-TFA:*n*-BuOH:*i*-PrOH *via* slow evaporation at 4 °C over two weeks. Full small molecule X-ray crystallographic data for NS1 (**10**) are reported in the *Supporting*

Information. ^{1}H NMR (600 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (40 μL)): δ 8.37 (s, 1H), 8.34 (s, 1H), 7.78 (td, *J* = 1.8, 0.6 Hz, 1H), 7.72 (ddd, *J* = 7.8, 1.9, 1.2 Hz, 1H), 7.52 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.40 (td, *J* = 7.8, 0.6 Hz, 1H), 6.01 (d, *J* = 5.1 Hz, 1H), 4.74 (t, *J* = 5.1 Hz, 1H), 4.38 (ddd, *J* = 10.3, 4.4, 3.2 Hz, 1H), 4.23 (t, *J* = 4.8 Hz, 1H), 4.02 (dd, *J* = 6.6, 5.8 Hz, 1H), 2.81 (ddt, *J* = 10.6, 9.1, 4.5 Hz, 1H), 2.17 (dddd, *J* = 14.4, 11.5, 6.7, 4.5 Hz, 1H), 2.10–1.97 (m, 2H), 1.95–1.89 (m, 1H), 1.81–1.72 (m, 1H), 1.61–1.52 (m, 1H); ^{13}C NMR (101 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (40 μL)): δ 172.1, 171.3, 151.0, 149.4, 145.2, 144.1, 135.8, 134.4, 131.6, 129.9, 128.1, 124.5, 120.1, 92.8, 90.0, 83.9, 83.4, 74.8, 74.5, 53.5, 38.9, 30.9, 29.6, 28.7; HRMS (ESI+): calcd. for $[C_{24}H_{28}N_7O_6]^+$ 510.2096, meas. 510.2082, Δ 2.6 ppm.

Synthesis of Compounds 11-41

(*S*)-2-amino-6-((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)hexanoic acid (11). To a solution of nucleoside **S11** (73 mg, 0.10 mmol) in a 4:2:1 mixture of MeOH, THF and water (35 mL) at RT was added $CsOH•xH_2O$ (100 mg). The resulting mixture was stirred for 16 h and a 10% AcOH aq. solution was added dropwise until neutral pH was reached. Volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 95% B' over 80 min) to afford the partially deprotected carbamate. To a solution of the carbamate intermediate in a 10:1 mixture of *i*-PrOAc and *t*-BuOH (25 mL) at RT, was added Pd/C (10%, 65 mg, 61 μmol). The reaction mixture

was purged with hydrogen (3 ×) and stirred at RT under a hydrogen atmosphere (balloon) for 2 h. The reaction rate appeared sluggish so $Pd(OH)_2$/C (20%, 105 mg, 150 μmol) was added as a suspension in a 1:1 mixture of *i*-PrOAc and AcOH (10 mL) and the resulting mixture was stirred at 50 °C for 2 h. The reaction remained slow, so EtOH (20 mL) was added. The temperature was lowered to 40 °C and the reaction media was stirred for 3 h, upon which complete cleavage of the Cbz protecting group was observed.[70] The flask head-space was purged with nitrogen under vigorous stirring. The contents were filtered over a pad of celite and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (2.5% → 27.5% B' over 80 min) to afford **11** (33 mg, 69 μmol, 66% over 2 steps) as the TFA salt, as a fluffy white powder. ^{1}H NMR (600 MHz, D_2O): δ 8.48 (s, 1H), 8.45 (s, 1H), 6.12 (d, J = 5.1 Hz, 1H), 4.82 (t, J = 5.2 Hz, 1H), 4.29 (t, J = 5.0 Hz, 1H), 4.21–4.16 (m, 1H), 4.00 (t, J = 6.2 Hz, 1H), 1.98 (app. ddt, J = 14.2, 10.9, 5.5 Hz, 1H), 1.94–1.86 (m, 1H), 1.88–1.77 (m, 2H), 1.59–1.39 (m, 4H); ^{13}C NMR (101 MHz, D_2O): δ 173.2, 150.3, 148.6, 144.9, 143.0, 119.2, 88.4, 85.1, 74.1, 73.4, 53.6, 32.5, 30.0, 24.7, 24.2; HRMS (ESI+): calcd. for $[C_{15}H_{23}N_6O_5]^+$ 367.1724, meas. 367.1728, Δ 1.1 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetra-hydrofuran-2-yl)methyl)hept-6-ynoic acid (12). To a solution of **8** (42 mg, 61 μmol) in THF (1.0 mL) at RT, was added a 0.5 M LiOH aq. solution (1.0 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 10% AcOH aq. solution was added dropwise until

pH 7 was reached. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 3 mL) at RT. The resulting mixture was stirred for 16 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (2% → 42% B over 30 min followed by 42% → 95% B over 5 min) to afford **12** (22 mg, 44 μmol, 72% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of sample preparation for NMR analysis. ^{1}H NMR (500 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.87 (s, 2H), 6.50 (d, J = 5.0 Hz, 1H), 5.19 (t, J = 5.1 Hz, 1H), 4.68 (t, J = 4.8 Hz, 1H), 4.49 (dd, J = 6.7, 5.8 Hz, 1H), 3.08 (dddd, J = 13.7, 7.2, 5.8, 4.3 Hz, 1H), 3.02 (d, J = 2.4 Hz, 1H), 2.59 (dddd, J = 14.2, 11.6, 6.8, 4.7 Hz, 1H), 2.53–2.45 (m, 1H), 2.41 (td, J = 10.0, 5.2 Hz, 1H), 2.33 (ddd, J = 14.0, 10.9, 3.4 Hz, 1H), 2.19 (tt, J = 12.3, 5.0 Hz, 1H), 1.99 (dddd, J = 13.4, 11.9, 9.1, 4.6 Hz, 1H); ^{13}C NMR (101 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.0, 150.9, 149.3, 145.2, 144.0, 120.0, 89.8, 86.1, 83.7, 74.8, 74.4, 73.1, 53.4, 38.8, 30.7, 28.8, 28.4; HRMS (ESI+): calcd. for $[C_{17}H_{23}N_6O_5]^+$ 391.1724, meas. 391.1722, Δ 0.7 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-phenylhept-6-ynoic acid (13). To a solution of **S12** (74 mg, 97 μmol) in THF (7.0 mL) at RT, was added a 0.5 M LiOH aq. solution (1.7 mL). The resulting mixture was stirred for 3 h, cooled to 0 °C, and a 10% AcOH aq. solution was added dropwise until pH 7 was reached. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 10 mL) at RT. The resulting mixture

was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 45 min followed by 45% → 95% B over 5 min) to afford **13** (35 mg, 60 μmol, 62% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, CD_3CN (350 μL)/**D_2O** (350 μL)/*d*-TFA (40 μL)): δ 8.84 (s, 1H), 8.82 (s, 1H), 7.84–7.80 (m, 2H), 7.78–7.74 (m, 3H), 6.47 (d, *J* = 5.1 Hz, 1H), 5.19 (t, *J* = 5.1 Hz, 1H), 4.83 (ddd, *J* = 10.5, 4.5, 3.2 Hz, 1H), 4.68 (t, *J* = 4.8 Hz, 1H), 4.48 (dd, *J* = 6.7, 5.7 Hz, 1H), 3.27 (ddt, *J* = 10.7, 9.2, 4.5 Hz, 1H), 2.63 (dddd, *J* = 14.3, 11.5, 6.8, 4.5 Hz, 1H), 2.56–2.44 (m, 2H), 2.38 (ddd, *J* = 13.9, 10.8, 3.3 Hz, 1H), 2.23 (tt, *J* = 12.6, 5.0 Hz, 1H), 2.03 (dddd, *J* = 13.4, 11.9, 9.2, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (40 μL)): δ 172.0, 151.0, 149.4, 145.2, 144.0, 132.4, 129.5, 129.2, 123.9, 120.1, 91.7, 89.9, 84.2, 83.9, 74.8, 74.5, 53.5, 39.0, 30.9, 29.5, 28.7; HRMS (ESI+): calcd. for $[C_{23}H_{26}N_6O_5Na]^+$ 489.1857, meas. 489.1841, Δ 3.2 ppm.

(2*S*,5*R*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoylphenyl)hept-6-ynoic acid (14). To a solution of **S19** (50 mg, 62 μmol) in THF (1.2 mL) at RT, was added a 0.5 M LiOH aq. solution (1.2 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.22 mL) was added until pH 7 was reached. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 5 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude

material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **14** (38 mg, 62 μmol, quant. over 2 steps) as the TFA salt upon treatment with *d*-TFA (50 μL), as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (50 μL)): δ 8.37 (s, 1H), 8.31 (s, 1H), 7.70 (ddd, *J* = 7.7, 1.7, 1.3 Hz, 1H), 7.67 (ddd, *J* = 1.7, 1.5, 0.4 Hz, 1H), 7.41 (ddd, *J* = 7.7, 1.5, 1.3 Hz, 1H), 7.36 (app td, *J* = 7.7, 0.4 Hz, 1H), 6.00 (d, *J* = 5.2 Hz, 1H), 4.79 (app t, *J* = 5.2 Hz, 1H), 4.28 (ddd, *J* = 8.6, 5.1, 4.0 Hz, 1H), 4.24 (dd, *J* = 5.2, 4.0 Hz, 1H), 4.01 (app t, *J* = 6.4 Hz, 1H), 2.81 (app tt, *J* = 8.6, 5.3 Hz, 1H), 2.21 (dddd, *J* = 14.2, 11.5, 6.4, 5.4 Hz, 1H), 2.08 (app dt, *J* = 13.9, 8.6 Hz, 1H), 2.03–1.94 (m, 2H), 1.76–1.63 (m, 2H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (50 μL)): δ 172.2, 171.6, 150.9, 149.4, 145.2, 144.1, 135.7, 134.2, 131.3, 129.9, 128.1, 124.5, 120.0, 93.8, 90.0, 84.6, 82.9, 74.7, 74.7, 53.6, 38.9, 30.7, 29.8, 28.6; HRMS (ESI+): calcd. for $[C_{24}H_{28}N_7O_6]^+$ 510.2096, meas. 510.2112, Δ 3.2 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoylphenyl)heptanoic acid (15). To a solution of **9** (20 mg, 25 μmol) in MeOH (1.3 mL) at RT was added Pd/C (10%, 8 mg, 7 μmol, 30 mol %). The vial headspace was evacuated and refilled with hydrogen (3 ×) and the resulting mixture was stirred vigorously at 50 °C under a hydrogen atmosphere (balloon) for 17 h and filtered through a nylon syringe filter (13 mm, 0.22 μm). The solids were washed with MeOH (5 × 1 mL) and the filtrates were concentrated. Crude alkane was used directly in

the next step without further purification. To a solution of crude alkane in THF (0.5 mL) at RT, was added a 0.5 M LiOH aq. solution (0.46 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C and a 1 M HCl aq. solution (0.11 mL) was added to reach pH 7. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4.5 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford NS1-alkane (**15**) (16 mg, 25 μmol, quant. over 3 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.32 (s, 1H), 8.31 (s, 1H), 7.57– 7.56 (m, 1H), 7.54 (app dt, J = 7.3, 1.7 Hz, 1H), 7.32 (app dt, J = 7.7, 1.7 Hz, 1H), 7.30 (ddd, J = 7.7, 7.3, 0.5 Hz, 1H), 5.93 (d, J = 4.7 Hz, 1H), 4.64 (app t, J = 4.7 Hz, 1H), 4.13–4.10 (m, 1H), 4.10 (dd, J = 5.3, 4.7 Hz, 1H), 3.94 (app t, J = 6.1 Hz, 1H), 2.65–2.53 (m, 2H), 1.93–1.87 (m, 1H), 1.86–1.78 (m, 1H), 1.76–1.70 (m, 2H), 1.68–1.60 (m, 1H), 1.60–1.53 (m, 2H), 1.52–1.45 (m, 1H), 1.45–1.38 (m, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.5, 172.3, 151.0, 149.4, 145.3, 144.2, 144.0, 133.8, 133.4, 129.7, 128.4, 125.9, 120.0, 89.9, 83.4, 74.8, 74.7, 53.9, 37.4, 34.8, 34.3, 32.8, 29.0, 27.9; HRMS (ESI+): calcd. for $[C_{24}H_{32}N_7O_6]^+$ 514.2414, meas. 514.2437, Δ 4.5 ppm.

(2*S*,5*R*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoylphenyl)heptanoic acid (16). To a solution

of **14** (10 mg, 16 μmol) in a 9:1 mixture of EtOH and water (2.0 mL) at RT was added Pd/C (10%, 5 mg, 5 μmol, 30 mol %). The vial headspace was evacuated and refilled with nitrogen (3 ×) followed by hydrogen (3 ×), and the resulting mixture was stirred vigorously at 50 °C under a hydrogen atmosphere (balloon) for 2.5 h and filtered through a nylon syringe filter (13 mm, 0.22 μm). The solids were washed with EtOH (3 × 1 mL) and the filtrates were concentrated. Crude material was purified by preparative HPLC (5% → 45% B' over 30 min followed by 45% → 95% B' over 5 min) to afford **16** (9 mg, 14 μmol, 89%) as the TFA salt. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)): δ 8.26 (s, 1H), 8.24 (s, 1H), 7.56–7.52 (m, 2H), 7.29–7.25 (m, 2H), 5.92 (d, J = 4.6 Hz, 1H), 4.64 (dd, J = 5.0, 4.6 Hz, 1H), 4.09 (app t, J = 5.0 Hz, 1H), 4.09–4.05 (m, 1H), 3.80 (app t, J = 6.1 Hz, 1H), 2.59–2.52 (m, 2H), 1.90–1.83 (m, 1H), 1.80–1.70 (m, 2H), 1.67 (ddd, J = 14.3, 7.6, 3.5 Hz, 1H), 1.64–1.51 (m, 3H), 1.48–1.37 (m, 2H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)): δ 173.1, 172.3, 152.4, 149.5, 147.5, 144.2, 143.0, 133.8, 133.2, 129.6, 128.3, 125.8, 119.9, 89.5, 83.6, 74.8, 74.6, 54.6, 37.7, 35.6, 34.3, 32.7, 28.1, 27.5; HRMS (ESI+): calcd. for $[C_{24}H_{32}N_7O_6]^+$ 514.2409, meas. 514.2423, Δ 2.9 ppm.

3-(4-((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)but-1-yn-1-yl)benzamide (17). To a vial charged with **S26** (58 mg, 97 μmol) was added a solution of ammonia in methanol (7 N, 8.0 mL) at RT. The resulting mixture was stirred for 17 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (10% → 45% B' over 35 min followed by 45% → 95% B' over 5 min) to afford **17**

(35 mg, 86 μmol, 88%) as a white fluffy solid. ^{1}H NMR (600 MHz, **CD$_3$CN** (400 μL)/D$_2$O (400 μL)): δ 8.36 (s, 1H), 8.31 (s, 1H), 7.70–7.69 (m, 1H), 7.71–7.67 (m, 1H), 7.46–7.43 (m, 1H), 7.38–7.35 (m, 1H), 5.98 (d, *J* = 5.1 Hz, 1H), 4.70 (dd, *J* = 5.1, 4.7 Hz, 1H), 4.22–4.18 (m, 2H), 2.53 (app dt, *J* = 17.3, 6.9 Hz, 1H), 2.49 (app dt, *J* = 17.3, 7.2 Hz, 1H), 2.03–1.96 (m, 2H); ^{13}C NMR (126 MHz, **CD$_3$CN** (400 μL)/D$_2$O (400 μL)): δ 171.3, 150.9, 149.4, 145.3, 143.8, 135.6, 134.2, 131.2, 129.9, 127.9, 124.6, 119.9, 91.6, 89.6, 84.8, 80.9, 74.8, 74.1, 32.6, 16.3; HRMS (ESI+): calcd. for $[C_{20}H_{20}N_6O_4Na]^+$ 431.1438, meas. 431.1453, Δ 3.4 ppm.

3-(((1*R*,2*R*)-2-((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)cyclopropyl)ethynyl)benzamide (18). To a vial charged with **S33** (50 mg, 82 μmol) was added a solution of ammonia in methanol (7 N, 6.0 mL) at RT. The resulting mixture was stirred for 17 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (10% → 45% B' over 30 min followed by 45% → 95% B' over 5 min) to afford **18** (32 mg, 76 μmol, 93%) as a white fluffy solid. ^{1}H NMR (500 MHz, **CD3CN** (350 μL)/D2O (350 μL)): δ 8.39 (s, 1H), 8.32 (s, 1H), 7.71 (td, *J* = 1.8, 0.6 Hz, 1H), 7.67 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H), 7.44 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H), 7.35 (td, *J* = 7.8, 0.6 Hz, 1H), 5.95 (d, *J* = 4.7 Hz, 1H), 4.73 (dd, *J* = 5.2, 4.7 Hz, 1H), 4.36 (dd, *J* = 5.2, 4.8 Hz, 1H), 3.45 (dd, *J* = 9.1, 4.8 Hz, 1H), 1.62 (dddd, *J* = 9.1, 8.8, 5.9, 4.4 Hz, 1H), 1.53 (ddd, *J* = 8.6, 5.4, 4.4 Hz, 1H), 1.05 (ddd, *J* = 8.8, 5.4, 4.8 Hz, 1H), 1.02 (ddd, *J* = 8.6, 5.9, 4.8 Hz, 1H); ^{13}C NMR (126 MHz, **CD3CN** (350 μL)/D2O (350 μL)): δ 171.2, 151.1, 149.3,

145.4, 143.8, 135.6, 134.3, 131.3, 129.8, 127.8, 124.5, 120.0, 93.6, 89.7, 88.5, 76.7, 74.9, 74.4, 25.2, 12.9, 6.4; HRMS (ESI+): calcd. for $[C_{21}H_{21}N_6O_4]^+$ 421.1619, meas. 421.1627, Δ 1.9 ppm.

(2*S*,5*S*)-2-amino-7-(3-carbamoylphenyl)-5-(((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)hept-6-ynoic acid (19). To a solution of **S35** (23 mg, 44 μmol) in THF (2.0 mL) at RT was added a 0.5 M LiOH aq. solution (2.0 mL). The reaction mixture was stirred at RT for 3 h and a 10% AcOH aq. solution was added slowly until pH 5 was obtained. Volatiles were removed *in vacuo* and the residue was purified by preparative HPLC (5% → 55% B' over 60 min followed by 55% → 95% B' over 5 min) to afford **19** (11 mg, 28 μmol, 63%) as the TFA salt. ^{1}H NMR (600 MHz, D_2O): δ 7.84 (t, *J* = 1.8 Hz, 1H), 7.74 (ddd, *J* = 7.9, 1.9, 1.0 Hz, 1H), 7.65 (ddt, *J* = 7.7, 1.6, 0.8 Hz, 1H), 7.47 (tt, *J* = 7.9, 0.6 Hz, 1H), 4.90 (d, *J* = 1.4 Hz, 1H), 4.31 (ddd, *J* = 10.2, 6.3, 3.0 Hz, 1H), 4.13 (dd, *J* = 6.3, 4.7 Hz, 1H), 4.11–4.06 (m, 2H), 3.40 (s, 3H), 2.97–2.89 (m, 1H), 2.25 (dddd, *J* = 14.2, 11.5, 6.7, 4.6 Hz, 1H), 2.16 (ddt, *J* = 14.4, 11.2, 5.5 Hz, 1H), 1.93 (ddd, *J* = 13.8, 10.9, 3.1 Hz, 1H), 1.85–1.80 (m, 1H), 1.77 (ddd, *J* = 13.7, 10.6, 4.5 Hz, 1H), 1.70–1.59 (m, 1H); ^{13}C NMR (101 MHz, D_2O): δ 172.6, 172.3, 135.3, 133.1, 130.5, 129.0, 127.2, 123.2, 108.2, 92.1, 82.7, 81.0, 74.6, 74.4, 55.2, 53.2, 39.5, 29.9, 29.1, 28.0; HRMS (ESI+): calcd. for $[C_{20}H_{27}N_2O_7]^+$ 407.1813, meas. 407.1809, Δ 1.0 ppm.

(*S*)-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoylphenyl)hept-6-ynoic acid (20). To a solution of **S41** (105

mg, 0.151 mmol) in a 2:1 mixture of MeOH and THF (24 mL) at 0 °C was added a 0.2 M LiOH aq. solution (7.5 mL, 3.01 mmol, 20 equiv.). The resulting mixture was stirred at RT for 24 h and organics were removed *in vacuo*. A 1 M HCl aq. solution (1.7 mL) was added and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (30% → 95% B over 35 min followed by 95% B over 5 min) to afford **20** (45 mg, 91 μmol, 60%) as a fluffy white solid. ^{1}H NMR (600 MHz, **CD$_3$CN** (400 μL)/D$_2$O (400 μL)/*d*-TFA (100 μL)): δ 8.36 (s, 1H), 8.34 (s, 1H), 7.76 (dd, *J* = 1.8, 1.2 Hz, 1H), 7.70 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H), 7.49 (app dt, *J* = 7.8, 1.2 Hz, 1H), 7.37 (app t, *J* = 7.8 Hz, 1H), 6.01 (d, *J* = 5.3 Hz, 1H), 4.81–4.77 (dd, *J* = 5.3, 5.0 Hz, 1H), 4.41 (ddd, *J* = 10.6, 4.0, 3.1 Hz, 1H), 4.25–4.21 (dd, *J* = 5.0, 4.0 Hz, 1H), 2.78–2.71 (m, 1H), 2.36–2.26 (m, 2H), 2.01 (ddd, *J* = 13.9, 10.6, 4.1 Hz, 1H), 1.87 (ddd, *J* = 13.9, 11.0, 3.1 Hz, 1H), 1.83–1.75 (m, 1H), 1.73–1.64 (m, 1H), 1.61–1.49 (m, 2H); ^{13}C NMR (126 MHz, **CD$_3$CN** (400 μL)/D$_2$O (400 μL)/*d*-TFA (100 μL)): δ 178.3, 171.9, 151.1, 149.5, 145.3, 144.4, 136.0, 134.2, 131.5, 130.0, 128.1, 124.9, 120.2, 94.0, 90.3, 84.5, 82.9, 74.9, 74.8, 39.2, 35.2, 34.5, 29.8, 23.5; HRMS (ESI+): calcd. for $[C_{24}H_{27}N_6O_6]^+$ 495.1987, meas. 495.1984, Δ 0.4 ppm.

3-((*S*)-6-amino-3-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)hex-1-yn-1-yl)benzamide (21). To a vial charged with **S47** (79 mg, 0.11 mmol) was added a solution of ammonia in methanol (7 N, 15 mL) at RT. The resulting mixture was stirred for 24 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95%

B over 5 min) to afford **21** (33 mg, 57 μmol, 54%) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.36 (s, 1H), 8.33 (s, 1H), 7.76 (ddd, *J* = 1.8, 1.5, 0.4 Hz, 1H), 7.71 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H), 7.50 (ddd, *J* = 7.8, 1.5, 1.2 Hz, 1H), 7.39 (app td, *J* = 7.8, 0.4 Hz, 1H), 6.00 (d, *J* = 5.0 Hz, 1H), 4.71 (dd, *J* = 5.2, 5.0 Hz, 1H), 4.36 (ddd, *J* = 10.3, 4.5, 3.4 Hz, 1H), 4.21 (dd, *J* = 5.2, 4.5 Hz, 1H), 2.97–2.89 (m, 2H), 2.80–2.74 (m, 1H), 1.98 (ddd, *J* = 14.1, 10.3, 4.3 Hz, 1H), 1.90 (ddd, *J* = 14.1, 10.7, 3.4 Hz, 1H), 1.89–1.82 (m, 1H), 1.78–1.70 (m, 1H), 1.61 (app ddt, *J* = 13.2, 10.7, 5.4 Hz, 1H), 1.54 (dddd, *J* = 13.2, 10.6, 9.1, 5.1 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 171.4, 150.9, 149.4, 145.2, 144.0, 135.7, 134.2, 131.4, 129.9, 128.0, 124.5, 120.0, 93.3, 89.8, 83.9, 83.1, 74.8, 74.4, 40.2, 38.9, 32.3, 29.5, 25.8; HRMS (ESI+): calcd. for $[C_{23}H_{28}N_7O_4]^+$ 466.2197, meas. 466.2219, Δ 4.7 ppm.

3-((*S*)-7-amino-3-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-oxohept-1-yn-1-yl)benzamide (22). To a vial charged with **S41** (105 mg, 0.151 mmol) was added a solution of ammonia in methanol (7 N, 18 mL) at RT. The resulting mixture was stirred at 40 °C for 5 days and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **22** (57 mg, 0.12 mmol, 77%) as a white fluffy solid. ^{1}H NMR (600 MHz, **CD$_3$CN** (400 μL)/D$_2$O (400 μL)/*d*-TFA (50 μL)): δ 8.35 (s, 1H), 8.32 (s, 1H), 7.75 (app t, *J* = 1.5 Hz, 1H), 7.69 (app dt, *J* = 7.8, 1.5 Hz, 1H), 7.48

(app dt, J = 7.8, 1.5 Hz, 1H), 7.36 (app t, J = 7.8 Hz, 1H), 5.99 (d, J = 5.2 Hz, 1H), 4.75 (app t, J = 5.2 Hz, 1H), 4.38 (ddd, J = 10.6, 4.1, 3.1 Hz, 1H), 4.21 (dd, J = 5.2, 4.1 Hz, 1H), 2.73 (dddd, J = 10.9, 9.2, 5.4, 4.0 Hz, 1H), 2.23 (ddd, J = 14.6, 8.0, 7.2 Hz, 1H), 2.20 (ddd, J = 14.6, 7.8, 7.1 Hz, 1H), 1.99 (ddd, J = 13.8, 10.6, 4.1 Hz, 1H), 1.85 (ddd, J = 13.8, 11.0, 3.1 Hz, 1H), 1.82–1.73 (m, 1H), 1.71–1.62 (m, 1H), 1.59–1.47 (m, 2H); ^{13}C NMR (126 MHz, **CD_3CN** (400 μL)/D_2O (400 μL)/*d*-TFA (50 μL)): δ 179.5, 171.4, 150.9, 149.4, 145.2, 144.2, 135.8, 134.2, 131.4, 129.9, 128.0, 124.7, 120.1, 93.9, 90.1, 84.3, 82.8, 74.8, 74.6, 39.1, 35.6, 35.1, 29.7, 24.2; HRMS (ESI+): calcd. for $[C_{24}H_{27}N_7O_5Na]^+$ 516.1966, meas. 516.1986, Δ 3.9 ppm.

Methyl (2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoylphenyl)hept-6-ynoate (23). To a solution of **S51** (32 mg, 34 μmol) in a 1:1 mixture of MeCN and CH_2Cl_2 (2.0 mL) at RT was added piperidine (0.50 mL). The resulting mixture was stirred for 20 min and volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4.5 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B' over 35 min followed by 45% → 95% B' over 5 min) to afford **23** (12 mg, 19 μmol, 27% over 2 steps) as the TFA salt. ^{1}H NMR (600 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)): δ 8.33 (s, 1H), 8.30 (s, 1H), 7.77 (ddd, J = 1.9, 1.5, 0.5 Hz, 1H), 7.72 (ddd, J = 7.8, 1.9, 1.2 Hz, 1H), 7.51 (ddd, J = 7.8, 1.5, 1.2 Hz, 1H), 7.40 (app td, J = 7.8, 0.5 Hz, 1H), 5.98 (d, J = 5.0 Hz,

1H), 4.68 (dd, *J* = 5.2, 5.0 Hz, 1H), 4.34 (ddd, *J* = 10.0, 4.7, 3.4 Hz, 1H), 4.19 (dd, *J* = 5.2, 4.7 Hz, 1H), 4.04 (dd, *J* = 6.6, 6.1 Hz, 1H), 3.73 (s, 3H), 2.82–2.75 (m, 1H), 2.13 (dddd, *J* = 14.3, 11.6, 6.6, 4.6 Hz, 1H), 2.06–1.95 (m, 2H), 1.91 (ddd, *J* = 13.9, 10.5, 3.4 Hz, 1H), 1.71 (app ddt, *J* = 12.8, 11.6, 5.1 Hz, 1H), 1.52 (dddd, *J* = 12.8, 11.7, 9.7, 4.6 Hz, 1H); ^{13}C NMR (126 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)): δ 171.2, 170.9, 151.5, 149.4, 146.2, 143.6, 135.7, 134.3, 131.4, 129.9, 128.1, 124.3, 119.9, 92.7, 89.7, 83.6, 83.4, 74.7, 74.3, 54.3, 53.5, 38.7, 30.7, 29.5, 28.7; HRMS (ESI+): calcd. for $[C_{25}H_{30}N_7O_6]^+$ 524.2252, meas. 524.2271, Δ 3.6 ppm.

3-((3*S*,6*S*)-6,7-diamino-3-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-oxohept-1-yn-1-yl)benzamide (24). To a vial charged with **9** (44 mg, 54 μmol) was added a solution of ammonia in methanol (7 N, 15 mL) at RT. The resulting mixture was stirred at 40 °C for 36 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **24** (31 mg, 50 μmol, 91%) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (50 μL)): δ 8.36 (s, 1H), 8.33 (s, 1H), 7.78–7.76 (m, 1H), 7.71 (ddd, *J* = 7.8, 1.9, 1.2 Hz, 1H), 7.51 (ddd, *J* = 7.8, 1.5, 1.2 Hz, 1H), 7.39 (app td, *J* = 7.8, 0.6 Hz, 1H), 6.00 (d, *J* = 5.1 Hz, 1H), 4.73 (app t, *J* = 5.1 Hz, 1H), 4.37 (ddd, *J* = 10.3, 4.5, 3.4 Hz, 1H), 4.22 (dd, *J* = 5.1, 4.5 Hz, 1H), 3.96 (app t, *J* = 6.4 Hz, 1H), 2.79 (dddd, *J* = 10.6, 9.2, 4.5, 4.3 Hz, 1H), 2.13 (dddd, *J* = 14.2, 12.4, 6.4, 4.4

Hz, 1H), 2.00 (ddd, *J* = 14.3, 10.3, 4.3 Hz, 1H), 1.98–1.89 (m, 2H), 1.67 (dddd, *J* = 13.1, 12.4, 4.8, 4.5 Hz, 1H), 1.56 (dddd, *J* = 13.1, 12.7, 9.2, 4.4 Hz, 1H); ^{13}C NMR (126 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (50 μL)): δ 172.4, 171.6, 151.0, 149.4, 145.2, 144.1, 135.9, 134.2, 131.6, 130.0, 128.1, 124.5, 120.1, 92.9, 90.0, 84.0, 83.4, 74.9, 74.5, 53.8, 38.8, 30.7, 29.8, 29.7; HRMS (ESI+): calcd. for $[C_{24}H_{28}N_8O_5Na]^+$ 531.2075, meas. 531.2089, Δ 2.7 ppm.

3-((*S*)-3-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-5-ureidopent-1-yn-1-yl)benzamide (25). To a vial charged with **S58** (31 mg, 45 μmol) was added a solution of ammonia in methanol (7 N, 5.0 mL) at RT. The resulting mixture was stirred for 17 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 35 min followed by 45% → 95% B over 5 min) to afford **25** (18 mg, 36 μmol, 80%) as a fluffy white solid. ^{1}H NMR (600 MHz, CD_3CN (350 μL)/**D_2O** (350 μL)/*d*-TFA (40 μL)): δ 8.54 (s, 1H), 8.51 (s, 1H), 7.96 (t, *J* = 1.7 Hz, 1H), 7.90 (ddd, *J* = 7.8, 1.9, 1.1 Hz, 1H), 7.70 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.58 (dd, *J* = 7.8, 0.6 Hz, 1H), 6.18 (d, *J* = 5.2 Hz, 1H), 4.93 (t, *J* = 5.2 Hz, 1H), 4.56 (dt, *J* = 10.6, 3.5 Hz, 1H), 4.40 (t, *J* = 4.7 Hz, 1H), 3.50 (ddd, *J* = 13.3, 7.7, 5.4 Hz, 1H), 3.43 (dt, *J* = 13.7, 7.5 Hz, 1H), 2.98 (tt, *J* = 9.7, 4.5 Hz, 1H), 2.19 (ddd, *J* = 14.4, 10.5, 4.2 Hz, 1H), 2.08 (ddd, *J* = 13.9, 10.9, 3.2 Hz, 1H), 2.00–1.91 (m, 1H), 1.86 (dddd, *J* = 13.2, 9.7, 7.4, 5.4 Hz, 1H); ^{13}C NMR (126 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (40 μL)): δ 171.6, 161.7, 151.0, 149.5, 145.2, 144.3, 135.9, 134.3, 131.5, 130.0, 128.2, 124.6, 120.1,

93.0, 90.1, 84.1, 83.2, 74.8, 74.6, 39.5, 39.0, 35.3, 27.6; HRMS (ESI+): calcd. for $[C_{23}H_{26}N_8O_5Na]^+$ 517.1918, meas. 517.1912, Δ 1.2 ppm.

(2*S*,6*S*)-2-amino-6-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-8-(3-carbamoylphenyl)oct-7-ynoic acid (26). To a solution of **S66** (69 mg, 84 μmol) in THF (1.5 mL) at RT was added a 0.5 M LiOH aq. solution (1.5 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.20 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4.5 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 25% B' over 30 min followed by 25% → 95% B' over 5 min) to afford **26** (9 mg, 14 μmol, 17% over 2 steps) as the TFA salt. ^{1}H NMR (600 MHz, D_2O (700 μL)): δ 8.42 (s, 1H), 8.30 (s, 1H), 7.70–7.65 (m, 1H), 7.51–7.48 (m, 1H), 7.37–7.33 (m, 2H), 6.08 (d, *J* = 5.0 Hz, 1H), 4.95 (dd, *J* = 5.1, 5.0 Hz, 1H), 4.47 (ddd, *J* = 9.2, 4.6, 3.2 Hz, 1H), 4.40 (dd, *J* = 5.1, 4.6 Hz, 1H), 3.95 (app t, *J* = 6.1 Hz, 1H), 2.89–2.84 (m, 1H), 2.08 (ddd, *J* = 14.4, 9.2, 3.7 Hz, 1H), 2.00 (ddd, *J* = 14.4, 9.8, 3.2 Hz, 1H), 1.96–1.91 (m, 2H), 1.72–1.60 (m, 3H), 1.56–1.49 (m, 1H); ^{13}C NMR (126 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)): δ 172.5, 171.3, 151.2, 149.4, 145.7, 143.8, 135.7, 134.2, 131.4, 129.9, 127.9, 124.5, 120.0, 93.7, 89.7, 83.9, 82.9, 74.7, 74.4, 53.7, 38.9, 34.9, 30.5, 29.5, 23.2; HRMS (ESI+): calcd. for $[C_{25}H_{30}N_7O_6]^+$ 524.2252, meas. 524.2277, Δ 4.7 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(4-carbamoylphenyl)hept-6-ynoic acid (27). To a solution of **S67** (18 mg, 22 μmol) in THF (0.41 mL) at RT was added a 0.5 M LiOH aq. solution (0.41 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.10 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4.5 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B' over 30 min followed by 45% → 95% B' over 5 min) to afford **27** (7 mg, 11 μmol, 50% over 2 steps) as the TFA salt. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)): δ 8.32 (s, 1H), 8.30 (s, 1H), 7.73–7.70 (m, 2H), 7.43–7.39 (m, 2H), 5.97 (d, *J* = 5.0 Hz, 1H), 4.71 (dd, *J* = 5.3, 5.0 Hz, 1H), 4.33 (ddd, *J* = 10.3, 4.5, 3.3 Hz, 1H), 4.19 (dd, *J* = 5.3, 4.5 Hz, 1H), 3.90 (dd, *J* = 6.8, 5.7 Hz, 1H), 2.83–2.76 (m, 1H), 2.10 (dddd, *J* = 14.2, 11.5, 6.8, 4.6 Hz, 1H), 2.03–1.96 (m, 2H), 1.94–1.88 (m, 1H), 1.73 (dddd, *J* = 12.8, 12.0, 5.4, 4.6 Hz, 1H), 1.55 (m, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)): δ 172.6, 171.4, 151.7, 149.4, 146.5, 143.6, 133.2, 132.5, 128.6, 127.7, 120.0, 94.7, 89.7, 83.7, 83.4, 74.7, 74.4, 53.9, 38.6, 30.9, 29.6, 28.8; HRMS (ESI+): calcd. for $[C_{24}H_{28}N_7O_6]^+$ 510.2096, meas. 510.2097, Δ 0.2 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(2-carbamoylphenyl)hept-6-ynoic acid (28). To a solution of alkyne **S68** (18 mg, 22 μmol) in THF (0.41 mL) at RT was added a 0.5 M LiOH aq.

solution (0.41 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.10 mL) was added. Volatiles were removed in vacuo. The residue was dissolved in a solution of ammonia in methanol (7 N, 4.5 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed in vacuo. Crude material was purified by preparative HPLC (5% → 45% B' over 30 min followed by 45% → 95% B' over 5 min) to afford **28** (8 mg, 13 μmol, 58% over 2 steps) as the TFA salt. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)): δ 8.32 (s, 1H), 8.29 (s, 1H), 7.56–7.52 (m, 1H), 7.44–7.41 (m, 1H), 7.40 (app td, J = 7.6, 1.5 Hz, 1H), 7.37 (ddd, J = 7.6, 7.1, 2.0 Hz, 1H), 5.97 (d, J = 5.1 Hz, 1H), 4.71 (app t, J = 5.1 Hz, 1H), 4.34 (ddd, J = 10.1, 4.5, 3.4 Hz, 1H), 4.20–4.18 (m, 1H), 3.88 (dd, J = 6.7, 5.9 Hz, 1H), 2.84–2.76 (m, 1H), 2.09 (dddd, J = 14.2, 11.7, 6.7, 4.6 Hz, 1H), 2.01–1.95 (m, 2H), 1.95–1.89 (m, 1H), 1.72 (app ddt, J = 13.4, 11.7, 4.9 Hz, 1H), 1.60–1.52 (m, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)): δ 172.8, 151.7, 149.4, 146.5, 143.6, 137.3, 134.1, 131.7, 129.4, 128.8, 121.3, 120.0, 97.1, 89.7, 83.7, 81.8, 74.6, 74.4, 54.1, 38.5, 30.9, 29.7, 28.9; HRMS (ESI+): calcd. for $[C_{24}H_{28}N_7O_6]^+$ 510.2096, meas. 510.2095, Δ 0.2 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-sulfamoylphenyl)hept-6-ynoic acid (29). To a solution of **S69** (41 mg, 49 μmol) in THF (1.3 mL) at RT was added a 0.5 M LiOH aq. solution (1.1 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.20 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a

solution of ammonia in methanol (7 N, 5 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **29** (26 mg, 39 μmol, 81% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.36 (s, 1H), 8.34 (s, 1H), 7.82 (dd, *J* = 1.9, 1.5 Hz, 1H), 7.77 (ddd, *J* = 7.8, 1.9, 1.2 Hz, 1H), 7.57 (ddd, *J* = 7.8, 1.5, 1.2 Hz, 1H), 7.48 (app t, *J* = 7.8 Hz, 1H), 6.00 (d, *J* = 5.1 Hz, 1H), 4.73 (dd, *J* = 5.2, 5.1 Hz, 1H), 4.36 (ddd, *J* = 10.3, 4.5, 3.3 Hz, 1H), 4.22 (dd, *J* = 5.2, 4.5 Hz, 1H), 4.01 (dd, *J* = 6.7, 5.8 Hz, 1H), 2.86–2.78 (m, 1H), 2.16 (dddd, *J* = 14.4, 11.7, 6.7, 4.5 Hz, 1H), 2.10–2.02 (m, 1H), 2.01 (ddd, *J* = 14.3, 10.3, 4.1 Hz, 1H), 1.96–1.90 (m, 1H), 1.77 (app ddt, *J* = 13.0, 11.7, 5.1 Hz, 1H), 1.58 (dddd, *J* = 13.0, 11.7, 9.6, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.1, 151.0, 149.4, 145.3, 144.1, 143.7, 136.4, 130.6, 129.6, 126.4, 125.2, 120.1, 93.8, 90.1, 83.9, 82.8, 74.9, 74.5, 53.6, 38.8, 30.9, 29.6, 28.7; HRMS (ESI+): calcd. for $[C_{23}H_{28}N_7O_7S_1]^+$ 546.1765, meas. 546.1764, Δ 0.3 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoyl-4-fluorophenyl)hept-6-ynoic acid (30). To a solution of **S71** (44 mg, 53 μmol) in THF (1 mL) at RT, was added a 0.5 M LiOH aq. solution (1 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl

aq. solution (0.20 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 5 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **30** (17 mg, 26 μmol, 50% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.36 (s, 1H), 8.34 (s, 1H), 7.76 (dd, *J* = 7.1, 2.3 Hz, 1H), 7.51 (ddd, *J* = 8.6, 4.8, 2.3 Hz, 1H), 7.15 (dd, *J* = 11.1, 8.6 Hz, 1H), 6.00 (d, *J* = 5.1 Hz, 1H), 4.72 (app t, *J* = 5.1 Hz, 1H), 4.35 (ddd, *J* = 10.3, 4.5, 3.3 Hz, 1H), 4.21 (dd, *J* = 5.1, 4.5 Hz, 1H), 4.01 (dd, *J* = 6.7, 5.8 Hz, 1H), 2.82–2.76 (m, 1H), 2.15 (dddd, *J* = 14.4, 11.7, 6.7, 4.5 Hz, 1H), 2.07–2.02 (m, 1H), 1.99 (ddd, *J* = 13.9, 10.3, 4.5 Hz, 1H), 1.91 (ddd, *J* = 13.9, 10.8, 3.3 Hz, 1H), 1.75 (app ddt, *J* = 12.9, 11.7, 5.0 Hz, 1H), 1.56 (dddd, *J* = 12.9, 11.9, 9.5, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.2, 167.5 (d, $J_{C\text{-}F}$ = 2.0 Hz), 160.7 (d, $J_{C\text{-}F}$ = 253 Hz), 151.0, 149.4, 145.3, 144.1, 137.7 (d, $J_{C\text{-}F}$ = 9.5 Hz), 134.8 (d, $J_{C\text{-}F}$ = 2.7 Hz), 122.4 (d, $J_{C\text{-}F}$ = 14 Hz), 120.9 (d, $J_{C\text{-}F}$ = 3.7 Hz), 120.1, 117.9 (d, $J_{C\text{-}F}$ = 25 Hz), 92.5, 90.0, 84.0, 82.4, 74.9, 74.5, 53.6, 38.9, 30.9, 29.6, 28.8; ^{19}F NMR (471 MHz, CD$_3$CN (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL), HFB IStd): δ –113.6; HRMS (ESI+): calcd. for $[C_{24}H_{26}F_1N_7O_6Na]^+$ 550.1821, meas. 550.1821, Δ0.0 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoyl-4-methylphenyl)hept-6-ynoic acid (31). To

a solution of **S73** (26 mg, 32 μmol) in THF (1.5 mL) at RT was added a 0.5 M LiOH aq. solution (0.9 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.17 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 5 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **31** (11 mg, 17 μmol, 55% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.36 (s, 1H), 8.33 (s, 1H), 7.37 (br d, J = 1.8 Hz, 1H), 7.29 (dd, J = 7.9, 1.8 Hz, 1H), 7.17 (br d, J = 7.9 Hz, 1H), 5.99 (d, J = 5.1 Hz, 1H), 4.73 (app t, J = 5.1 Hz, 1H), 4.35 (ddd, J = 10.3, 4.5, 3.2 Hz, 1H), 4.21 (dd, J = 5.1, 4.5 Hz, 1H), 4.00 (dd, J = 6.7, 5.8 Hz, 1H), 2.82–2.75 (m, 1H), 2.32 (s, 3H), 2.14 (dddd, J = 14.3, 11.7, 6.7, 4.5 Hz, 1H), 2.07–2.00 (m, 1H), 1.99 (ddd, J = 13.9, 10.3, 4.3 Hz, 1H), 1.90 (ddd, J = 13.9, 10.7, 3.2 Hz, 1H), 1.78–1.70 (app ddt, J = 13.0, 11.7, 5.1 Hz, 1H), 1.55 (dddd, J = 13.0, 11.8, 9.3, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 174.5, 172.2, 151.0, 149.4, 145.2, 144.1, 136.9, 136.5, 133.9, 132.1, 130.9, 121.4, 120.1, 92.1, 90.0, 83.9, 83.5, 74.8, 74.5, 53.6, 38.9, 30.9, 29.6, 28.8, 19.8; HRMS (ESI+): calcd. for $[C_{25}H_{29}N_7O_6Na]^+$ 546.2072, meas. 546.2074, Δ 0.5 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoyl-4-(trifluoromethyl)phenyl)hept-6-ynoic

acid (32). To a solution of **S75** (51 mg, 58 μmol) in THF (5.0 mL) at RT was added a 0.5 M LiOH aq. solution (1.5 mL). The resulting mixture was stirred for 3 h, cooled to 0 °C, and a 10% AcOH aq. solution was added dropwise until pH 7 was reached. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 8 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 35% B over 30 min followed by 35% → 95% B over 5 min) to afford **32** (22 mg, 32 μmol, 55% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, CD_3CN (350 μL)/**D_2O** (350 μL)/*d*-TFA (40 μL)): δ 8.82 (s, 1H), 8.80 (s, 1H), 8.12 (d, J = 8.2 Hz, 1H), 8.01 (ddt, J = 8.2, 1.8, 1.0 Hz, 1H), 8.00–7.97 (m, 1H), 6.45 (d, J = 5.0 Hz, 1H), 5.16 (t, J = 5.1 Hz, 1H), 4.82–4.79 (m, 1H), 4.65 (t, J = 4.8 Hz, 1H), 4.46 (dd, J = 6.7, 5.8 Hz, 1H), 3.32–3.25 (m, 1H), 2.60 (dddd, J = 14.3, 11.5, 6.7, 4.5 Hz, 1H), 2.54–2.43 (m, 2H), 2.41–2.35 (m, 1H), 2.27–2.18 (m, 1H), 2.03 (dddd, J = 13.4, 11.8, 9.3, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (40 μL)): δ 172.0, 171.9, 151.0, 149.4, 145.2, 144.0, 136.0, 133.8, 132.0, 128.3, 127.8, 127.8, 127.7, 126.6, 126.3, 125.5, 123.4, 120.0, 117.9, 115.6, 113.3, 95.7, 89.9, 83.8, 82.3, 74.8, 74.4, 53.5, 38.7, 30.7, 29.6, 28.7; HRMS (ESI+): calcd. for $[C_{25}H_{26}F_3N_7O_6Na]^+$ 600.1789, meas. 600.1776, Δ 2.2 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(3-carbamoyl-4-chlorophenyl)hept-6-ynoic acid (33). To a

solution of **S77** (45 mg, 53 μmol) in THF (1 mL) at RT was added a 0.5 M LiOH aq. solution (1 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.20 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 5 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **33** (35 mg, 53 μmol, quant. over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.36 (s, 1H), 8.34 (s, 1H), 7.48–7.47 (m, 1H), 7.38–7.36 (m, 2H), 5.99 (d, J = 5.1 Hz, 1H), 4.72 (app t, J = 5.1 Hz, 1H), 4.34 (ddd, J = 10.4, 4.5, 3.2 Hz, 1H), 4.20 (dd, J = 5.1, 4.5 Hz, 1H), 4.00 (dd, J = 6.7, 5.8 Hz, 1H), 2.79 (app ddt, J = 10.7, 9.2, 4.5 Hz, 1H), 2.14 (dddd, J = 14.3, 11.6, 6.7, 4.5 Hz, 1H), 2.07–2.01 (m, 1H), 2.00 (ddd, J = 14.1, 10.4, 4.5 Hz, 1H), 1.91 (ddd, J = 14.1, 10.7, 3.2 Hz, 1H), 1.75 (dddd, J = 13.1, 11.6, 5.0, 4.5 Hz, 1H), 1.55 (dddd, J = 13.1, 11.8, 9.2, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.1, 171.1, 151.0, 149.4, 145.2, 144.1, 136.0, 135.1, 132.7, 131.3, 131.0, 123.1, 120.1, 93.9, 90.0, 83.9, 82.4, 74.8, 74.5, 53.5, 38.8, 30.8, 29.6, 28.7; HRMS (ESI+): calcd. for $[C_{24}H_{26}Cl_1N_7O_6Na]^+$ 566.1525, meas. 566.1528, Δ 0.4 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(1-oxo-1,2,3,4-tetrahydroisoquinolin-7-yl)hept-6-ynoic

acid (34). To a solution of **S78** (29 mg, 35 μmol) in THF (0.65 mL) at RT was added a 0.5 M LiOH aq. solution (0.65 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.14 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 5 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **34** (23 mg, 35 μmol, quant. over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.36 (s, 1H), 8.33 (s, 1H), 7.08 (br d, J = 7.7 Hz, 1H), 6.96 (dd, J = 7.7, 1.6 Hz, 1H), 6.82 (d, J = 1.6 Hz, 1H), 5.99 (d, J = 5.1 Hz, 1H), 4.73 (app t, J = 5.1 Hz, 1H), 4.35 (ddd, J = 10.4, 4.5, 3.2 Hz, 1H), 4.21 (dd, J = 5.1, 4.5 Hz, 1H), 4.00 (dd, J = 6.7, 5.6 Hz, 1H), 2.89–2.85 (m, 2H), 2.78 (dddd, J = 10.8, 9.3, 4.7, 4.3 Hz, 1H), 2.53–2.48 (m, 2H), 2.13 (dddd, J = 14.3, 11.8, 6.7, 4.6 Hz, 1H), 2.03 (dddd, J = 14.3, 11.7, 5.6, 5.2 Hz, 1H), 1.99 (ddd, J = 14.0, 10.4, 4.3 Hz, 1H), 1.89 (ddd, J = 14.0, 10.8, 3.2 Hz, 1H), 1.74 (dddd, J = 13.0, 11.8, 5.2, 4.7 Hz, 1H), 1.54 (dddd, J = 13.0, 11.7, 9.3, 4.6 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 174.6, 172.2, 151.0, 149.4, 145.2, 144.1, 138.1, 129.2, 127.5, 125.6, 122.8, 120.1, 119.1, 91.7, 90.0, 83.9, 83.8, 74.8, 74.5, 53.5, 38.9, 30.9, 30.7, 29.5, 28.7, 25.4; HRMS (ESI+): calcd. for $[C_{26}H_{30}N_7O_6]^+$ 536.2252, meas. 536.2268, Δ 3.0 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetra-hydrofuran-2-yl)methyl)-7-(3-oxoisoindolin-5-yl)hept-6-ynoic acid (35). To a solution of **8** (107 mg, 0.155 mmol) and 6-iodoisoindolin-1-one (101 mg, 0.388 mmol, 2.5 equiv.) in a 5:3:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 4.7 mL) at RT were added CuI (7 mg, 0.04 mmol, 25 mol %) and $Pd(PPh_3)_4$ (9 mg, 8 μmol, 5 mol %) rapidly. The resulting mixture was stirred at 70 °C for 3 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 85:15) to afford the desired crude reaction product which was used directly in the next step without further purification. To a solution of the crude reaction product in THF (3 mL) at RT was added a 0.5 M LiOH aq. solution (3 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.60 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 18 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **35** (35 mg, 55 μmol, 36% over 3 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, $\mathbf{CD_3CN}$ (350 μL)/D_2O (350 μL)/*d*-TFA (40 μL)): δ 8.36 (s, 1H), 8.33 (s, 1H), 7.65 (br s, 1H), 7.54 (dd, J = 7.9, 1.5 Hz, 1H), 7.48–7.45 (m, 1H), 6.01 (d, J = 5.1 Hz, 1H), 4.74 (app t, J = 5.1 Hz, 1H), 4.40 (br s, 2H), 4.38 (ddd, J = 10.2, 4.4, 3.3 Hz, 1H), 4.23 (app t, J = 4.8 Hz, 1H), 4.03 (app t, J = 6.2

Hz, 1H), 2.82 (app ddt, *J* = 10.7, 9.2, 4.5 Hz, 1H), 2.18 (dddd, *J* = 14.4, 11.4, 6.7, 4.5 Hz, 1H), 2.06 (app ddt, *J* = 14.5, 11.5, 5.6 Hz, 1H), 2.01 (ddd, *J* = 14.1, 10.2, 4.2 Hz, 1H), 1.96–1.90 (m, 1H), 1.77 (app ddt, *J* = 12.9, 11.5, 5.0 Hz, 1H), 1.57 (dddd, *J* = 13.4, 11.7, 9.4, 4.4 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.8, 172.2, 150.9, 149.4, 145.2, 145.1, 144.1, 136.1, 132.7, 127.0, 124.9, 123.8, 120.0, 92.7, 90.0, 84.0, 83.4, 74.8, 74.5, 53.6, 47.0, 38.8, 30.9, 29.6, 28.8; HRMS (ESI+): calcd. for $[C_{25}H_{28}N_7O_6]^+$ 522.2096, meas. 522.2103, Δ 1.4 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(6-carbamoylbenzo[*d*][1,3]dioxol-4-yl)hept-6-ynoic acid (36). To a solution of **S81** (27 mg, 32 μmol) in THF (0.60 mL) at RT was added a 0.5 M LiOH aq. solution (0.60 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. Solution (90 μL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4.5 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed in vacuo. Crude material was purified by preparative HPLC (5% → 40% B' over 40 min followed by 40% → 95% B' over 5 min) to afford **36** (16 mg, 24 μmol, 76% over 2 steps) as the TFA salt. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)): δ 8.31 (s, 1H), 8.29 (s, 1H), 7.32 (d, *J* = 1.8 Hz, 1H), 7.19 (d, *J* = 1.8 Hz, 1H), 6.05 (d, *J* = 1.0 Hz, 1H), 6.04 (d, *J* = 1.0 Hz, 1H), 5.96 (d, *J* = 5.1 Hz, 1H), 4.71 (app t, *J* = 5.1 Hz, 1H), 4.32 (ddd, *J* = 10.4, 4.4, 3.2 Hz, 1H), 4.19 (dd, *J* = 5.1, 4.4 Hz, 1H), 3.88 (dd, *J* = 6.7, 5.8 Hz, 1H), 2.84–2.78 (m, 1H), 2.08 (dddd, *J* =

14.2, 11.7, 6.7, 4.7 Hz, 1H), 2.03–1.97 (m, 2H), 1.90 (ddd, *J* = 13.9, 10.7, 3.2 Hz, 1H), 1.72 (app ddt, *J* = 13.1, 11.7, 5.0 Hz, 1H), 1.55 (dddd, *J* = 13.1, 11.7, 9.2, 4.7 Hz, 1H); ^{13}C NMR (101 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.4, 171.4, 152.8, 151.1, 149.5, 149.1, 145.4, 144.4, 127.8, 126.9, 120.1, 108.6, 105.4, 104.0, 97.2, 90.3, 84.1, 77.5, 74.9, 74.6, 53.8, 38.7, 30.9, 29.9, 28.9; HRMS (ESI+): calcd. for $[C_{25}H_{28}N_7O_8]^+$ 554.1994, meas. 554.2004, Δ 1.8 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(6-carbamoylpyridin-2-yl)hept-6-ynoic acid (37). To a solution of **S82** (28 mg, 35 μmol) in THF (0.66 mL) at RT was added a 0.5 M LiOH aq. solution (0.66 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.13 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative (5% → 20% B over 30 min followed by 20% → 95% B over 5 min) to afford **37** (19 mg, 30 μmol, 88% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.37 (s, 1H), 8.34 (s, 1H), 7.99 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.96 (dd, *J* = 7.9, 7.5 Hz, 1H), 7.63 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.00 (d, *J* = 5.0 Hz, 1H), 4.74 (dd, *J* = 5.2, 5.0 Hz, 1H), 4.37 (ddd, *J* = 10.1, 4.5, 3.4 Hz, 1H), 4.23 (dd, *J* = 5.2, 4.5 Hz, 1H), 4.02 (dd, *J* = 6.7, 5.8 Hz, 1H), 2.91–2.85 (m, 1H), 2.15 (dddd, *J* = 14.3, 11.6, 6.7,

4.6 Hz, 1H), 2.10–2.02 (m, 2H), 1.98 (ddd, J = 13.9, 10.6, 3.4 Hz, 1H), 1.80 (app ddt, J = 13.1, 11.6, 5.0 Hz, 1H), 1.64 (dddd, J = 13.1, 11.8, 9.2, 4.6 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.1, 167.9, 151.0, 149.6, 149.4, 145.2, 144.2, 142.3, 140.6, 131.6, 123.1, 120.1, 95.1, 90.0, 83.8, 82.2, 74.8, 74.4, 53.5, 38.3, 30.6, 29.6, 28.7; HRMS (ESI+): calcd. for $[C_{23}H_{27}N_8O_6]^+$ 511.2048, meas. 511.2059, Δ 2.1 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(2-carbamoylpyridin-4-yl)hept-6-ynoic acid (38). To a solution of **S83** (35 mg, 43 μmol) in THF (0.8 mL) at RT was added a 0.5 M LiOH aq. solution (0.8 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.16 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4 mL) at RT. The resulting mixture was stirred for 18 h and volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 20% B over 30 min followed by 20% → 95% B over 5 min) to afford **38** (19 mg, 30 μmol, 70% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.68 (dd, J = 5.8, 0.6 Hz, 1H), 8.37 (s, 1H), 8.35 (s, 1H), 8.27 (dd, J = 1.6, 0.6 Hz, 1H), 7.90 (dd, J = 5.8, 1.6 Hz, 1H), 6.01 (d, J = 5.0 Hz, 1H), 4.73 (dd, J = 5.2, 5.0 Hz, 1H), 4.36 (ddd, J = 10.5, 4.4, 3.3 Hz, 1H), 4.22 (dd, J = 5.2, 4.4 Hz, 1H), 4.01 (dd, J = 6.6, 5.9 Hz, 1H), 2.95 (app ddt, J = 10.9, 9.3, 4.8 Hz, 1H), 2.14 (dddd, J = 14.3,

11.6, 6.6, 4.6 Hz, 1H), 2.09–2.02 (m, 2H), 1.99 (ddd, *J* = 14.0, 10.9, 3.3 Hz, 1H), 1.82 (app ddt, *J* = 13.3, 11.6, 4.8 Hz, 1H), 1.64 (dddd, *J* = 13.3, 11.9, 9.3, 4.6 Hz, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.1, 165.6, 151.0, 149.4, 145.5, 145.3, 145.2, 144.2, 141.1, 131.6, 127.6, 120.1, 105.9, 90.1, 83.6, 81.0, 74.8, 74.5, 53.5, 38.3, 30.5, 30.2, 28.7; HRMS (ESI+): calcd. for $[C_{23}H_{27}N_8O_6]^+$ 511.2048, meas. 511.2050, Δ 0.4 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(5-carbamoylpyridin-3-yl)hept-6-ynoic acid (39). To a solution of **S84** (26 mg, 32 μmol) in THF (0.6 mL) at RT was added a 0.5 M LiOH aq. solution (0.6 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.13 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4.5 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 20% B over 30 min followed by 20% → 95% B over 5 min) to afford **39** (16 mg, 26 μmol, 80% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (500 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 9.04 (d, *J* = 1.8 Hz, 1H), 8.89 (d, *J* = 1.8 Hz, 1H), 8.81 (app t, *J* = 1.8 Hz, 1H), 8.37 (s, 1H), 8.36 (s, 1H), 6.01 (d, *J* = 4.9 Hz, 1H), 4.72 (app t, *J* = 4.9 Hz, 1H), 4.37 (ddd, *J* = 10.3, 4.9, 3.1 Hz, 1H), 4.22 (app t, *J* = 4.9 Hz, 1H), 4.01 (app t, *J* = 6.3 Hz, 1H), 2.95–2.86 (m, 1H), 2.15 (dddd, *J* = 14.3, 11.6, 6.3, 4.8 Hz, 1H), 2.10–2.01

(m, 2H), 1.98 (ddd, J = 13.7, 10.7, 3.1 Hz, 1H), 1.85–1.77 (m, 1H), 1.68–1.59 (m, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 172.1, 165.8, 151.1, 149.4, 147.7, 147.0, 145.3, 144.1, 141.1, 133.9, 125.1, 120.1, 101.4, 90.1, 83.7, 77.4, 74.9, 74.5, 53.5, 38.4, 30.6, 30.0, 28.7; HRMS (ESI+): calcd. for $[C_{23}H_{27}N_8O_6]^+$ 511.2048, meas. 511.2069, Δ 4.0 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(4-carbamoylpyridin-2-yl)hept-6-ynoic acid (40). To a solution of **S85** (22 mg, 27 μmol) in THF (1.0 mL) at RT was added a 0.5 M LiOH aq. solution (1.0 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.20 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 20% B over 30 min followed by 20% → 95% B over 5 min) to afford **40** (14 mg, 22 μmol, 82% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of the sample preparation for NMR analysis. ^{1}H NMR (500 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)): δ 8.73 (dd, J = 6.1, 0.7 Hz, 1H), 8.37 (s, 1H), 8.35 (s, 1H), 8.22 (dd, J = 1.9, 0.7 Hz, 1H), 8.14 (dd, J = 6.1, 1.9 Hz, 1H), 6.01 (d, J = 4.9 Hz, 1H), 4.73 (dd, J = 5.1, 4.9 Hz, 1H), 4.35 (ddd, J = 10.2, 4.8, 3.4 Hz, 1H), 4.23 (dd, J = 5.1, 4.8 Hz, 1H), 4.01 (app t, J = 6.3 Hz, 1H), 3.03–2.96 (m, 1H), 2.18–1.99 (m, 4H), 1.89–1.80 (m, 1H), 1.74–1.64 (m, 1H); ^{13}C NMR (126 MHz, **CD$_3$CN** (350 μL)/D$_2$O (350 μL)/*d*-TFA (40 μL)):

δ 172.1, 166.8, 151.0, 150.0, 149.4, 145.3, 144.8, 144.2, 137.5, 129.7, 124.8, 120.1, 106.3, 90.1, 83.4, 76.1, 74.8, 74.4, 53.5, 38.0, 30.3, 30.2, 28.6; HRMS (ESI+): calcd. for $[C_{23}H_{27}N_8O_6]^+$ 511.2048, meas. 511.2055, Δ 1.3 ppm.

(2*S*,5*S*)-2-amino-5-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-7-(5-aminonaphthalen-1-yl)hept-6-ynoic acid (41). To a solution of **S86** (34 mg, 41 μmol) in THF (0.76 mL) at RT was added a 0.5 M LiOH aq. solution (0.76 mL). The resulting mixture was stirred for 1 h, cooled to 0 °C, and a 1 M HCl aq. solution (0.17 mL) was added. Volatiles were removed *in vacuo*. The residue was dissolved in a solution of ammonia in methanol (7 N, 4.5 mL) at RT. The resulting mixture was stirred for 18 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 45% B over 30 min followed by 45% → 95% B over 5 min) to afford **41** (20 mg, 26 μmol, 64% over 2 steps) as the TFA salt upon treatment with *d*-TFA as part of sample preparation for NMR analysis. ^{1}H NMR (600 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (40 μL)): δ 8.33 (s, 1H), 8.29 (app dt, *J* = 8.4, 1.0 Hz, 1H), 8.29 (s, 1H), 7.89 (app dt, *J* = 8.5, 1.0 Hz, 1H), 7.70 (dd, *J* = 7.2, 1.0 Hz, 1H), 7.63 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.61 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.57 (dd, *J* = 8.4, 7.5 Hz, 1H), 6.00 (d, *J* = 5.0 Hz, 1H), 4.77 (dd, *J* = 5.2, 5.0 Hz, 1H), 4.44 (ddd, *J* = 10.0, 4.6, 3.3 Hz, 1H), 4.28 (dd, *J* = 5.2, 4.6 Hz, 1H), 4.06 (dd, *J* = 6.7, 5.6 Hz, 1H), 3.01–2.95 (m, 1H), 2.23 (dddd, *J* = 14.4, 11.6, 6.7, 4.6 Hz, 1H), 2.17–2.10 (m, 1H), 2.11 (ddd, *J* = 14.0, 10.0, 4.5 Hz, 1H), 2.05 (ddd, *J* = 14.0, 10.3, 3.3 Hz, 1H), 1.84 (app ddt, *J* = 13.1, 11.6, 5.1 Hz, 1H), 1.72–1.64 (m,

1H); ^{13}C NMR (126 MHz, **CD_3CN** (350 μL)/D_2O (350 μL)/*d*-TFA (40 μL)): δ 172.1, 150.8, 149.4, 145.1, 144.2, 134.6, 132.5, 128.5, 128.4, 127.5, 127.5, 127.4, 123.0, 122.7, 122.0, 119.9, 98.5, 90.1, 83.9, 81.3, 74.7, 74.4, 53.5, 38.6, 30.8, 29.7, 28.8; HRMS (ESI+): calcd. for $[C_{27}H_{30}N_7O_5]^+$ 532.2303, meas. 532.2325, Δ 4.2 ppm.

Synthesis of Compounds S2-S86

Methyl 2-(dimethoxyphosphoryl)-2-(2,2,2-trifluoroacetamido)acetate (S2).[67] To a solution of (±)-Z-α-phosphonoglycine trimethyl ester (**S1**) (3.31 g, 9.99 mmol) in CH_2Cl_2 (50 mL) at RT, was added Pd/C (10%, 0.53 g, 0.50 mmol, 5 mol %). The flask headspace was evacuated and refilled with hydrogen (3 ×) and the reaction mixture was stirred vigorously under a hydrogen atmosphere (balloon) for 24 h. The flask headspace was evacuated and refilled with nitrogen, and the contents were filtered through a pad of celite. The filtrate was cooled to 0 ℃ and TFAA (3.47 mL, 25.0 mmol, 2.5 equiv.) was added dropwise. The reaction mixture was stirred at RT for 12 h and the solution was concentrated to yield an orange residue. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 100%) to afford TFA protected phosphonate **S2** (2.05 g, 6.99 mmol, 70% over 2 steps). R_f = 0.41 (EtOAc); FTIR (thin film), cm−1: ν_{max} (cm^{-1}): 3197, 3046, 2964, 2861, 1757, 1722, 1559, 1433, 1360, 1307, 1257, 1212, 1180, 1150, 1035; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.23 (br s, 1H), 5.14 (dd, $^2J_{H\text{-}P}$ = 21.3, J = 8.7 Hz, 1H), 3.89 (s, 3H), 3.87 (d, $^3J_{H\text{-}P}$, J = 11.2 Hz, 3H), 3.83 (d, $^3J_{H\text{-}P}$, J = 11.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ

165.5, 157.2 (qd, $^{2}J_{C\text{-}F}$ = 38 Hz, $^{3}J_{C\text{-}P}$ = 5.9 Hz), 115.7 (q, $^{1}J_{C\text{-}F}$ = 287 Hz), 54.5 (d, $^{2}J_{C\text{-}P}$ = 6.6 Hz), 54.1 (d, $^{2}J_{C\text{-}P}$ = 6.9 Hz), 53.3, 50.3 (d, $^{1}J_{C\text{-}P}$ = 152 Hz); ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.5; ^{31}P NMR (162 MHz, $CDCl_3$): δ 16.1; HRMS (ESI+): calcd. for $[C_7H_{12}F_3N_1O_6P_1]^+$ 294.0360, meas. 294.0349, Δ 3.7 ppm.

Methyl (2*S*,5*S*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)hept-6-ynoate (S3). To a vial charged with **7** (182 mg, 0.307 mmol) was added DMF (3 mL) at RT under a nitrogen atmosphere. A separate vial was charged with TASF (253 mg, 0.920 mmol, 3.0 equiv.) in a glovebox, put under a nitrogen atmosphere, and DMF (2 mL) was added at RT. The resulting solution was added to the vial containing alkyne **7** and the reaction mixture was stirred at 60 °C for 2 h. Volatiles were removed *in vacuo*. The residue was partitioned between Et_2O (40 mL) and a 1:1 mixture of brine and 5% LiCl aq. solution (40 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 5 → 50%) to yield **S3** (128 mg, 0.293 mmol, 95%) as a colorless oil. R_f = 0.35 (hexanes/EtOAc, 60:40); FTIR (neat), ν_{max} (cm^{-1}): 3292, 3089, 2990, 2954, 2938, 2836, 1747, 1719, 1553, 1455, 1440, 1383, 1275, 1209, 1160, 1104, 1060; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.94 (d, *J* = 7.9 Hz, 1H), 4.93 (s, 1H), 4.64 (td, *J* = 7.8, 4.9 Hz, 1H), 4.59 (d, *J* = 6.0 Hz, 1H), 4.55 (dd, *J* = 6.0, 1.0 Hz, 1H), 4.45 (ddd, *J* = 11.2, 3.9, 0.9 Hz, 1H), 3.80 (s, 3H), 3.31 (s, 3H), 2.66–2.58 (m,

1H), 2.14 (d, J = 2.4 Hz, 1H), 2.10 (ddq, J = 14.0, 10.8, 5.2 Hz, 1H), 2.05–1.94 (m, 1H), 1.67 (ddd, J = 13.3, 11.2, 4.0 Hz, 1H), 1.55 (dddd, J = 21.7, 13.2, 10.9, 4.6 Hz, 2H), 1.47 (s, 3H), 1.40 (dddd, J = 16.5, 14.4, 8.7, 3.9 Hz, 1H), 1.30 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 157.0 (q, $^2J_{C\text{-}F}$ = 38 Hz), 115.1 (q, $^1J_{C\text{-}F}$ = 287 Hz), 112.5, 110.1, 85.6, 85.1, 85.0, 84.5, 71.6, 55.3, 53.1, 52.6, 52.5, 40.4, 30.7, 29.8, 28.6, 26.6, 25.1; HRMS (ESI+): calcd. for $[C_{19}H_{26}F_3N_1O_7Na]^+$ 460.1554, meas. 460.1569, Δ 3.3 ppm.

(3*R*,4*R*,5*R*)-5-((2*S*,5*S*)-2-ethynyl-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-2,3,4-triyl triacetate (S4). To a solution of acetonide **S3** (1.21 g, 2.77 mmol) in CH_2Cl_2 (16.5 mL) at RT, was added a 2:1 mixture of TFA and water (51 mL). The reaction mixture was stirred at RT for 21 h and volatiles were removed *in vacuo*. The residue was resuspended in Ac_2O (6.0 mL, 64 mmol, 23 equiv.) and pyridine (12.0 mL, 149 mmol, 54 equiv.). The resulting mixture was stirred at RT for 22 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 40%) to afford triacetate **S4** (735 mg, 1.44 mmol, 52% over 2 steps) as a 1:1.7 mixture of α and β anomers, as a light yellow oil. R_f = 0.37 (α-anomer) / 0.46 (β-anomer) (hexanes/EtOAc, 50:50); FTIR (thin-film), ν_{max} (cm^{-1}): 3290, 2956, 1748, 1724, 1553, 1438, 1373, 1219, 1178, 1112, 1049, 1013; ^{1}H NMR (600 MHz, $CDCl_3$): δ α-anomer: 6.92 (d, J = 7.8 Hz, 1H), 6.36 (d, J = 4.6 Hz, 1H), 5.22 (dd, J = 6.8, 4.6 Hz, 1H), 5.05 (dd, J = 6.8, 3.7 Hz, 1H), 4.63 (td, J = 7.6, 5.1 Hz, 1H), 4.43 (dt, J = 10.2, 3.6 Hz, 1H), 3.80 (s, 3H), 2.62 (dqd, J = 13.8, 4.6, 2.4 Hz, 1H), 2.13 (d, J = 2.4 Hz, 1H), 2.12 (s, 3H), 2.08 (s,

3H), 2.08–2.04 (m, 1H), 2.07 (s, 3H), 2.01 (dddd, J = 18.5, 9.1, 5.3, 2.9 Hz, 1H), 1.84 (ddd, J = 14.1, 10.8, 3.5 Hz, 1H), 1.65–1.59 (m, 1H), 1.59–1.53 (m, 1H), 1.40 (dddd, J = 13.1, 11.0, 9.5, 4.8 Hz, 1H); β-anomer: 7.02 (d, J = 8.1 Hz, 1H), 6.10 (s, 1H), 5.31 (dd, J = 4.9, 0.9 Hz, 1H), 5.16 (dd, J = 7.2, 4.7 Hz, 1H), 4.64 (td, J = 8.2, 4.6 Hz, 1H), 4.33 (ddd, J = 10.1, 7.1, 2.7 Hz, 1H), 3.81 (s, 3H), 2.66 (ttd, J = 9.6, 4.9, 2.4 Hz, 1H), 2.13 (d, J = 2.4 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 2.08–2.04 (m, 1H), 2.06 (s, 3H), 2.05–1.97 (m, 1H), 1.84 (ddd, J = 13.1, 9.9, 2.8 Hz, 1H), 1.69–1.61 (m, 1H), 1.60–1.55 (m, 1H), 1.47 (dddd, J = 13.2, 11.3, 8.9, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ α-anomer: 171.1, 169.9, 169.5, 169.3, 156.9 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 93.7, 84.6, 81.2, 72.5, 71.5, 69.7, 52.9, 52.3, 38.9, 30.4, 29.1, 28.4, 21.0, 20.5, 20.2; β-anomer: 171.1, 170.1, 169.7, 169.4, 156.9 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 115.6 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 98.3, 84.9, 79.8, 74.3, 73.8, 71.3, 52.9, 52.3, 39.6, 30.6, 29.5, 28.0, 21.0, 20.6, 20.2; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –75.8; HRMS (ESI+): calcd. for $[C_{21}H_{26}F_3N_1O_{10}Na]^+$ 532.1401, meas. 532.1407, Δ 1.2 ppm.

Methyl 6-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)hexanoate (S6).[68] To a 3-necked flask charged with pyridine (10 mL) were added NaI (1.49 g, 10.0 mmol, 1.0 equiv.) and $FeCl_3$•$6H_2O$ (1.35 g, 5.00 mmol, 50 mol %) sequentially, both in one portion. Zinc powder (3.92 g, 60.0 mmol, 6.0 equiv.) was added portionwise to the reaction mixture (Caution, exothermic), monitoring the temperature so that it remains below 40 °C. Upon complete addition of the zinc powder, the resulting mixture was allowed to cool gradually to RT and a solution of riboside **S5**[69] (3.14 g, 10.0

mmol) in methyl acrylate (6.0 mL) was added over 10 min *via* syringe. The reaction mixture was stirred at RT for 2 h, before the addition of toluene (100 mL) and filtration over celite. Volatiles were removed *in vacuo* to give methyl ester **S6** (2.52 g, 9.25 mmol, 93%) which was of suitable purity to be used in the next step without further purification. R_f = 0.42 (cyclohexane/EtOAc, 67:33); FTIR (thin film), ν_{max} (cm^{-1}): 2989, 2940, 1735, 1437, 1372, 1270, 1239, 1208, 1194, 1159, 1090, 1057, 1011; ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.94 (s, 1H), 4.59 (d, *J* = 5.9 Hz, 1H), 4.52 (dd, *J* = 5.9, 1.1 Hz, 1H), 4.13 (ddd, *J* = 8.9, 6.4, 1.1 Hz, 1H), 3.67 (s, 3H), 3.34 (s, 2H), 2.35 (td, *J* = 7.4, 1.4 Hz, 2H), 1.86 – 1.76 (m, 1H), 1.71 (ddtd, *J* = 13.2, 10.1, 7.3, 5.7 Hz, 1H), 1.62 (dddd, *J* = 14.1, 10.1, 8.9, 5.1 Hz, 1H), 1.53 (ddt, *J* = 13.4, 10.4, 6.0 Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.5, 112.1, 109.5, 86.7, 85.5, 84.1, 54.9, 51.4, 34.4, 33.5, 26.4, 24.9, 21.7; HRMS (ESI+): calcd. for $[C_{13}H_{22}O_6Na]^+$ 297.1309, meas. 297.1307, Δ 0.7 ppm.

4-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)butanal (S7). To an oversized flask (250 mL) containing a solution of methyl ester **S6** (2.15 g, 7.83 mmol) in toluene (20 mL) at –85 °C (*i*-PrOH/liquid nitrogen bath) was added *i*-Bu_2AlH (1.0 M in hexanes, 8.0 mL, 8.0 mmol, 1.02 equiv.) over 40 min *via* syringe pump, down the side-wall of the flask. Upon complete addition of *i*-Bu_2AlH, the reaction mixture was stirred at –85 °C for 1 h, before the slow addition of MeOH (4 mL) over 20 min down the side-wall of the flask, followed by a potassium sodium tartrate sat. aq. solution (40 mL) and EtOAc (50 mL). The reaction mixture was removed from the cooling bath and allowed

to warm gradually to RT. Water (10 mL) and brine (10 mL) were added, and the biphasic mixture was stirred for another 10 min. The layers were separated and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (200 mL), dried over $MgSO_4$, filtered and concentrated to give aldehyde **S7** (1.80 g, 7.37 mmol, 94%) as a light-yellow oil which was of suitable purity to be used in the next step without further purification. R_f = 0.31 (cyclohexane/EtOAc, 67:33); FTIR (thin film), ν_{max} (cm^{-1}): 2988, 2938, 2832, 2723, 1724, 1457, 1412, 1381, 1372, 1273, 1240, 1209, 1193, 1160, 1087, 1058, 1018; ^1H NMR (600 MHz, $CDCl_3$): δ 9.73 (t, J = 1.6 Hz, 1H), 4.89 (s, 1H), 4.55 (d, J = 6.0 Hz, 1H), 4.48 (dd, J = 6.0, 1.1 Hz, 1H), 4.09 (ddd, J = 9.0, 6.3, 1.1 Hz, 1H), 3.30 (s, 3H), 2.45 (dddd, J = 8.7, 7.1, 3.4, 1.6 Hz, 2H), 1.81–1.71 (m, 1H), 1.72–1.63 (m, 1H), 1.59 (dtd, J = 13.6, 9.2, 5.2 Hz, 1H), 1.49 (ddt, J = 13.5, 10.1, 6.1 Hz, 1H), 1.43 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 202.0, 112.3, 109.6, 86.8, 85.5, 84.1, 55.1, 43.4, 34.4, 26.6, 25.0, 18.9.

Methyl (*Z*)-2-(((benzyloxy)carbonyl)amino)-6-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)hex-2-enoate (S8). To a stirred suspension of (±)-Cbz-α-phosphonoglycine trimethyl ester (**S1**) (343 mg, 1.04 mmol, 1.2 equiv.) in THF (3.0 mL) at –78 °C was added 1,1,3,3-tetramethylguanidine (0.12 mL, 0.95 mmol, 1.1 equiv.). The resulting mixture was stirred at –78 °C for 15 min, before the addition of a solution of aldehyde **S7** (211 mg, 0.864 mmol) in THF (2.0 mL). The reaction mixture was stirred at –78 °C for 30 min and then placed in an ice/water bath and stirred for 1 h.

The reaction was quenched by the addition of a 10% AcOH aq. solution (2 mL) and water (10 mL) was added. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated. ^{1}H NMR analysis of the crude revealed a 15:1 mixture of *Z* and *E* isomers. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 50%). *Z* and *E* isomers separated and only fractions containing *Z* isomers were collected to afford enamide **S8** (353 mg, 0.785 mmol, 91 %) as a clear viscous oil. R_f = 0.44 (cyclohexane/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3316, 2988, 2942, 1716, 1657, 1500, 1455, 1437, 1381, 1373, 1270, 1213, 1160, 1105, 1089, 1047; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41–7.30 (m, 5H), 6.61 (t, *J* = 7.2 Hz, 1H), 5.14 (s, 2H), 4.93 (s, 1H), 4.58 (d, *J* = 5.9 Hz, 1H), 4.49 (d, *J* = 5.9 Hz, 1H), 4.12 (dd, *J* = 8.3, 6.3 Hz, 1H), 3.75 (s, 3H), 3.33 (s, 3H), 2.31–2.18 (m, 2H), 1.68–1.48 (m, 4H), 1.47 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 165.1, 137.5, 136.1, 128.6, 128.3, 128.2, 112.3, 109.6, 86.9, 85.6, 84.2, 67.4, 55.0, 52.5, 34.7, 28.1, 26.6, 25.1; HRMS (ESI+): calcd. for $[C_{23}H_{32}NO_8]^+$ 450.2122, meas. 450.2124, Δ 0.3 ppm.

Methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-6-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)hexanoate (S9). To a flask charged with enamide **S8** (179 mg, 0.398 mmol, exclusively *Z* isomer) was added MeOH (5 mL) at RT under a nitrogen atmosphere. The contents were stirred until full dissolution was noted. Separately, $[Rh(nbd)_2]BF_4$ (49 mg, 0.12 mmol, 30 mol %) and (*S*,*S*)-Me-DUPHOS (43 mg,

0.14 mmol, 35 mol %) were weighed out into the same vial in a glove box. The vial was removed from the glove box, placed under a nitrogen atmosphere and MeOH (10 mL) was added. The flask headspace was evacuated and backfilled with hydrogen (3 ×). The methanolic solution of enamide **S8** was transferred to the vial containing the catalyst (2 mL of MeOH used for vial rinse) and the contents were stirred vigorously at RT under a hydrogen atmosphere (balloon) for 1 h. Volatiles were removed *in vacuo* and crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 50%) to afford protected amino acid **S9** (172 mg, 0.381 mmol, 96%) as a clear viscous oil and as a single diastereomer. R_f = 0.32 (cyclohexane/EtOAc, 67:33); FTIR (thin film), ν_{max} (cm^{-1}): 3338, 2988, 2939, 2862, 1720, 1523, 1455, 1439, 1381, 1373, 1348, 1259, 1239, 1209, 1161, 1106, 1087, 1056, 1026; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.38–7.28 (m, 5H), 5.31 (d, J = 8.4 Hz, 1H), 5.09 (s, 2H), 4.92 (s, 1H), 4.57 (d, J = 5.9 Hz, 1H), 4.48 (d, J = 6.0 Hz, 1H), 4.37 (td, J = 8.0, 5.1 Hz, 1H), 4.10 (dd, J = 8.9, 5.8 Hz, 1H), 3.73 (s, 3H), 3.31 (s, 3H), 1.88–1.79 (m, 1H), 1.64 (app. h, J = 8.1 Hz, 1H), 1.58 (app. q, J = 8.8 Hz, 1H), 1.49–1.43 (m, 2H), 1.46 (s, 3H), 1.40–1.32 (m, 3H), 1.30 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.0, 155.9, 136.3, 128.5, 128.2, 128.1, 112.2, 109.4, 87.0, 85.6, 84.1, 67.0, 54.9, 53.8, 52.3, 34.8, 32.6, 26.5, 25.8, 25.0; HRMS (ESI+): calcd. for $[C_{23}H_{34}NO_8]^+$ 452.2280, meas. 452.2285, Δ 1.1 ppm.

(3*R*,4*R*,5*R*)-5-((*S*)-5-(((benzyloxy)carbonyl)amino)-6-methoxy-6-oxohexyl)tetrahydrofuran-2,3,4-triyl triacetate (S10). To a vial charged with acetonide **S9** (171 mg, 0.378

mmol) was added a 4:1 mixture of acetic acid and water (7.5 mL) at RT. The resulting mixture was stirred at 85 °C for 8 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with benzene (1 × 10 mL). Crude material was used directly in the next step without further purification. To a solution of the crude triol in CH_2Cl_2 (4.0 mL) at RT, were added pyridine (0.50 mL, 6.2 mmol, 16 equiv.), Ac_2O (0.50 mL, 5.3 mmol, 14 equiv.) and DMAP (5 mg, 0.04 mmol, 0.11 equiv.) sequentially. The resulting mixture was stirred at RT for 2 h and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 100%) to afford triacetate **S10** (152 mg, 0.290 mmol, 77% over 2 steps) as a *ca*. 1:1 mixture of α and β anomers as a colorless oil. R_f = 0.28 (α-anomer)/0.37 (β-anomer) (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3359, 2950, 1743, 1721, 1523, 1455, 1437, 1371, 1216, 1110, 1047, 1010; ^{1}H NMR (600 MHz, $CDCl_3$): δ α-anomer: 7.38–7.27 (m, 5H), 6.34 (d, *J* = 4.6 Hz, 1H), 5.29 (d, *J* = 8.2 Hz, 1H), 5.18 (dd, *J* = 6.8, 4.6 Hz, 1H), 5.09 (s, 2H), 5.01 (dd, *J* = 6.8, 3.6 Hz, 1H), 4.35 (td, *J* = 7.8, 5.1 Hz, 1H), 4.20–4.15 (m, 1H), 3.73 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.83–1.77 (m, 1H), 1.69–1.54 (m, 3H), 1.47–1.42 (m, 1H), 1.39–1.28 (m, 3H); β-anomer: 7.38–7.28 (m, 5H), 6.10 (d, *J* = 1.1 Hz, 1H), 5.31 (d, *J* = 8.1 Hz, 1H), 5.29 (dd, *J* = 4.8, 1.2 Hz, 1H), 5.14 (dd, *J* = 6.9, 4.8 Hz, 1H), 5.10 (s, 2H), 4.36 (td, *J* = 7.8, 5.0 Hz, 1H), 4.12 (td, *J* = 7.4, 5.2 Hz, 1H), 3.73 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.85–1.78 (m, 1H), 1.68–1.55 (m, 2H), 1.51–1.42 (m, 1H), 1.40–1.29 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ mixture of α and β-anomers: 172.9, 170.2, 169.8,

169.4, 155.9, 136.3, 128.6, 128.2, 128.2, 98.3, 94.0, 83.3, 81.4, 74.6, 73.6, 72.3, 69.9, 67.0, 53.8, 52.4, 33.8, 33.1, 32.5, 25.0, 25.0, 24.9, 24.8, 21.1, 21.1, 20.7, 20.6, 20.6, 20.4; HRMS (ESI+): calcd. for $[C_{25}H_{34}NO_{11}]^+$ 524.2126, meas. 524.2134, Δ 1.5 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((*S*)-5-(((benzyloxy)carbonyl)amino)-6-methoxy-6-oxohexyl)tetrahydrofuran-3,4-diyl diacetate (S11). To a suspension of N^6-benzoyladenine (149 mg, 0.624 mmol, 2.15 equiv.) in propionitrile (dried over 4 Å M.S., 6.0 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (0.21 mL, 0.87 mmol, 3.00 equiv.). The resulting mixture was stirred at 95 °C for 10 min, upon which complete solubilization of N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S10** (152 mg, 0.290 mmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (21 mg, 58 μmol, 20 mol %) was added propionitrile (6.0 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained. This clear solution was added to the solution of silylated N^6-benzoyladenine. The reaction mixture was stirred at 95 °C for 2.5 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 100% then EtOAc/*i*-PrOH, 0 → 15%) to afford nucleoside **S11** (148 mg, 0.211 mmol, 73%) as a white amorphous solid. R_f = 0.28 (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3330, 2950, 1744, 1703, 1609, 1581, 1510, 1487, 1454, 1408, 1373, 1330, 1239, 1214, 1177, 1095, 1047, 1028; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.28 (br s, 1H), 8.74 (s, 1H), 8.11 (s, 1H), 8.01–7.97 (m, 2H), 7.59–7.53 (m, 1H), 7.49–7.44 (m, 2H), 7.33–7.24 (m, 5H), 6.12 (d, *J* = 5.3 Hz, 1H), 5.94

(t, J = 5.5 Hz, 1H), 5.45 (d, J = 3.5 Hz, 1H), 5.44 (t, J = 5.3 Hz, 1H), 5.07 (s, 2H), 4.34 (td, J = 8.0, 5.1 Hz, 1H), 4.15 (dt, J = 8.8, 4.9 Hz, 1H), 3.69 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 1.85–1.70 (m, 3H), 1.65–1.57 (m, 1H), 1.51–1.42 (m, 1H), 1.41–1.30 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.9, 169.7, 169.5, 165.0, 155.9, 152.7, 151.8, 149.9, 141.9, 136.3, 133.6, 132.8, 128.8, 128.5, 128.2, 128.1, 128.0, 123.9, 86.5, 82.4, 73.3, 73.1, 67.0, 53.7, 52.4, 32.9, 32.5, 24.9, 24.9, 20.6, 20.4; HRMS (ESI+): calcd. for $[C_{35}H_{38}N_6O_{10}Na]^+$ 725.2542, meas. 725.2573, Δ 4.3 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-6-methoxy-6-oxo-2-(phenylethynyl)-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S12). To a vial charged with nucleoside **8** (82 mg, 0.12 mmol) were added iodobenzamide (49 mg, 0.24 mmol, 2.0 equiv.), CuI (5 mg, 0.02 mmol, 20 mol %) and $Pd(PPh_3)_4$ (7 mg, 6 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 3.5 mL) was added at RT. The resulting mixture was stirred at 70 °C for 3 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 5%) to afford alkyne **S12** (76 mg, 99 μmol, 83%) as a white amorphous solid. R_f = 0.48 (EtOAc); FTIR (thin-film), ν_{max} (cm^{-1}): 3297, 3082, 2954, 1745, 1717, 1610, 1582, 1509, 1489, 1455, 1373, 1328, 1239, 1212, 1176, 1159, 1096, 1072, 1047, 1029; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.95 (s, 1H), 8.81 (s, 1H), 8.13 (s, 1H), 8.01 (d, J = 7.6 Hz, 2H), 7.61 (t, J = 7.2 Hz, 1H), 7.52 (t, J = 7.5 Hz,

2H), 7.40 (ddt, J = 5.3, 2.7, 1.4 Hz, 2H), 7.31 (tt, J = 3.8, 2.2 Hz, 3H), 6.92 (d, J = 5.8 Hz, 1H), 6.14 (d, J = 5.4 Hz, 1H), 6.09 (t, J = 5.4 Hz, 1H), 5.59 (t, J = 4.9 Hz, 1H) 4.66 (td, J = 7.7, 5.0 Hz, 1H), 4.53 (ddd, J = 10.8, 4.5, 2.9 Hz, 1H), 3.78 (s, 3H), 2.82 (ddt, J = 10.9, 9.1, 4.5 Hz, 1H), 2.16 (s, 3H), 2.19–2.09 (m, 1H), 2.07 (s, 3H), 2.06–1.92 (m, 2H), 1.70–1.60 (m, 2H), 1.58–1.46 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.2, 169.8, 169.6, 164.8, 157.0 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 152.9, 151.7, 149.8, 142.4, 133.5, 132.9, 131.8, 128.4, 128.2, 128.0, 124.0, 123.2, 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 90.0, 87.1, 83.8, 81.0, 77.3, 73.7, 73.0, 53.1, 52.4, 38.8, 31.1, 30.0, 28.8, 20.7, 20.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.7; HRMS (ESI+): calcd. for $[C_{37}H_{36}F_3N_6O_9]^+$ 765.2490, meas. 765.2504, Δ 1.8 ppm.

(*S*)-3-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-5-(triisopropylsilyl)pent-4-ynal (S13). A 50 mL flask was charged with $[Rh(OAc)(C_2H_4)_2]_2$ (60 mg, 0.14 mmol, 2.5 mol %) and (*R*)-DTBMSegPhos (389 mg, 0.329 mmol, 6.0 mol %). The flask headspace was purged with nitrogen for 10 min followed by the addition of MeOH (14 mL). The resulting orange suspension was stirred vigorously at RT, until a clear solution was obtained (15 min), followed by the sequential addition of enal **4** (1.33 g, 5.49 mmol) in MeOH (4 mL) and TIPS-acetylene (2.46 mL, 11.0 mmol, 2.0 equiv.). The resulting red mixture was stirred at 40 °C for 15 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/acetone, 0 → 30%) to afford aldehyde **S13** (2.00 g, 4.71 mmol, 86 %) as a single diastereomer, as a light yellow oil. R_f = 0.31 (hexanes/acetone, 85:15); FTIR (thin film), ν_{max} (cm^{-1}): 2942, 2866,

2169, 1729, 1464, 1382, 1211, 1160, 1108; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.83 (dd, J = 2.1, 1.7 Hz, 1H), 4.95 (s, 1H), 4.60 (s, 2H), 4.39 (app t, J = 7.7 Hz, 1H), 3.34 (s, 3H), 3.09 (app tdd, J = 7.7, 6.5, 6.0 Hz, 1H), 2.65 (ddd, J = 16.6, 6.0, 1.7 Hz, 1H), 2.60 (ddd, J = 16.6, 7.7, 2.1 Hz, 1H), 1.89 (app dt, J = 13.6, 7.7 Hz, 1H), 1.73 (ddd, J = 13.6, 7.7, 6.5 Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H), 1.06–1.03 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 200.8, 112.6, 109.6, 108.8, 85.6, 84.8, 83.9, 83.8, 55.2, 47.8, 39.9, 26.7, 25.3, 24.2, 18.7, 11.3; HRMS (ESI+): calcd. for $[C_{23}H_{40}O_5Si_1Na]^+$ 447.2537, meas. 447.2523, Δ 3.2 ppm.

Methyl (*R*,*Z*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)-7-(triisopropylsilyl)hept-2-en-6-ynoate (S14). To a suspension of NaH (95%, 45 mg, 1.8 mmol, 1.5 equiv.) in THF (2.5 mL) at 0 °C, was added a solution of phosphonate **S2** (517 mg, 1.76 mmol, 1.5 equiv.) in THF (2.5 mL) dropwise. The resulting cloudy mixture was stirred at 0 °C for 30 min and a solution of aldehyde **S13** (499 mg, 1.18 mmol) in THF (2.5 mL) was added in one portion. The yellow reaction mixture was stirred at 40 °C for 1.5 h and cooled to 0 °C, before the addition of an aqueous pH 7 buffer (6 mL) and Et_2O (6 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 6 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 1 → 35%) to afford olefin **S14** (651 mg, 1.10 mmol, 94%) as a 6:1 mixture of *Z* and *E* isomers as a colorless oil. R_f = 0.28 (hexanes/EtOAc, 80:20); FTIR (thin film), ν_{max} (cm^{-1}): 3305, 2943, 2865, 2168, 1721, 1665,

1536, 1463, 1439, 1382, 1274, 1210, 1160, 1107, 1061; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78 (s, 1H), 7.09 (dd, J = 7.5, 7.2 Hz, 1H), 4.95 (s, 1H), 4.60 (d, J = 6.0 Hz, 1H), 4.55 (dd, J = 6.0, 0.9 Hz, 1H), 4.37 (app td, J = 7.5, 0.9 Hz, 1H), 3.81 (s, 3H), 3.34 (s, 3H), 2.81 (dddd, J = 9.0, 7.5, 7.0, 4.6 Hz, 1H), 2.45 (ddd, J = 15.7, 7.2, 4.6 Hz, 1H), 2.29 (ddd, J = 15.7, 9.0, 7.5 Hz, 1H), 1.89 (app dt, J = 13.7, 7.5 Hz, 1H), 1.70 (ddd, J = 13.7, 7.5, 7.0 Hz, 1H), 1.46 (s, 3H), 1.30 (s, 3H), 1.06–1.04 (m, 21H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.8, 155.2 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 137.7, 123.7, 115.8 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 112.6, 109.5, 109.3, 85.6, 84.8, 83.9, 55.1, 52.9, 40.0, 33.8, 28.8, 26.6, 25.2, 18.7, 11.3; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.3, –77.2; HRMS (ESI+): calcd. for $[C_{28}H_{44}F_3N_1O_7Si_1Na]^+$ 614.2731, meas. 614.2735, Δ 0.7 ppm.

Methyl (2*S*,5*R*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)-7-(triisopropylsilyl)hept-6-ynoate (S15). To a flask charged with enamide **S14** (6:1 mixture of *Z* and *E* isomers, 651 mg, 1.10 mmol) was added MeOH (32 mL) under a nitrogen atmosphere. The contents were stirred until full solubilization was observed. In a glove box, a separate vial was charged with (*S*,*S*)-MeBPE-Rh (31 mg, 55 μmol, 5 mol %). The vial was removed from the glove box, placed under a nitrogen atmosphere and MeOH (4 mL) was added. The resulting orange solution was transferred to the flask containing the methanolic solution of enamide **S14** *via* syringe. The flask headspace was evacuated and refilled with hydrogen

(3 ×), and the reaction mixture was stirred vigorously under a hydrogen atmosphere (balloon) for 1 h. Volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 1 → 50%) to afford protected amino acid **S15** (627 mg, 1.06 mmol, 96%) as a single diastereomer, as a colorless oil. R_f = 0.30 (hexanes/EtOAc, 85:15); FTIR (thin film), ν_{max} (cm^{-1}): 3319, 2942, 2865, 2166, 1749, 1720, 1549, 1461, 1382, 1208, 1159, 1106, 1092, 1061; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.90 (d, J = 7.5 Hz, 1H), 4.93 (s, 1H), 4.64 (app td, J = 7.5, 5.2 Hz, 1H), 4.59 (d, J = 5.9 Hz, 1H), 4.53 (dd, J = 5.9, 0.8 Hz, 1H), 4.36 (app td, J = 7.8, 0.8 Hz, 1H), 3.79 (s, 3H), 3.33 (s, 3H), 2.58–2.51 (m, 1H), 2.29 (dddd, J = 13.9, 11.4, 5.2, 4.7 Hz, 1H), 1.90–1.81 (m, 2H), 1.63 (ddd, J = 13.6, 7.8, 6.7 Hz, 1H), 1.59–1.44 (m, 2H), 1.46 (s, 3H), 1.30 (s, 3H), 1.06–1.02 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4, 156.9 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 112.6, 109.6, 109.5, 85.6, 84.9, 83.9, 83.5, 55.1, 53.1, 52.7, 40.3, 30.4, 30.1, 29.3, 26.6, 25.2, 18.7, 11.3; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{28}H_{46}F_3N_1O_7Si_1Na]^+$ 616.2888, meas. 616.2890, Δ 0.3 ppm.

Methyl (2*S*,5*R*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)hept-6-ynoate (S16). To a solution of TIPS alkyne **S15** (108 mg, 0.182 mmol) in DMF (4 mL) at RT, was added a solution of TASF (0.15 g, 0.55 mmol, 3.0 equiv.) in DMF (3.3 mL). The resulting mixture was stirred at 60 °C for 2.5 h and volatiles were removed *in vacuo*. The residue was partitioned between Et_2O (7 mL) and a 1:1 mixture of brine and 5% LiCl aq. solution (7 mL). The

layers were separated and the aqueous phase was extracted with Et_2O (3 × 7 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 5 → 50%) to afford alkyne **S16** (80 mg, 0.18 mmol, quant.) as a colorless oil. R_f = 0.26 (hexanes/EtOAc, 70:30); FTIR (thin film), ν_{max} (cm^{-1}): 3288, 2936, 1747, 1719, 1552, 1456, 1440, 1383, 1374, 1209, 1160, 1106, 1091, 1059; ^1H NMR (500 MHz, $CDCl_3$): δ 6.96 (d, J = 7.5 Hz, 1H), 4.93 (s, 1H), 4.63 (ddd, J = 7.5, 7.0, 5.8 Hz, 1H), 4.59 (d, J = 5.9 Hz, 1H), 4.51 (dd, J = 5.9, 1.0 Hz, 1H), 4.33 (app td, J = 7.5, 1.0 Hz, 1H), 3.80 (s, 3H), 3.33 (s, 3H), 2.50 (ddddd, J = 9.5, 7.3, 6.8, 4.6, 2.4 Hz, 1H), 2.22 (dddd, J = 14.0, 11.2, 5.8, 4.8 Hz, 1H), 2.13 (d, J = 2.4 Hz, 1H), 1.90–1.82 (m, 2H), 1.64 (ddd, J = 13.7, 7.5, 6.8 Hz, 1H), 1.60–1.44 (m, 2H), 1.47 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 156.9 (q, $^2J_{C\text{-}F}$ = 38 Hz), 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 112.6, 109.8, 85.6, 85.4, 84.6, 84.0, 71.2, 55.2, 53.1, 52.6, 39.8, 29.8, 29.6, 28.2, 26.6, 25.1; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{19}H_{26}F_3N_1O_7Na]^+$ 460.1554, meas. 460.1562, Δ 1.9 ppm.

(3*R*,4*R*,5*R*)-5-((2*R*,5*S*)-2-ethynyl-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-2,3,4-triyl triacetate (S17). To a solution of acetonide **S16** (80 mg, 0.18 mmol) in CH_2Cl_2 (0.8 mL) at 0 °C, was added a 4:1 mixture of TFA and water (3.6 mL). The resulting mixture was stirred at RT for 5.5 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with benzene (2 × 0.8 mL). Crude material was used directly in the next step without further purification. To a solution

of the crude triol in CH_2Cl_2 (3.6 mL) at RT, were added pyridine (0.15 mL, 1.8 mmol, 10 equiv.), Ac_2O (0.17 mL, 1.8 mmol, 10 equiv.) and DMAP (16 mg, 0.13 mmol, 0.70 equiv.) sequentially. The resulting mixture was stirred at RT for 15 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 5 → 70%) to afford triacetate **S17** (60 mg, 0.12 mmol, 64% over 2 steps) as a 2:1 mixture of diastereomers, as a colorless oil. R_f = 0.35/0.43 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3290, 2930, 1746, 1722, 1554, 1438, 1372, 1214, 1111, 1011; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.12 (d, J = 8.1 Hz, 2H), 6.98 (d, J = 8.0 Hz, 1H), 6.35 (d, J = 4.5 Hz, 1H), 6.06 (d, J = 1.0 Hz, 2H), 5.30 (dd, J = 4.8, 1.0 Hz, 2H), 5.25–5.20 (m, 3H), 5.17 (dd, J = 6.9, 3.5 Hz, 1H), 4.66–4.59 (m, 3H), 4.38–4.32 (m, 3H), 3.80 (s, 3H), 3.79 (s, 6H), 2.60–2.50 (m, 3H), 2.25–2.15 (m, 2H), 2.14 (d, J = 2.5 Hz, 1H), 2.13 (d, J = 2.5 Hz, 2H), 2.12 (s, 6H), 2.11 (s, 3H), 2.11 (s, 3H), 2.09 (s, 6H), 2.07 (s, 6H), 2.06 (s, 3H), 1.93–1.83 (m, 4H), 1.83–1.74 (m, 6H), 1.61–1.47 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.2, 170.2, 170.0, 169.9, 169.7, 169.6, 169.5, 157.1 (q, $^2J_{C\text{-}F}$ = 38 Hz), 156.9 (q, $^2J_{C\text{-}F}$ = 37 Hz), 115.8 (q, $^1J_{C\text{-}F}$ = 287 Hz), 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 98.5, 93.9, 85.2, 85.0, 81.3, 79.2, 74.4, 73.7, 72.0, 71.6, 71.2, 69.9, 53.1, 53.1, 52.6, 52.5, 38.4, 37.9, 29.7, 29.6, 29.5, 27.8, 27.3, 21.2, 21.1, 20.8, 20.6, 20.6, 20.4; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.75, –76.76; HRMS (ESI+): calcd. for $[C_{21}H_{26}F_3N_1O_{10}Na]^+$ 532.1401, meas. 532.1416, Δ 2.8 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*R*,5*S*)-2-ethynyl-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S18). To a

suspension of N^6-benzoyladenine (113 mg, 0.471 mmol, 4.0 equiv.) in propionitrile (dried over 4 Å M.S., 2.4 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (0.14 mL, 0.59 mmol, 5.0 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S17** (dried by azeotropic distillation with benzene (4 ×), 60 mg, 0.12 mmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (21 mg, 59 μmol, 50 mol %) was added propionitrile (2.4 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 5.5 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 30 → 100%) to afford nucleoside **S18** (53 mg, 77 μmol, 65%) as a colorless oil. R_f = 0.33 (hexanes/EtOAc, 20:80); FTIR (thin film), ν_{max} (cm^{-1}): 3283, 3084, 2929, 1745, 1720, 1612, 1583, 1511, 1487, 1456, 1374, 1244, 1218, 1075, 1029; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.07 (s, 1H), 8.79 (s, 1H), 8.11 (s, 1H), 8.04–7.98 (m, 2H), 7.63–7.57 (m, 1H), 7.54–7.49 (m, 2H), 7.04 (br d, *J* = 7.6 Hz, 1H), 6.12 (d, *J* = 5.5 Hz, 1H), 6.04 (app t, *J* = 5.5 Hz, 1H), 5.58 (dd, *J* = 5.5, 4.5 Hz, 1H), 4.60 (ddd, *J* = 7.6, 7.0, 5.4 Hz, 1H), 4.38 (ddd, *J* = 7.5, 5.8, 4.5 Hz, 1H), 3.76 (s, 3H), 2.57 (dddd, *J* = 14.0, 8.2, 6.4, 2.4 Hz, 1H), 2.23–2.12 (m, 2H), 2.15 (s, 3H), 2.15 (d, *J* = 2.4 Hz, 1H), 2.06 (s, 3H), 2.00–1.93 (m, 1H), 1.86–1.77 (m, 1H), 1.54–1.48 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 169.8, 169.6, 164.8, 156.9 (q, $^2J_{C\text{-}F}$ = 38 Hz), 153.0, 151.8, 149.9, 142.0, 133.5, 133.0, 129.0, 128.0, 124.0,

115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 86.7, 85.1, 80.6, 73.3, 72.8, 71.5, 53.2, 52.5, 37.7, 29.6, 29.6, 27.5, 20.7, 20.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.7; HRMS (ESI+): calcd. for $[C_{31}H_{32}F_3N_6O_9]^+$ 689.2177, meas. 689.2178, Δ 0.1 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*R*,5*S*)-2-((3-carbamoylphenyl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S19). To a solution of nucleoside **S18** (53 mg, 77 μmol) and 3-iodobenzamide (48 mg, 0.19 mmol, 2.5 equiv.) in a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 3.9 mL) at RT, were introduced CuI (4 mg, 0.02 mmol, 25 mol %) and $Pd(PPh_3)_4$ (4 mg, 4 μmol, 5 mol %) rapidly. The resulting mixture was stirred at 60 °C for 2 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 5%) to afford alkyne **S19** (50 mg, 62 μmol, 80%) as a colorless oil. R_f = 0.43 (EtOAc/*i*-PrOH, 95:5); FTIR (thin film), ν_{max} (cm^{-1}): 3284, 2932, 1746, 1716, 1668, 1611, 1580, 1455, 1375, 1242, 1215, 1183, 1160, 1075; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.23 (s, 1H), 8.75 (s, 1H), 8.18 (s, 1H), 8.04–7.98 (m, 2H), 7.84–7.82 (m, 1H), 7.80 (ddd, *J* = 7.7, 1.9, 1.3 Hz, 1H), 7.61–7.56 (m, 1H), 7.52–7.47 (m, 2H), 7.46 (app dt, *J* = 7.7, 1.3 Hz, 1H), 7.36 (br d, *J* = 7.3 Hz, 1H), 7.34 (app t, *J* = 7.7 Hz, 1H), 6.91 (br s, 1H), 6.16 (d, *J* = 5.7 Hz, 1H), 6.13 (dd, *J* = 5.7, 5.4 Hz, 1H), 5.99 (br s, 1H), 5.76 (dd, *J* = 5.4, 4.3 Hz, 1H), 4.68–4.63 (m, 1H), 4.43 (ddd, *J* = 7.0, 5.5, 4.3 Hz, 1H), 3.76 (s, 3H), 2.87–2.81 (m, 1H), 2.33–2.26 (m, 1H), 2.26 (ddd, *J* = 14.2, 8.4, 7.0 Hz, 1H), 2.14 (s, 3H), 2.06 (s, 3H),

2.05–2.01 (m, 1H), 1.97–1.89 (m, 1H), 1.61–1.55 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 170.0, 169.6, 168.8, 165.0, 157.1 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 152.9, 151.9, 149.9, 142.2, 134.5, 133.6, 133.5, 133.0, 130.6, 129.0, 128.8, 128.1, 127.7, 124.1, 123.3, 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 91.7, 86.7, 82.9, 81.1, 73.2, 72.7, 532, 52.7, 37.6, 30.2, 29.6, 28.0, 20.8, 20.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.7; HRMS (ESI+): calcd. for $[C_{38}H_{37}F_3N_7O_{10}]^+$ 808.2549, meas. 808.2541, Δ 0.9 ppm.

3-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)propan-1-ol (S21).[71] To a solution of known α,β-unsaturated methyl ester **S20**[72] (1.22 g, 4.72 mmol) in a 10:1 mixture of Et_2O and MeOH (22 mL) at 0 °C, was added a solution of $LiBH_4$ (2.0 M in THF, 9.45 mL, 18.9 mmol, 4.0 equiv.) dropwise. The resulting mixture was allowed to warm gradually to RT and stirred for 17 h, before the addition of a 1:1 mixture of a NH_4Cl sat. aq. solution and water (35 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 35 mL). The combined organic extracts were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated to give alcohol **S21** (1.10 g, 4.72 mmol, quant.) as a colorless oil. R_f = 0.24 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3422, 2989, 2937, 1449, 1373, 1210, 1160, 1088, 1054, 1015; ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.95 (s, 1H), 4.61 (d, J = 5.9 Hz, 1H), 4.54 (dd, J = 5.9, 1.0 Hz, 1H), 4.20–4.16 (m, 1H), 3.70–3.67 (m, 2H), 3.36 (s, 3H), 1.76–1.59 (m, 4H), 1.48 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 112.4, 109.7, 87.2, 85.5, 84.3, 62.3, 55.1, 31.6, 29.6, 26.6, 25.1; HRMS (ESI+): calcd. for $[C_{11}H_{20}O_5Na]^+$ 255.1203, meas. 255.1196, Δ 2.6 ppm.

3-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)propanal (S22).[73] To a solution of alcohol **S21** (400 mg, 1.72 mmol) in CH_2Cl_2 (11.5 mL) at RT, was added DMP (876 mg, 2.07 mmol, 1.2 equiv.) in one portion. The resulting mixture was stirred at RT for 1 h, before the addition of a 1:1 mixture of a $Na_2S_2O_3$ sat. aq. solution and a $NaHCO_3$ sat. aq. solution (10 mL). The biphasic mixture was stirred vigorously at RT for 10 min and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 10 mL) and the combined organic extracts were washed with brine (40 mL), dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (pentane/EtOAc, 2 → 50%) to afford aldehyde **S22** (324 mg, 1.41 mmol, 82%) as a colorless oil. R_f = 0.32 (hexanes/EtOAc, 75:25); FTIR (thin film), ν_{max} (cm^{-1}): 2988, 2936, 2834, 2724, 1721, 1373, 1209, 1160, 1090, 1058; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.79 (t, *J* = 1.3 Hz, 1H), 4.93 (s, 1H), 4.59 (d, *J* = 5.9 Hz, 1H), 4.52 (dd, *J* = 5.9, 1.1 Hz, 1H), 4.14 (ddd, *J* = 9.2, 6.5, 1.1 Hz, 1H), 3.32 (s, 3H), 2.62 (dddd, *J* = 18.1, 7.9, 6.6, 1.3 Hz, 1H), 2.57 (dddd, *J* = 18.1, 8.2, 6.9, 1.3 Hz, 1H), 1.89–1.82 (m, 2H), 1.45 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 201.4, 112.5, 109.9, 86.3, 85.6, 84.1, 55.3, 40.8, 27.5, 26.6, 25.1; HRMS (ESI+): calcd. for $[C_{11}H_{18}O_5Na]^+$ 253.1046, meas. 253.1036, Δ 4.0 ppm.

(3a*R*,4*R*,6*R*,6a*R*)-4-(but-3-yn-1-yl)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole (S23).[74] To a solution of aldehyde **S22** (324 mg, 1.41 mmol) in MeOH (6.0 mL) at RT, was added K_2CO_3 (583 mg, 4.22 mmol, 3.0 equiv.), followed by a solution of

dimethyl-1-diazo-2-oxopropyl phosphonate (338 mg, 1.76 mmol, 1.25 equiv.) in MeOH (3.0 mL) under vigorous stirring. The resulting yellow suspension was stirred at RT for 3 h. The reaction was quenched by the addition of a NH_4Cl sat. aq. solution (15 mL) and Et_2O (25 mL) was added. The layers were separated and the aqueous phase was extracted with Et_2O (3 × 25 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (hexane/Et_2O, 2 → 50%) to afford alkyne **S23** (254 mg, 1.12 mmol, 80%) as a colorless oil. R_f = 0.50 (hexanes/EtOAc, 75:25); ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.95 (s, 1H), 4.60 (d, J = 5.9 Hz, 1H), 4.56 (dd, J = 5.9, 1.0 Hz, 1H), 4.30 (ddd, J = 9.8, 5.8, 1.0 Hz, 1H), 3.34 (s, 3H), 2.36 (dddd, J = 16.8, 7.8, 5.9, 2.6 Hz, 1H), 2.31 (dddd, J = 16.8, 7.8, 7.3, 2.6 Hz, 1H), 1.98 (t, J = 2.6 Hz, 1H), 1.80 (dddd, J = 13.4, 9.8, 7.3, 5.9 Hz, 1H), 1.74 (app dtd, J = 13.4, 7.8, 5.8 Hz, 1H), 1.48 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 112.5, 109.8, 85.9, 85.7, 84.1, 83.3, 69.1, 55.3, 33.9, 26.6, 25.1, 15.6; HRMS (ESI+): calcd. for $[C_{12}H_{18}O_4Na]^+$ 249.1097, meas. 249.1118, Δ 8.4 ppm.

(3*R*,4*R*,5*R*)-5-(but-3-yn-1-yl)tetrahydrofuran-2,3,4-triyl triacetate (S24). To a vial charged with acetonide **S23** (50 mg, 0.22 mmol) was added a 4:1 mixture of acetic acid and water (2.5 mL) at RT. The resulting mixture was stirred at 80 °C for 5 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with benzene (2 × 1.0 mL). Crude material was used directly in the next step without further purification. To a solution of the crude triol in CH_2Cl_2 (2.5 mL) at RT, were added pyridine (0.18 mL, 2.2

mmol, 10 equiv.), Ac_2O (0.21 mL, 2.2 mmol, 10 equiv.) and DMAP (19 mg, 0.15 mmol, 0.70 equiv.) sequentially. The resulting mixture was stirred at RT for 2 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 2 → 60%) to afford triacetate **S24** (58 mg, 0.19 mmol, 88% over 2 steps) as a 1:1 mixture of diastereomers, as a colorless oil; R_f = 0.42/0.50 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3285, 2958, 2920, 2850, 2117, 1742, 1433, 1371, 1214, 1107, 1010; ^1H NMR (600 MHz, $CDCl_3$): δ 6.36 (d, J = 4.6 Hz, 1H), 6.12 (d, J = 1.0 Hz, 1H), 5.30 (dd, J = 4.8, 1.0 Hz, 1H), 5.21 (dd, J = 6.8, 4.6 Hz, 1H), 5.21 (dd, J = 7.0, 4.8 Hz, 1H), 5.10 (dd, J = 6.8, 3.6 Hz, 1H), 4.33 (ddd, J = 8.5, 4.9, 3.6 Hz, 1H), 4.27 (ddd, J = 8.5, 7.0, 4.6 Hz, 1H), 2.39–2.25 (m, 4H), 2.11 (s, 6H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.97 (t, J = 2.8 Hz, 1H), 1.96 (t, J = 2.6 Hz, 1H), 1.95–1.86 (m, 2H), 1.86–1.78 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2, 170.0, 169.8, 169.6, 169.5, 169.2, 98.3, 93.9, 83.0, 82.8, 82.1, 80.3, 74.7, 73.5, 72.1, 70.0, 69.4, 69.2, 33.2, 32.3, 21.2, 21.2, 20.8, 20.7, 20.6, 20.4, 14.9, 14.8; HRMS (ESI+): calcd. for $[C_{14}H_{18}O_7Na]^+$ 321.0945, meas. 321.0952, Δ 2.3 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-(but-3-yn-1-yl)tetrahydrofuran-3,4-diyl diacetate (S25). To a suspension of N^6-benzoyladenine (160 mg, 0.670 mmol, 4.0 equiv.) in propionitrile (dried over 4 Å M.S., 5.0 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (0.20 mL, 0.84 mmol, 5.0 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of N^6-benzoyladenine was observed.

To a separate flask charged with triacetate **S24** (dried by azeotropic distillation with benzene (4 ×), 50 mg, 0.17 mmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (30 mg, 84 μmol, 50 mol %) was added propionitrile (5.0 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 17 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 100%) to afford nucleoside **S25** (73 mg, 0.15 mmol, 91%) as a colorless oil. R_f = 0.31 (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3291, 3091, 2934, 1747, 1699, 1609, 1582, 1510, 1486, 1455, 1373, 1239, 1217, 1099, 1073, 1053; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.12 (s, 1H), 8.78 (s, 1H), 8.09 (s, 1H), 8.04–7.98 (m, 2H), 7.62–7.57 (m, 1H), 7.54–7.48 (m, 2H), 6.13 (d, *J* = 5.4 Hz, 1H), 6.04 (dd, *J* = 5.6, 5.4 Hz, 1H), 5.57 (dd, *J* = 5.6, 4.7 Hz, 1H), 4.37 (ddd, *J* = 9.2, 4.7, 4.3 Hz, 1H), 2.38 (dddd, *J* = 17.0, 7.0, 5.6, 2.6 Hz, 1H), 2.31 (dddd, *J* = 17.0, 8.5, 6.8, 2.6 Hz, 1H), 2.15 (s, 3H), 2.16–2.09 (m, 1H), 2.06 (s, 3H), 2.06–2.00 (m, 1H), 1.99 (t, *J* = 2.6 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 169.8, 169.6, 164.8, 153.0, 151.7, 149.9, 142.1, 133.6, 133.0, 129.0, 128.0, 124.0, 86.9, 82.8, 81.3, 73.3, 73.1, 69.6, 31.8, 20.7, 20.5, 14.8; HRMS (ESI+): calcd. for $[C_{24}H_{24}N_5O_6]^+$ 478.1721, meas. 478.1728, Δ 1.5 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-(4-(3-carbamoylphenyl)but-3-yn-1-yl)tetrahydrofuran-3,4-diyl diacetate (S26). To a vial charged with alkyne **S25** (72 mg, 0.15 mmol) were added 3-iodobenzamide (75 mg, 0.30 mmol, 2.0 equiv.), CuI (7 mg, 38

μmol, 25 mol %) and $Pd(PPh_3)_4$ (9 mg, 8 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 6.0 mL) was added at RT. The resulting mixture was stirred at 80 °C for 1 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 20%) to afford alkyne **S26** (58 mg, 97 μmol, 64%) as a colorless oil. R_f = 0.24 (EtOAc/*i*-PrOH, 90:10); FTIR (thin film), ν_{max} (cm^{-1}): 3187, 2931, 1747, 1664, 1609, 1578, 1511, 1486, 1454, 1373, 1239, 1215, 1097, 1049; ^1H NMR (600 MHz, $CDCl_3$): δ 9.30 (s, 1H), 8.76 (s, 1H), 8.17 (s, 1H), 8.04–7.97 (m, 2H), 7.83 (app t, *J* = 1.5 Hz, 1H), 7.74 (app dt, *J* = 7.8, 1.5 Hz, 1H), 7.59–7.55 (m, 1H), 7.50–7.45 (m, 3H), 7.33 (app t, *J* = 7.8 Hz, 1H), 6.64 (br s, 1H), 6.17 (d, *J* = 5.6 Hz, 1H), 6.13 (br s, 1H), 6.11 (app t, *J* = 5.6 Hz, 1H), 5.71 (dd, *J* = 5.6, 4.3 Hz, 1H), 4.40 (ddd, *J* = 8.1, 4.9, 4.3 Hz, 1H), 2.61 (app dt, *J* = 17.2, 6.3 Hz, 1H), 2.53 (ddd, *J* = 17.2, 8.4, 6.1 Hz, 1H), 2.23–2.17 (m, 1H), 2.14 (s, 3H), 2.16–2.09 (m, 1H), 2.05 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.0, 169.7, 168.9, 165.0, 152.9, 151.9, 149.9, 142.2, 134.7, 133.7, 133.5, 133.0, 130.6, 128.9, 128.7, 128.1, 127.1, 124.1, 124.0, 89.7, 86.7, 81.8, 80.8, 73.3, 73.0, 31.7, 20.8, 20.5, 15.6; HRMS (ESI+): calcd. for $[C_{31}H_{29}N_6O_7]^+$ 597.2092, meas. 597.2097, Δ 0.8 ppm.

((1*R*,2*R*)-2-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)cyclopropyl)methanol (S28). The following protocol was adapted from an early

publication reporting the asymmetric cyclopropanation of allylic alcohols with dioxaborolane ligands.[75] To a stirred solution of diethylzinc in hexanes (1.0 M in hexanes, 4.46 mL, 4.46 mmol, 2.23 equiv.) was added CH_2Cl_2 (8.0 mL). The mixture was cooled to 0 °C and diiodomethane (0.72 mL, 8.9 mmol, 4.45 equiv.) was added dropwise over 5 min. The reaction mixture was stirred at 0 °C for 15 min (white precipitate was formed), and a pre-cooled (0 °C) solution of butylboronic acid *N*,*N*,*N'*,*N'*-tetramethyl-D-tartaric acid diamide ester (360 mg, 2.28 mmol, 1.14 equiv.) and allylic alcohol **S27**[72] (457 mg, 2.00 mmol) in CH_2Cl_2 (12 mL) was rapidly added *via* syringe. The resulting mixture was stirred at RT for 1 h, cooled to 0 °C and a NH_4Cl sat. aq. solution (15 mL) was added slowly. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were passed through a phase separator (Biotage Isolute), dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 100%) to afford cyclopropyl alcohol **S28** (382 mg, 1.56 mmol, 78%) as a 12:1 mixture of cyclopropyl diastereomers (major=desired), as a colorless oil. R_f = 0.24 (cyclohexane/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3446, 2991, 2935, 2251, 2041, 1458, 1382, 1373, 1274, 1210, 1194, 1159, 1105, 1089, 1053, 1030; ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.95 (s, 1H), 4.74 (d, J = 5.9 Hz, 1H), 4.66 (d, J = 5.9 Hz, 1H), 3.51–3.43 (m, 3H), 3.38 (s, 3H), 1.45 (s, 3H), 1.31 (s, 3H), 1.21 (dddd, J = 12.0, 8.4, 6.9, 5.1 Hz, 1H), 0.94 (ddt, J = 10.4, 9.0, 4.7 Hz, 1H), 0.53 (ddt, J = 17.2, 8.4, 5.1 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 112.1, 109.1, 91.1, 85.3, 84.0, 65.5, 54.4, 26.3,

24.8, 20.8, 20.4, 8.1; HRMS (ESI+): calcd. for $[C_{12}H_{20}O_3Na]^+$ 267.1203, meas. 267.1202, Δ 0.2 ppm.

(1*R*,2*R*)-2-((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)cyclopropane-1-carbaldehyde (S29). To a solution of cyclopropyl alcohol **S28** (245 mg, 1.00 mmol) in CH_2Cl_2 (20 mL) open to atmosphere at RT was added DMP (510 mg, 1.20 mmol, 1.2 equiv.) portionwise over 5 min. The reaction mixture was stirred for 2 h and was diluted with Et_2O (10 mL). The suspension was filtered and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 50%) to yield aldehyde **S29** (182 mg, 0.751 mmol, 75%) as a clear oil. In our hands, aldehyde **S29** was best prepared, purified and used immediately in the next reaction. R_f = 0.39 (cyclohexane/EtOAc, 67:33); ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.16 (d, J = 4.9 Hz, 1H), 4.96 (s, 1H), 4.75 (d, J = 5.9 Hz, 1H), 4.66 (d, J = 5.8 Hz, 1H), 3.55 (d, J = 10.0 Hz, 1H), 3.28 (s, 3H), 2.02 (app dtd, J = 7.8, 4.9, 3.9 Hz, 1H), 1.79 (app tdd, J = 10.1, 8.9, 6.4, 4.0 Hz, 1H), 1.45 (s, 3H), 1.33 (dd, J = 8.9, 4.9 Hz, 1H), 1.31 (s, 3H), 1.07 (ddd, J = 8.3, 6.4, 4.9 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 199.9, 112.5, 109.2, 89.3, 85.4, 84.0, 54.6, 28.9, 26.4, 26.1, 25.0, 12.7.

(3a*R*,4*R*,6*R*,6a*R*)-4-((1*R*,2*R*)-2-ethynylcyclopropyl)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole (S30). To a solution of aldehyde **S29** (182 mg, 0.751 mmol) in MeOH (4.0 mL) at RT was added K_2CO_3 (312 mg, 2.26 mmol, 3.0 equiv.) followed by a solution of dimethyl-1-diazo-2-oxopropyl phosphonate (180 mg, 0.939 mmol, 1.25 equiv.)

in MeOH (3.0 mL) under vigorous stirring. The resulting yellow suspension was stirred at RT for 3 h. The reaction was quenched by the addition of a NH_4Cl sat. aq. solution (10 mL) and CH_2Cl_2 (15 mL) was added. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (cyclohexane/EtOAc, 0 → 50%) to afford alkyne **S30** (98 mg, 0.41 mmol, 55 %) as a colorless oil. Alkyne **S30** crystallized when stored as a neat oil at –20 °C for 1 week. A small molecule X-ray structure of **S30** was obtained, confirming its absolute configuration as shown in the *Supporting Information*. R_f = 0.68 (cyclohexane/EtOAc, 67:33); FTIR (thin film), ν_{max} (cm^{-1}): 3283, 2989, 2935, 2834, 2116, 1458, 1407, 1382, 1373, 1307, 1273, 1233, 1209, 1194, 1159, 1104, 1090, 1056, 1034, 1023; ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.99 (s, 1H), 4.73 (d, *J* = 5.9 Hz, 1H), 4.66 (d, *J* = 5.9 Hz, 1H), 3.42 (s, 3H), 3.39–3.34 (m, 1H), 1.80 (d, *J* = 2.0 Hz, 1H), 1.45 (s, 3H), 1.44–1.38 (m, 1H), 1.38–1.32 (m, 1H), 1.3 (s, 3H), 0.94 (app dt, *J* = 8.5, 5.0 Hz, 1H), 0.79–0.74 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 112.4, 109.1, 90.5, 85.9, 85.6, 84.1, 64.8, 54.6, 26.5, 26.1, 25.0, 13.2, 6.1; HRMS (ESI+): calcd. for $[C_{13}H_{19}O_4]^+$ 239.1278, meas. 239.1288, Δ 4.4 ppm.

(3*R*,4*R*,5*R*)-5-((1*R*,2*R*)-2-ethynylcyclopropyl)tetrahydrofuran-2,3,4-triyl triacetate (S31). To a vial charged with acetonide **S30** (97 mg, 0.41 mmol) was added a 4:1 mixture of acetic acid and water (5.0 mL) at RT. The resulting mixture was stirred at 80 °C for 6 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with

benzene (2 × 1.0 mL). Crude material was used directly in the next step without further purification. To a solution of the crude triol in CH_2Cl_2 (5.0 mL) at RT, were added pyridine (0.99 mL, 12 mmol, 30 equiv.), Ac_2O (0.50 mL, 5.3 mmol, 13 equiv.) and DMAP (10 mg, 0.082 mmol, 0.20 equiv.) sequentially. The resulting mixture was stirred at RT for 2 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 100%) to afford triacetate **S31** (63 mg, 0.20 mmol, 50% over 2 steps) as a 4:5 mixture of α and β anomers, as a colorless oil. R_f = 0.60 (α-anomer) / 0.74 (β-anomer) (cyclohexane/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3284, 2580, 1742, 1431, 1370, 1210, 1103, 1044, 1009; ^{1}H NMR (400 MHz, $CDCl_3$): δ α-anomer: 6.38 (dd, *J* = 3.9, 0.8 Hz, 1H), 5.26–5.19 (m, 2H), 3.85 (dd, *J* = 6.8, 2.9 Hz, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 1.82 (d, *J* = 2.1 Hz, 1H), 1.42 (dddd, *J* = 8.8, 6.7, 6.0, 4.5 Hz, 1H), 1.31 (app. dtd, *J* = 9.5, 5.0, 2.1 Hz, 1H), 0.97 (app. dt, *J* = 8.7, 5.1 Hz, 1H), 0.91 (ddd, *J* = 8.9, 6.1, 4.8 Hz, 1H); β-anomer: 6.12 (d, *J* = 1.1 Hz, 1H), 5.33 (dd, *J* = 4.8, 1.1 Hz, 1H), 5.30 (dd, *J* = 6.6, 4.8 Hz, 1H), 3.75 (t, *J* = 6.8 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 1.82 (d, *J* = 2.1 Hz, 1H), 1.39 (dddd, *J* = 8.8, 7.0, 6.0, 4.4 Hz, 1H), 1.37–1.26 (m, 1H), 0.95 (app. dt, *J* = 8.7, 5.0 Hz, 1H), 0.85 (ddd, *J* = 8.8, 6.0, 4.8 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ α-anomer: 170.1, 169.7, 169.4, 93.9, 85.2, 84.5, 72.6, 70.0, 65.3, 23.7, 21.1, 20.8, 20.4, 11.7, 4.6; β-anomer: 169.8, 169.5, 169.3, 98.1, 82.8, 74.6, 73.8, 65.1, 55.5, 24.0, 21.2, 20.7, 20.6, 11.7, 4.4; HRMS (ESI+): calcd. for $[C_{15}H_{18}O_7Na]^+$ 333.0945, meas. 333.0970, Δ 7.5 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((1*R*,2*R*)-2-ethynylcyclopropyl)tetrahydrofuran-3,4-diyl diacetate (S32). To a suspension of N^6-benzoyladenine (114 mg, 0.478 mmol, 2.15 equiv.) in propionitrile (dried over 4 Å M.S., 6.0 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (0.16 mL, 0.67 mmol, 3.00 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S31** (dried by azeotropic distillation with benzene (4 ×), 69 mg, 0.22 mmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (16 mg, 44 μmol, 20 mol %) was added propionitrile (6.0 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 2.5 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 90:10) to afford alkyne **S32**, which was further purified by preparative HPLC (5% → 95% B' over 40 min) to afford alkyne **S32** (63 mg, 0.13 mmol, 58%) as a white amorphous solid. R_f = 0.19 (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3295, 3020, 2931, 1748, 1696, 1650, 1612, 1583, 1512, 1488, 1458, 1375, 1240, 1215, 1072, 1050; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.80 (s, 1H), 8.24 (s, 1H), 8.07–8.02 (m, 2H), 7.65–7.59 (m, 1H), 7.56–7.50 (m, 2H), 6.13 (d, *J* = 5.4 Hz, 1H), 6.07 (app t, *J* = 5.4 Hz, 1H), 5.66 (dd, *J* = 5.4, 4.3 Hz, 1H), 3.68 (dd, *J* = 8.3, 4.3 Hz, 1H), 2.15 (s, 3H), 2.08 (s, 3H), 1.83 (d, *J* = 2.1 Hz, 1H), 1.78–1.71 (m, 1H), 1.45–1.39 (m, 1H), 1.06 (app dt, *J* = 8.8, 5.2 Hz, 1H), 0.92 (ddd, *J* = 8.8, 5.8, 5.0 Hz, 1H); ^{13}C NMR (126 MHz,

$CDCl_3$): δ 169.7, 169.5, 165.1, 152.0, 151.9, 149.7, 142.5, 133.2, 133.1, 129.0, 128.3, 123.7, 87.1, 85.2, 84.9, 73.6, 73.2, 65.5, 23.6, 20.7, 20.5, 12.1, 5.3; HRMS (ESI+): calcd. for $[C_{25}H_{24}N_5O_6]^+$ 490.1721, meas. 490.1725, Δ 0.8 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((1*R*,2*R*)-2-((3-carbamoylphenyl)ethynyl)cyclopropyl)tetrahydrofuran-3,4-diyl diacetate (S33). To a vial charged with alkyne **S32** (48 mg, 98 μmol) were added 3-iodobenzamide (61 mg, 0.25 mmol, 2.5 equiv.), CuI (5 mg, 0.03 mmol, 25 mol %) and $Pd(PPh_3)_4$ (6 mg, 5 μmol, 5 mol %). The vial headspace was purged with nitrogen for 10 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 5.0 mL) was added at RT. The resulting mixture was stirred at 60 °C for 1.5 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 75:25) to afford benzamide **S33**, which was further purified by preparative HPLC (15% → 95% B' over 30 min) to afford benzamide **S33** (50 mg, 82 μmol, 84%) as a white amorphous solid. R_f = 0.35 (EtOAc/*i*-PrOH, 95:5); FTIR (thin film), ν_{max} (cm^{-1}): 3351, 3199, 3067, 2934, 1748, 1664, 1611, 1583, 1514, 1457, 1375, 1241, 1215, 1072; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.80 (s, 1H), 8.35 (s, 1H), 8.03–7.99 (m, 2H), 7.76–7.74 (m, 1H), 7.74–7.71 (m, 1H), 7.62–7.57 (m, 1H), 7.52–7.46 (m, 3H), 7.33 (t, *J* = 7.7 Hz, 1H), 6.88 (br s, 1H), 6.80 (br s, 1H), 6.28 (dd, *J* = 5.8, 5.3 Hz, 1H), 6.17 (d, *J* = 5.8 Hz, 1H), 5.70 (dd, *J* = 5.3, 3.5 Hz, 1H), 3.69 (dd, *J* = 9.0, 3.5 Hz, 1H), 2.17 (s, 3H), 2.08 (s, 3H), 2.00–1.94 (m, 1H), 1.61–1.56 (m, 1H), 1.17 (app dt, *J* = 8.7, 5.2 Hz, 1H), 1.02

(app dt, J = 8.7, 5.4 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2, 169.8, 169.6, 165.4, 151.9, 151.8, 149.6, 143.2, 135.4, 133.4, 132.8, 132.7, 130.7, 129.0, 128.9, 128.3, 127.2, 124.1, 123.6, 92.1, 87.5, 86.3, 76.3, 74.1, 72.9, 24.3, 20.8, 20.5, 13.0, 6.4; HRMS (ESI+): calcd. for $[C_{32}H_{29}N_6O_7]^+$ 609.2092, meas. 609.2114, Δ 3.6 ppm.

Methyl (2*S*,5*S*)-7-(3-carbamoylphenyl)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)hept-6-ynoate (S34). To a vial charged with alkyne **S3** (140 mg, 0.320 mmol) were added 3-iodobenzamide (197 mg, 0.799 mmol, 2.5 equiv.), CuI (15 mg, 80 μmol, 25 mol %) and $Pd(PPh_3)_4$ (19 mg, 16 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 7.1 mL) was added at RT. The resulting mixture was stirred at 60 °C for 1 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 100%) to afford alkyne **S34** (72 mg, 0.13 mmol, 40%) as a colorless solid. R_f = 0.43 (EtOAc); FTIR (thin-film), ν_{max} (cm^{-1}): 3307, 3066, 2935, 2253, 1719, 1665, 1612, 1575, 1438, 1382, 1274, 1209, 1158; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (td, J = 1.8, 0.6 Hz, 1H), 7.75 (ddd, J = 7.8, 1.9, 1.2 Hz, 1H), 7.51 (dt, J = 7.7, 1.4 Hz, 1H), 7.41–7.30 (m, 2H), 6.47 (br. s, 1H), 5.97 (br. s, 1H), 4.95 (s, 1H), 4.69 (td, J = 7.6, 5.2 Hz, 1H), 4.61 (s, 2H), 4.50 (dd, J = 10.8, 4.2 Hz, 1H), 3.80 (s, 3H), 3.34 (s, 3H), 2.87 (ddt, J = 10.6, 9.0, 4.4 Hz, 1H), 2.26–2.04 (m, 2H), 1.81–1.50 (m, 4H), 1.48 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.5,

168.8, 157.2 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 134.9, 133.5, 130.8, 128.7, 127.2, 123.9, 115.8 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 112.5, 110.2, 91.7, 85.6, 85.3, 84.5, 83.0, 55.4, 53.2, 52.7, 40.5, 30.9, 29.7, 29.4, 26.6, 25.0; ^{19}F NMR (376 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{26}H_{31}F_3N_2O_8Na]^+$ 579.1925, meas. 579.1928, Δ 0.5 ppm.

Methyl (2*S*,5*S*)-7-(3-carbamoylphenyl)-5-(((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-2-(2,2,2-trifluoroacetamido)hept-6-ynoate (S35). To a vial charged with acetonide **S34** (38 mg, 68 μmol) was added a 2:1 mixture of MeOH and TFA (6.0 mL) at RT. The vial was sealed tightly and the reaction mixture was stirred at 85 °C for 6 h. Volatiles were removed *in vacuo* and the residue was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 15%) to afford, in order of elution, β-anomer **S35** (15 mg, 29 μmol, 42%) and the α-anomer counterpart (14 mg, 27 μmol, 40%) as off-white solids. Only β-anomer **S35** was used in the subsequent step. R_f = 0.37 (β-anomer) (EtOAc/MeOH, 91:9); FTIR (thin-film), ν_{max} (cm^{-1}): 3349, 3076, 2921, 1719, 1665, 1613, 1598, 1573, 1438, 1383, 1250, 1211, 1180, 1161, 1127, 1103, 1063, 1029; ^{1}H NMR (600 MHz, $(CD_3)_2CO$): δ β-anomer: 8.82 (d, *J* = 8.1 Hz, 1H), 7.96 (t, *J* = 1.8 Hz, 1H), 7.88 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.56 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.54 (br. s, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 6.70 (br. s, 1H), 4.74 (s, 1H), 4.62 (ddd, *J* = 9.6, 8.0, 4.9 Hz, 1H), 4.23 (d, *J* = 4.1 Hz, 1H), 4.18 (ddd, *J* = 10.5, 6.5, 2.8 Hz, 1H), 4.12 (d, *J* = 7.2 Hz, 1H), 3.98 (td, *J* = 6.7, 4.8 Hz, 1H), 3.91 (t, *J* = 4.4 Hz, 1H), 3.74 (s, 3H), 3.29 (s, 3H), 2.98 (ddd, *J* = 15.3, 9.5, 4.7 Hz, 1H), 2.23 (dddd, *J* = 13.7, 10.9, 6.2, 4.8 Hz, 1H), 2.15 (ddt, *J* = 13.8, 9.6, 5.0 Hz, 1H),

1.91 (ddd, J = 13.4, 10.6, 2.9 Hz, 1H), 1.80 (dddd, J = 13.1, 10.0, 6.2, 4.9 Hz, 1H), 1.75–1.67 (m, 1H), 1.65 (ddd, J = 13.3, 10.6, 4.4 Hz, 1H); ^{13}C NMR (101 MHz, $(CD_3)_2CO$): δ β-anomer: 171.9, 168.3, 135.7, 135.1, 131.5, 129.4, 127.9, 124.9, 109.6, 93.3, 83.1, 82.0, 76.4, 76.2, 54.9, 53.6, 52.9, 42.1, 32.3; ^{19}F NMR (471 MHz, **$CDCl_3$**(500 μL)/$(CD_3)_2CO$ (50 μL), BTF IStd): δ β-anomer: –76.6; HRMS (ESI+): calcd. for $[C_{23}H_{28}F_3N_2O_8]^+$ 517.1792, meas. 517.1788, Δ 0.8 ppm.

Methyl (*S*,*E*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-7-(triisopropylsilyl)hept-2-en-6-ynoate (S36). To a solution of **5** (728 mg, 1.71 mmol) in PhMe (16 mL) at RT, was added methyl (triphenylphosphoranylidene)acetate (1.06 g, 3.18 mmol, 1.85 equiv.) in one portion. The resulting mixture was stirred at 50 °C for 2 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 2 → 40%) to afford **S36** (747 mg, 1.55 mmol, 91%) as a 13:1 mixture of *E* and *Z* isomers, as a colorless oil. R_f = 0.35 (hexanes/Et_2O, 70:30); FTIR (thin film), ν_{max} (cm^{-1}): 2942, 2865, 2169, 1726, 1660, 1463, 1436, 1381, 1371, 1270, 1210, 1159, 1093, 1060; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.00 (app dt, J = 15.6, 7.3 Hz, 1H), 5.88 (app dt, J = 15.6, 1.4 Hz, 1H), 4.93 (s, 1H), 4.61 (dd, J = 5.9, 1.1 Hz, 1H), 4.57 (d, J = 5.9 Hz, 1H), 4.45 (ddd, J = 10.6, 4.3, 1.1 Hz, 1H), 3.71 (s, 3H), 3.31 (s, 3H), 2.80 (dddd, J = 11.2, 7.5, 6.0, 3.7 Hz, 1H), 2.43–2.34 (m, 2H), 1.69 (ddd, J = 13.3, 10.6, 3.7 Hz, 1H), 1.60 (ddd, J = 13.3, 11.2, 4.3 Hz, 1H), 1.46 (s, 3H), 1.30 (s, 3H), 1.06–1.02 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.7, 145.9, 123.3, 112.5, 109.8, 109.3,

85.6, 85.5, 84.5, 83.7, 55.2, 51.5, 40.6, 38.4, 29.7, 26.7, 25.4, 18.8, 11.3; HRMS (ESI+): calcd. for $[C_{26}H_{44}O_6Si_1Na]^+$ 503.2799, meas. 503.2787, Δ 2.5 ppm.

Methyl (*S*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-7-(triisopropylsilyl)hept-6-ynoate (S37). To a vial charged with **S36** (747 mg, 1.55 mmol) were added PhMe (degassed by sparging with nitrogen for 15 min, 16 mL) and *t*-BuOH (degassed by sparging with nitrogen for 15 min, 0.45 mL, 4.7 mmol, 3.0 equiv.) at RT, followed by the addition of (BDP)CuH (1.0 M in PhMe, 3.1 mL, 3.1 mmol, 2.0 equiv.). The resulting mixture was stirred for 4.5 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 1 → 25%) to afford **S37** (750 mg, 1.55 mmol, quant.) as a colorless oil. R_f = 0.37 (hexanes/EtOAc, 80:20); FTIR (thin film), ν_{max} (cm^{-1}): 2942, 2865, 2165, 1741, 1462, 1381, 1371, 1209, 1160, 1092, 1059; ^1H NMR (600 MHz, $CDCl_3$): δ 4.93 (s, 1H), 4.63 (dd, J = 5.9, 1.1 Hz, 1H), 4.57 (d, J = 5.9 Hz, 1H), 4.46 (ddd, J = 10.4, 4.6, 1.1 Hz, 1H), 3.65 (s, 3H), 3.31 (s, 3H), 2.63 (app dtd, J = 11.0, 7.1, 3.7 Hz, 1H), 2.38–2.29 (m, 2H), 1.90–1.82 (m, 1H), 1.82–1.73 (m, 1H), 1.67 (ddd, J = 13.3, 10.4, 3.7 Hz, 1H), 1.59 (ddd, J = 13.3, 11.0, 4.6 Hz, 1H), 1.51–1.46 (m, 2H), 1.46 (s, 3H), 1.30 (s, 3H), 1.07–1.03 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.9, 112.4, 110.5, 109.8, 85.8, 85.6, 84.6, 82.8, 55.2, 51.6, 41.1, 35.2, 33.9, 30.0, 26.7, 25.4, 22.8, 18.8, 11.4; HRMS (ESI+): calcd. for $[C_{26}H_{46}O_6Si_1Na]^+$ 505.2956, meas. 505.2961, Δ 0.9 ppm.

Methyl (*S*)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)hept-6-ynoate (S38). To a solution of TIPS alkyne **S37** (750 mg, 1.55 mmol) in THF (16 mL) at 0 °C, was added TBAF (1.0 M in THF, 1.94 mL, 1.94 mmol, 1.25 equiv.) slowly. The resulting mixture was stirred at RT for 1 h, followed by the addition of water (15 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 1 → 30%) to afford alkyne **S38** (468 mg, 1.43 mmol, 92%) as a colorless oil. R_f = 0.35 (hexanes/EtOAc, 75:25); FTIR (thin film), ν_{max} (cm^{-1}): 3273, 2959, 2926, 1738, 1438, 1373, 1258, 1089, 1014; ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.93 (s, 1H), 4.59 (d, J = 6.0 Hz, 1H), 4.57 (dd, J = 6.0, 0.8 Hz, 1H), 4.48 (ddd, J = 11.1, 3.9, 0.8 Hz, 1H), 3.66 (s, 3H), 3.32 (s, 3H), 2.64–2.57 (m, 1H), 2.37–2.29 (m, 2H), 2.11 (d, J = 2.4 Hz, 1H), 1.88–1.80 (m, 1H), 1.79–1.72 (m, 1H), 1.69 (ddd, J = 13.3, 11.2, 3.9 Hz, 1H), 1.56 (ddd, J = 13.3, 11.1, 4.0 Hz, 1H), 1.52–1.48 (m, 2H), 1.48 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.9, 112.4, 110.1, 85.9, 85.7, 85.2, 84.6, 70.9, 55.3, 51.6, 40.4, 34.8, 33.8, 28.7, 26.6, 25.2, 22.6; HRMS (ESI+): calcd. for $[C_{17}H_{26}O_6Na]^+$ 349.1622, meas. 349.1621, Δ 0.3 ppm.

(3*R*,4*R*,5*R*)-5-((*S*)-2-ethynyl-6-methoxy-6-oxohexyl)tetrahydrofuran-2,3,4-triyl tri-acetate (S39). To a vial charged with acetonide **S38** (468 mg, 1.43 mmol) at 0 °C, was added a 4:1 mixture of TFA and water (10 mL). The resulting mixture was stirred at RT for 5.5 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation

with benzene (2 × 2 mL). Crude material was used directly in the next step without further purification. To a solution of the crude triol in CH_2Cl_2 (14 mL) at RT, were added pyridine (1.16 mL, 14.3 mmol, 10 equiv.), Ac_2O (1.36 mL, 14.3 mmol, 10 equiv.) and DMAP (123 mg, 1.00 mmol, 0.70 equiv.) sequentially. The resulting mixture was stirred at RT for 1 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 2 → 60%) to afford triacetate **S39** (200 mg, 0.500 mmol, 35% over 2 steps) as a 2:1 mixture of diastereomers, as a colorless oil; R_f = 0.27/0.32 (hexanes/EtOAc, 60:40); FTIR (thin film), ν_{max} (cm^{-1}): 3280, 2952, 2923, 1739, 1436, 1371, 1216, 1110, 1011; ^1H NMR (600 MHz, $CDCl_3$): δ 6.35 (d, J = 4.6 Hz, 1H), 6.13 (d, J = 1.1 Hz, 2H), 5.31 (dd, J = 4.8, 1.1 Hz, 2H), 5.22 (dd, J = 6.8, 4.6 Hz, 1H), 5.18 (dd, J = 6.9, 4.8 Hz, 2H), 5.06 (dd, J = 6.8, 3.7 Hz, 1H), 4.46 (app dt, J = 10.3, 3.7 Hz, 1H), 4.40 (ddd, J = 10.3, 6.9, 3.1 Hz, 2H), 3.66 (s, 9H), 2.63–2.57 (m, 3H), 2.37–2.28 (m, 6H), 2.11 (s, 6H), 2.11 (s, 3H), 2.10 (s, 3H), 2.07 (s, 6H), 2.06 (s, 6H), 2.05 (s, 3H), 1.89–1.80 (m, 3H), 1.80– 1.69 (m, 6H), 1.65 (ddd, J = 13.9, 10.3, 4.2 Hz, 2H), 1.62 (ddd, J = 13.4, 10.3, 4.0 Hz, 1H), 1.55–1.45 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.9, 173.9, 170.2, 170.0, 169.8, 169.6, 169.5, 169.2, 98.5, 93.9, 85.7, 85.4, 81.5, 79.9, 74.7, 74.0, 72.7, 71.1, 70.9, 69.9, 51.7, 40.2, 39.2, 34.7, 34.7, 33.8, 33.8, 28.5, 28.2, 22.5, 22.5, 21.2, 21.2, 20.8, 20.7, 20.7, 20.4; HRMS (ESI+): calcd. for $[C_{19}H_{26}O_9Na]^+$ 421.1469, meas. 421.1490, Δ 4.9 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((*S*)-2-ethynyl-6-methoxy-6-oxohexyl)tetrahydrofuran-3,4-diyl diacetate (S40). To a suspension of N^6-benzoyladenine (240 mg, 1.00 mmol, 4.0 equiv.) in propionitrile (dried over 4 Å M.S., 4.2 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (0.31 mL, 1.3 mmol, 5.0 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S39** (dried by azeotropic distillation with benzene (4 ×), 100 mg, 0.251 mmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (45 mg, 0.13 mmol, 50 mol %) was added propionitrile (4.2 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 2.5 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (PhMe/MeCN, 2 → 60%) to afford nucleoside **S40** (102 mg, 0.177 mmol, 70%) as a colorless oil. R_f = 0.44 (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3285, 2952, 2925, 2855, 1744, 1703, 1610, 1582, 1510, 1486, 1455, 1241, 1219, 1096, 1074, 1047; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.98 (br s, 1H), 8.80 (s, 1H), 8.07 (s, 1H), 8.04–8.00 (m, 2H), 7.63–7.59 (m, 1H), 7.55–7.50 (m, 2H), 6.14–6.11 (m, 2H), 5.59–5.54 (m, 1H), 4.53 (ddd, J = 11.0, 4.4, 2.8 Hz, 1H), 3.66 (s, 3H), 2.59–2.51 (m, 1H), 2.35–2.25 (m, 2H), 2.16 (s, 3H), 2.16–2.10 (m, 1H), 2.13 (d, J = 2.4 Hz, 1H), 2.07 (s, 3H), 1.88–1.80 (m, 2H), 1.75–1.67 (m, 1H), 1.55–1.48 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.8, 169.8, 169.5, 164.7, 152.9, 151.7, 149.9, 142.4, 133.6, 133.0, 129.0, 128.0, 124.1, 87.2, 85.5,

81.0, 73.8, 72.9, 71.2, 51.7, 38.4, 34.7, 33.8, 28.1, 22.5, 20.8, 20.5; HRMS (ESI+): calcd. for $[C_{29}H_{32}N_5O_8]^+$ 578.2245, meas. 578.2242, Δ 0.5 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((*S*)-2-((3-carbamoylphenyl)ethynyl)-6-methoxy-6-oxohexyl)tetrahydrofuran-3,4-diyl diacetate (S41). To a solution of alkyne **S40** (102 mg, 0.177 mmol) and 3-iodobenzamide (131 mg, 0.530 mmol, 3.0 equiv.) in a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 9.0 mL) at RT, were introduced CuI (8 mg, 0.04 mmol, 25 mol %) and $Pd(PPh_3)_4$ (10 mg, 8.8 μmol, 5 mol %) rapidly. The resulting mixture was stirred at 60 °C for 2.5 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 10%) to afford benzamide **S41** (105 mg, 0.151 mmol, 85%) as a white amorphous solid. R_f = 0.39 (EtOAc/*i*-PrOH, 95:5); FTIR (thin film), ν_{max} (cm^{-1}): 3340, 3198, 2925, 2855, 1747, 1669, 1611, 1581, 1513, 1487, 1456, 1375, 1244, 1221, 1097, 1047; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.12 (s, 1H), 8.79 (s, 1H), 8.11 (s, 1H), 8.03–8.00 (m, 2H), 7.87–7.86 (m, 1H), 7.75 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H), 7.62–7.57 (m, 1H), 7.54–7.47 (m, 3H), 7.36 (app t, *J* = 7.8 Hz, 1H), 6.51 (br s, 1H), 6.18 (dd, *J* = 5.9, 5.2 Hz, 1H), 6.15 (d, *J* = 5.9 Hz, 1H), 5.98 (br s, 1H), 5.66 (dd, *J* = 5.2, 3.8 Hz, 1H), 4.53 (app dt, *J* = 10.0, 3.8 Hz, 1H), 3.65 (s, 3H), 2.76 (app dtd, *J* = 11.1, 7.0, 3.7 Hz, 1H), 2.39–2.29 (m, 2H), 2.20 (ddd, *J* = 13.6, 10.0, 3.7 Hz, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 1.98 (ddd, *J* = 13.6, 11.1, 3.8 Hz, 1H), 1.92–1.84 (m, 1H), 1.81–1.73 (m, 1H), 1.63–1.57 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ

173.9, 170.0, 169.7, 168.8, 164.8, 152.9, 151.8, 149.9, 142.4, 134.9, 133.6, 133.6, 133.0, 130.8, 129.0, 128.7, 128.0, 127.0, 124.3, 124.0, 92.3, 86.9, 82.5, 81.5, 74.0, 72.8, 51.7, 38.7, 35.0, 33.8, 29.0, 22.7, 20.8, 20.6; HRMS (ESI+): calcd. for $[C_{36}H_{36}N_6O_9Na]^+$ 719.2436, meas. 719.2452, Δ 2.3 ppm.

Triisopropyl((*S*,*E*)-3-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydro-furo[3,4-*d*][1,3]dioxol-4-yl)methyl)-6-nitrohex-5-en-1-yn-1-yl)silane (S42). To a solution of aldehyde **5** (385 mg, 0.907 mmol) in $MeNO_2$ (9 mL) at 0 °C, was added Et_3N (0.13 mL, 0.91 mmol, 1.00 equiv.). The resulting mixture was stirred at RT for 17 h and volatiles were removed *in vacuo*. To a solution of the residue in CH_2Cl_2 (9 mL) at –78 °C were added *i*-Pr_2NEt (0.40 mL, 2.3 mmol, 2.50 equiv.) and MsCl (90 μL, 1.1 mmol, 1.25 equiv.) sequentially. The resulting mixture was stirred at –78 °C for 2 h, before the addition of a NH_4Cl sat. aq. solution (8 mL). The biphasic mixture was allowed to warm to RT and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 8 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 2 → 25%) to afford **S42** (319 mg, 0.682 mmol, 75% over 2 steps) as a colorless oil. R_f = 0.46 (hexanes/EtOAc, 75:25); FTIR (thin film), ν_{max} (cm^{-1}): 2941, 2865, 2168, 1655, 1528, 1463, 1351, 1210, 1092; ^1H NMR (600 MHz, $CDCl_3$): δ 7.33 (ddd, *J* = 13.4, 7.9, 7.5 Hz, 1H), 7.06 (app dt, *J* = 13.4, 1.4 Hz, 1H), 4.95 (s, 1H), 4.62 (dd, *J* = 5.9, 1.1 Hz, 1H), 4.59 (d, *J* = 5.9 Hz, 1H), 4.46 (ddd, *J* = 10.6, 4.3, 1.1 Hz, 1H), 3.34 (s, 3H), 2.89 (dddd, *J* = 10.9, 7.9, 5.5, 4.0 Hz, 1H),

2.49 (dddd, J = 14.6, 7.5, 5.5, 1.4 Hz, 1H), 2.41 (app dtd, J = 14.6, 7.9, 1.4 Hz, 1H), 1.72 (ddd, J = 13.3, 10.6, 4.0 Hz, 1H), 1.66 (ddd, J = 13.3, 10.9, 4.3 Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H), 1.07–1.03 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 141.0, 139.2, 112.7, 110.0, 108.0, 85.5, 85.3, 85.1, 84.4, 55.4, 40.8, 34.4, 29.6, 26.7, 25.4, 18.7, 11.3; HRMS (ESI+): calcd. for $[C_{24}H_{41}N_1O_6Si_1Na]^+$ 490.2595, meas. 490.2580, Δ 3.2 ppm.

2,2,2-trifluoro-*N*-((*S*)-4-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydro-furo[3,4-*d*][1,3]dioxol-4-yl)methyl)-6-(triisopropylsilyl)hex-5-yn-1-yl)acetamide (S43). To a solution of **S42** (319 mg, 0.682 mmol) in THF (6.8 mL) at 0 °C, was added $LiAlH_4$ (1.0 M in Et_2O, 4.8 mL, 4.8 mmol, 7.0 equiv.) dropwise. The resulting mixture was stirred at RT for 4 h. The reaction was quenched by the dropwise addition of water (0.18 mL), a 15% NaOH aq. solution (0.18 mL) and water (0.54 mL), sequentially, at 0 °C and Na_2SO_4 was added. The mixture was stirred vigorously for 20 min and filtered through a cotton plug to remove the white solids. The filtrate was concentrated and the crude material was used directly in the next step without further purification. To a solution of the crude amine in THF (6.8 mL) at 0 °C, were added Et_3N (5.7 mL, 41 mmol, 60 equiv.) and ethyl trifluoroacetate (2.4 mL, 21 mmol, 30 equiv.) sequentially. The resulting mixture was stirred at RT for 17 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 2 → 50%) to afford trifluoroacetamide **S43** (252 mg, 0.470 mmol, 69% over 2 steps) as a colorless oil. R_f = 0.47 (hexanes/EtOAc, 65:35); FTIR (thin film), ν_{max} (cm^{-1}): 3306, 2941, 2865, 2166, 1705, 1559, 1463, 1381, 1209,

1161, 1106, 1061; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.27 (br s, 1H), 4.94 (s, 1H), 4.62 (dd, J = 5.9, 0.7 Hz, 1H), 4.58 (d, J = 5.9 Hz, 1H), 4.45 (ddd, J = 10.3, 4.7, 0.7 Hz, 1H), 3.44–3.39 (m, 2H), 3.32 (s, 3H), 2.69–2.63 (m, 1H), 1.88–1.80 (m, 1H), 1.76 (app ddq, J = 13.4, 9.9, 6.7 Hz, 1H), 1.68 (ddd, J = 13.4, 10.3, 4.1 Hz, 1H), 1.62 (ddd, J = 13.4, 10.7, 4.7 Hz, 1H), 1.56–1.48 (m, 2H), 1.47 (s, 3H), 1.31 (s, 3H), 1.08–1.03 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 157.3 (q, $^{2}J_{C\text{-}F}$ = 37 Hz), 116.0 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 112.5, 110.1, 109.8, 85.7, 85.6, 84.5, 83.4, 55.2, 41.2, 39.8, 32.8, 30.0, 26.9, 26.7, 25.4, 18.8, 11.3; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.9; HRMS (ESI+): calcd. for $[C_{26}H_{44}F_3N_1O_5Si_1Na]^+$ 558.2833, meas. 558.2858, Δ 4.4 ppm.

2,2,2-trifluoro-*N*-((*S*)-4-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)hex-5-yn-1-yl)acetamide (S44). To a solution of TIPS alkyne **S43** (196 mg, 0.366 mmol) in DMF (3 mL) at RT, was added a solution of TASF (0.30 g, 1.2 mmol, 3.0 equiv.) in DMF (4 mL). The resulting mixture was stirred at 60 °C for 2.5 h and volatiles were removed *in vacuo*. The residue was partitioned between Et_2O (7 mL) and a 1:1 mixture of brine and 5% LiCl aq. solution (7 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 7 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 5 → 50%) to afford alkyne **S44** (117 g, 0.308 mmol, 84%) as an amorphous white solid. R_f = 0.32 (hexanes/EtOAc, 65:35); FTIR (thin film), ν_{max} (cm^{-1}): 3298, 2937, 1706, 1558, 1454, 1382, 1209, 1159, 1104, 1059; ^{1}H

NMR (600 MHz, $CDCl_3$): δ 6.43 (br s, 1H), 4.94 (s, 1H), 4.59 (d, J = 6.0 Hz, 1H), 4.57 (dd, J = 6.0, 0.9 Hz, 1H), 4.47 (ddd, J = 11.1, 4.0, 0.9 Hz, 1H), 3.42–3.37 (m, 2H), 3.32 (s, 3H), 2.66–2.60 (m, 1H), 2.14 (d, J = 2.4 Hz, 1H), 1.86–1.78 (m, 1H), 1.74 (app ddt, J = 14.0, 10.3, 7.0 Hz, 1H), 1.69 (ddd, J = 13.3, 11.1, 4.0 Hz, 1H), 1.59 (ddd, J = 13.3, 10.9, 4.0 Hz, 1H), 1.53–1.49 (m, 2H), 1.48 (s, 3H), 1.32–1.30 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 157.4 (q, $^2J_{C\text{-}F}$ = 37 Hz), 116.0 (q, $^1J_{C\text{-}F}$ = 288 Hz), 112.5, 110.1, 85.6, 85.6, 85.1, 84.5, 71.3, 55.3, 40.5, 39.8, 32.3, 28.6, 26.8, 26.6, 25.1; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.9; HRMS (ESI+): calcd. for $[C_{17}H_{24}F_3N_1O_5Na]^+$ 402.1499, meas. 402.1510, Δ 2.7 ppm.

(3*R*,4*R*,5*R*)-5-((*S*)-2-ethynyl-5-(2,2,2-trifluoroacetamido)pentyl)tetrahydrofuran-2,3,4-triyl triacetate (S45). To a solution of **S44** (117 mg, 0.308 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C, was added a 4:1 mixture of TFA and water (6.0 mL). The resulting mixture was stirred at RT for 15 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with benzene (2 × 2 mL). Crude material was used directly in the next step without further purification. To a solution of the crude triol in CH_2Cl_2 (6.0 mL) at RT, were added pyridine (0.37 mL, 4.6 mmol, 15.0 equiv.), Ac_2O (0.44 mL, 4.6 mmol, 15.0 equiv.) and DMAP (39 mg, 0.32 mmol, 1.05 equiv.) sequentially. The resulting mixture was stirred at RT for 2.5 h, before the addition of water (6 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 6 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by

silica gel chromatography (hexanes/EtOAc, 5 → 70%) to afford **S45** (74 mg, 0.16 mmol, 53% over 2 steps) as a 1:1 mixture of diastereomers, as a colorless oil. R_f = 0.30/0.41 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3288, 2933, 1749, 1720, 1558, 1435, 1373, 1218, 1180, 1158, 1012; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.70–6.62 (m, 2H), 6.34 (d, J = 4.5 Hz, 1H), 6.10 (d, J = 0.9 Hz, 1H), 5.30 (dd, J = 4.8, 0.9 Hz, 1H), 5.21 (dd, J = 6.7, 4.5 Hz, 1H), 5.16 (dd, J = 7.0, 4.8 Hz, 1H), 5.04 (dd, J = 6.7, 3.7 Hz, 1H), 4.44 (app dt, J = 10.1, 3.7 Hz, 1H), 4.36 (ddd, J = 10.1, 7.0, 3.0 Hz, 1H), 3.42– 3.33 (m, 4H), 2.66–2.59 (m, 2H), 2.13 (d, J = 2.4 Hz, 2H), 2.11 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.87–1.76 (m, 4H), 1.75–1.68 (m, 2H), 1.68–1.60 (m, 2H), 1.57–1.43 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.3, 170.1, 169.9, 169.7, 169.5, 169.4, 157.4 (q, $^2J_{C\text{-}F}$ = 37 Hz), 157.4 (q, $^2J_{C\text{-}F}$ = 37 Hz), 116.0 (q, $^1J_{C\text{-}F}$ = 288 Hz), 116.0 (q, $^1J_{C\text{-}F}$ = 288 Hz), 98.5, 93.8, 85.5, 85.2, 81.5, 79.9, 74.5, 73.9, 72.7, 71.4, 71.2, 69.9, 40.1, 39.7, 39.6, 39.3, 32.1, 32.0, 28.5, 28.1, 26.6, 26.3, 21.2, 21.2, 20.8, 20.7, 20.6, 20.4; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.88, –76.90; HRMS (ESI+): calcd. for $[C_{19}H_{24}F_3N_1O_8Na]^+$ 474.1346, meas. 474.1358, Δ 2.5 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((*S*)-2-ethynyl-5-(2,2,2-trifluoroacetamido)pentyl)tetrahydrofuran-3,4-diyl diacetate (S46). To a suspension of N^6-benzoyladenine (72 mg, 0.30 mmol, 4.0 equiv.) in propionitrile (dried over 4 Å M.S., 1.5 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (92 μL, 0.38 mmol, 5.0 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of

N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S45** (dried by azeotropic distillation with benzene (4 ×), 34 mg, 75 μmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (13 mg, 38 μmol, 50 mol %) was added propionitrile (1.5 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 4 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (PhMe/MeCN, 0 → 50%) to afford nucleoside **S46** (35 mg, 56 μmol, 74%) as a colorless oil. R_f = 0.31 (hexanes/EtOAc, 25:75); FTIR (thin film), ν_{max} (cm^{-1}): 3289, 2928, 1749, 1708, 1612, 1583, 1457, 1245, 1218, 1159, 1076, 1049; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.15 (s, 1H), 8.75 (s, 1H), 8.07 (s, 1H), 8.04–7.98 (m, 2H), 7.62–7.57 (m, 1H), 7.53–7.48 (m, 2H), 6.71–6.62 (m, 1H), 6.13–6.09 (m, 2H), 5.55 (app t, J = 4.3 Hz, 1H), 4.49 (ddd, J = 10.8, 4.3, 3.1 Hz, 1H), 3.34 (m, 2H), 2.59–2.52 (m, 1H), 2.16 (s, 3H), 2.15 (d, J = 2.4 Hz, 1H), 2.12–2.06 (ddd, J = 13.5, 10.8, 4.2 Hz, 1H), 2.06 (s, 3H), 1.87 (ddd, J = 13.5, 11.2, 3.1 Hz, 1H), 1.82–1.74 (m, 1H), 1.67 (app tdd, J = 13.4, 8.5, 6.5 Hz, 1H), 1.56–1.45 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.9, 169.6, 164.9, 157.4 (q, $^2J_{C\text{-}F}$ = 37 Hz), 152.8, 151.7, 149.9, 142.4, 133.5, 133.0, 129.0, 128.0, 124.1, 116.0 (q, $^1J_{C\text{-}F}$ = 288 Hz), 87.2, 85.2, 80.9, 73.7, 72.9, 71.5, 39.6, 38.5, 32.2, 28.0, 26.7, 20.8, 20.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.9; HRMS (ESI+): calcd. for $[C_{29}H_{30}F_3N_6O_7]^+$ 631.2123, meas. 631.2142, Δ 3.1 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((*S*)-2-((3-carbamoylphenyl)ethynyl)-5-(2,2,2-trifluoroacetamido)pentyl)tetrahydrofuran-3,4-diyl diacetate (S47). To a solution of **S46** (33 mg, 52 μmol) and 3-iodobenzamide (39 mg, 0.16 mmol, 3.0 equiv.) in a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 2.6 mL) at RT, were introduced CuI (3 mg, 0.01 mmol, 25 mol %) and $Pd(PPh_3)_4$ (3 mg, 3 μmol, 5 mol %) rapidly. The resulting mixture was stirred at 60 °C for 2 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 10%) to afford alkyne **S47** (32 mg, 43 μmol, 82%) as a white amorphous solid. R_f = 0.28 (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3306, 2928, 1749, 1709, 1667, 1611, 1581, 1456, 1376, 1245, 1217, 1157, 1098, 1049; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.32 (s, 1H), 8.75 (s, 1H), 8.12 (s, 1H), 8.04–7.99 (m, 2H), 7.89–7.87 (m, 1H), 7.76 (ddd, *J* = 7.7, 1.9, 1.2 Hz, 1H), 7.61–7.56 (m, 1H), 7.52–7.47 (m, 3H), 7.34 (app td, *J* = 7.7, 0.5 Hz, 1H), 7.19–7.16 (m, 1H), 6.80 (br s, 1H), 6.18 (dd, *J* = 5.8, 5.2 Hz, 1H), 6.14 (d, *J* = 5.8 Hz, 1H), 6.08 (br s, 1H), 5.63 (dd, *J* = 5.2, 3.8 Hz, 1H), 4.51 (ddd, *J* = 10.2, 3.8, 3.6 Hz, 1H), 3.43–3.31 (m, 2H), 2.76 (m, 1H), 2.15 (s, 3H), 2.19–2.12 (m, 1H), 2.06 (s, 3H), 1.96 (ddd, *J* = 13.3, 11.0, 3.6 Hz, 1H), 1.88–1.79 (m, 1H), 1.79–1.69 (m, 1H), 1.64–1.52 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.1, 169.7, 168.9, 165.1, 157.6 (q, $^2J_{C\text{-}F}$ = 37 Hz), 152.7, 151.8, 150.0, 142.6, 134.7, 133.6, 133.5, 133.0, 131.0, 129.0, 128.7, 128.1, 127.3, 124.3, 123.7, 116.0 (q, $^1J_{C\text{-}F}$ = 288 Hz), 91.8, 87.0, 82.9, 81.3, 73.9, 72.7, 39.9, 38.7, 32.5, 28.8, 26.7, 20.8, 20.6; ^{19}F NMR

(471 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{36}H_{34}F_3N_7O_8Na]^+$ 772.2313, meas. 772.2306, Δ 0.9 ppm.

Methyl (2*S*,5*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-5-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)hept-6-ynoate (S48). To a solution of acetonide **S3** (136 mg, 311 μmol) in THF (3.0 mL) was added a 0.5 M LiOH aq. solution (1.3 mL) at 0 °C. After 75 min, more 0.5 M LiOH aq. solution (1.5 mL) was added. The reaction mixture was allowed to warm gradually to RT and was stirred for 8 h. More 0.5 M LiOH aq. solution (1.5 mL) was added and the resulting mixture was stirred for 1 h. The reaction mixture was cooled to 0 °C and a 1 M HCl aq. solution was added dropwise until pH 6 was obtained. A $NaHCO_3$ sat. aq. solution (5.0 mL) was added slowly followed by 1,4-dioxane (3.0 mL). A solution of Fmoc-Cl in 1,4-dioxane (0.39 M, 1.0 mL, 0.39 mmol, 1.25 equiv.) was added dropwise over 5 min under vigorous stirring. After 1 h, more Fmoc-Cl in 1,4-dioxane (0.39 M, 2.0 mL, 0.78 mmol, 2.50 equiv.) was added and the reaction mixture was stirred for 2 h. The reaction was quenched by the slow addition of a 1 M HCl aq. solution (8 mL) at 0 °C followed by CH_2Cl_2 (8 mL). The layers were separated using a phase separator (Biotage Isolute) and the aqueous phase was extracted with CH_2Cl_2 (2 × 8 mL). The combined organic extracts were concentrated and the residue was dissolved in MeOH (20 mL). (Trimethylsilyl)diazomethane (2.0 M in hexanes, 5.0 mL) was added to the methanolic solution over 2 min and the reaction mixture was stirred for 1 h. More (trimethylsilyl)diazomethane (2.0 M in hexanes,

3.0 mL) was added and the resulting mixture was stirred for 1 h. The reaction was quenched by the dropwise addition of glacial AcOH (4 mL) over 10 min and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 100%) to afford carbamate **S48** (83 mg, 0.15 mmol, 47% over 3 steps) as an amorphous white solid. R_f = 0.56 (cyclohexane/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3300, 2987, 2947, 1723, 1526, 1447, 1377, 1341, 1212, 1163, 1099, 1061; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (dq, J = 7.5, 1.0 Hz, 2H), 7.60 (t, J = 6.8 Hz, 2H), 7.40 (tq, J = 7.4, 1.0 Hz, 2H), 7.32 (tt, J = 7.5, 1.4 Hz, 2H), 5.31 (d, J = 8.5 Hz, 1H), 4.94 (s, 1H), 4.58 (q, J = 6.0 Hz, 2H), 4.48 (dd, J = 11.1, 4.0 Hz, 1H), 4.46–4.33 (m, 3H), 4.23 (t, J = 7.1 Hz, 1H), 3.77 (s, 3H), 3.31 (s, 3H), 2.66–2.60 (m, 1H), 2.14 (d, J = 2.3 Hz, 1H), 2.06–1.95 (m, 1H), 1.95–1.85 (m, 1H), 1.69 (ddd, J = 14.8, 11.2, 4.0 Hz, 1H), 1.58 (ddd, J = 16.3, 12.3, 4.7 Hz, 2H), 1.52fz (m, 1H), 1.49 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.9, 156.1, 144.0, 143.8, 141.4, 127.8, 127.2, 125.2, 125.1, 120.1, 112.4, 110.1, 110.1, 85.6, 85.1, 84.5, 71.4, 67.2, 55.3, 53.8, 52.6, 47.3, 40.4, 31.1, 30.5, 28.7, 26.6, 25.1; HRMS (ESI+): calcd. for $[C_{32}H_{37}N_1O_8Na]^+$ 586.2411, meas. 586.2407, Δ 0.8 ppm.

(3*R*,4*R*,5*R*)-5-((2*S*,5*S*)-5-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-ethynyl-6-methoxy-6-oxohexyl)tetrahydrofuran-2,3,4-triyl triacetate (S49). To a vial charged with acetonide **S48** (43 mg, 76 μmol) was added a 4:1 mixture of acetic acid and water (3.5 mL) at RT. The resulting mixture was stirred at 80 °C for 10 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with benzene (2 × 1.0 mL).

Crude material was used directly in the next step without further purification. To a solution of the crude triol in CH_2Cl_2 (3.5 mL) at RT, were added pyridine (60 μL, 0.76 mmol, 10 equiv.), Ac_2O (70 μL, 0.76 mmol, 10 equiv.) and DMAP (7 mg, 53 μmol, 0.70 equiv.) sequentially. The resulting mixture was stirred at RT for 2 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 5 → 60%) to afford **S49** (42 mg, 66 μmol, 87% over 2 steps) as a 1:1 mixture of diastereomers, as a colorless oil. R_f = 0.30/0.38 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3355, 3294, 2954, 2924, 2853, 1744, 1722, 1526, 1451, 1372, 1218, 1107, 1050, 1012; ^1H NMR (500 MHz, $CDCl_3$): δ 7.78–7.75 (m, 4H), 7.63–7.58 (m, 4H), 7.42–7.38 (m, 4H), 7.34–7.29 (m, 4H), 6.36 (d, *J* = 4.6 Hz, 1H), 6.14 (d, *J* = 1.0 Hz, 1H), 5.38 (br d, *J* = 8.5 Hz, 1H), 5.34 (br d, *J* = 8.4 Hz, 1H), 5.32 (dd, *J* = 4.8, 1.0 Hz, 1H), 5.23 (dd, *J* = 6.8, 4.6 Hz, 1H), 5.18 (dd, *J* = 7.1, 4.8 Hz, 1H), 5.07 (dd, *J* = 6.8, 3.6 Hz, 1H), 4.46 (app dt, *J* = 10.2, 3.6 Hz, 1H), 4.42–4.36 (m, 7H), 4.23 (app t, *J* = 7.0 Hz, 2H), 3.76 (s, 6H), 2.69–2.57 (m, 2H), 2.13 (d, *J* = 2.4 Hz, 1H), 2.12 (d, *J* = 2.4 Hz, 1H), 2.12 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.07 (s, 6H), 2.06 (s, 3H), 2.00–1.75 (m, 6H), 1.70–1.43 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.9, 172.8, 170.2, 170.0, 169.8, 169.6, 169.5, 169.3, 156.1, 156.0, 144.0, 144.0, 143.9, 143.8, 141.4, 141.4, 127.8, 127.2, 125.2, 120.1, 120.1, 98.5, 93.9, 85.3, 85.1, 81.4, 79.9, 74.6, 73.9, 72.6, 71.5, 71.3, 69.9, 67.1, 53.8, 53.7, 52.6, 47.3, 40.1, 39.2, 31.0, 30.4, 30.3, 28.6, 28.2, 21.2, 21.2, 20.8, 20.7, 20.7, 20.4; HRMS (ESI+): calcd. for $[C_{34}H_{37}N_1O_{11}Na]^+$ 658.2259, meas. 658.2248, Δ 1.7 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-((2*S*,5*S*)-5-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-ethynyl-6-methoxy-6-oxohexyl)-5-(6-benzamido-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (S50). To a suspension of N^6-benzoyladenine (63 mg, 0.26 mmol, 4.0 equiv.) in propionitrile (dried over 4 Å M.S., 1.5 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (80 μL, 0.33 mmol, 5.0 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S49** (dried by azeotropic distillation with benzene (4 ×), 42 mg, 66 μmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (12 mg, 33 μmol, 50 mol %) was added propionitrile (1.5 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 90 °C for 4 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 30 → 100%) to afford nucleoside **S50** (44 mg, 54 μmol, 82%) as a colorless oil; R_f = 0.49 (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3300, 2953, 2926, 2854, 1745, 1704, 1610, 1582, 1510, 1452, 1241, 1217, 1098, 1073, 1047; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.79 (s, 1H), 8.13 (s, 1H), 8.02–7.97 (m, 2H), 7.76–7.72 (m, 2H), 7.62–7.57 (m, 3H), 7.53–7.49 (m, 2H), 7.40–7.35 (m, 2H), 7.31–7.27 (m, 2H), 6.13 (br d, *J* = 5.5 Hz, 1H), 6.05 (br t, *J* = 5.5 Hz, 1H), 5.55 (br t, *J* = 5.0 Hz, 1H), 5.35 (br d, *J* = 8.4 Hz, 1H), 4.54–4.48 (m, 1H), 4.46–4.35 (m, 3H), 4.23 (br t, *J* = 7.1 Hz, 1H), 3.74 (s, 3H), 2.67–2.58 (m, 1H), 2.16 (s, 3H), 2.15 (d, *J* = 2.4 Hz, 1H), 2.12–2.05 (m, 1H), 2.07 (s, 3H), 2.00–1.92

(m, 1H), 1.92–1.80 (m, 2H), 1.64–1.45 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.8, 169.8, 169.6, 164.8, 156.1, 152.8, 151.7, 149.8, 144.0, 143.8, 142.3, 141.4, 133.5, 133.0, 129.0, 128.0, 127.8, 127.2, 125.2, 123.9, 120.1, 87.1, 85.1, 80.9, 73.7, 73.0, 71.6, 67.1, 53.6, 52.6, 47.3, 38.5, 31.0, 30.5, 28.0, 20.8, 20.5; HRMS (ESI+): calcd. for $[C_{44}H_{43}N_6O_{10}]^+$ 815.3035, meas. 815.3028, Δ 0.9 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-((2*S*,5*S*)-5-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-((3-carbamoylphenyl)ethynyl)-6-methoxy-6-oxohexyl)-5-(6-benzamido-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (S51). To a vial charged with **S50** (61 mg, 75 μmol) were added 2-iodobenzamide (46 mg, 0.19 mmol, 2.5 equiv.), CuI (4 mg, 0.02 mmol, 25 mol %) and $Pd(PPh_3)_4$ (4 mg, 4 μmol, 5 mol %). The vial headspace was purged with nitrogen for 10 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 2.5 mL) was added at RT. The resulting mixture was stirred at 60 °C for 2 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 25%) to afford **S51** (68 mg, 73 μmol, 97%) as a light yellow oil. FTIR (thin film), ν_{max} (cm^{-1}): 3357, 3070, 2951, 2928, 1746, 1703, 1669, 1613, 1582, 1520, 1452, 1376, 1245, 1219, 1103, 1080, 1050; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.78 (s, 1H), 8.36 (s, 1H), 8.01 (br d, J = 7.7 Hz, 2H), 7.90–7.86 (m, 1H), 7.77 (dt, J = 7.9, 1.5 Hz, 1H), 7.74–7.69 (m, 2H), 7.63–7.58 (m, 1H), 7.57–7.52 (m, 3H), 7.52–7.48 (m, 2H), 7.39–7.33 (m, 3H), 7.28–7.24 (m, 2H), 6.92 (br s, 1H), 6.82 (br s, 1H), 6.17 (br d, J = 5.8 Hz, 1H), 6.10 (br t, J = 5.8 Hz, 1H),

5.71–5.65 (m, 1H), 5.51 (br d, J = 8.3 Hz, 1H), 4.56–4.50 (m, 1H), 4.49–4.41 (m, 1H), 4.41–4.32 (m, 2H), 4.18 (br t, J = 7.0 Hz, 1H), 3.75 (s, 3H), 2.89–2.80 (m, 1H), 2.16 (s, 3H), 2.19–2.12 (m, 1H), 2.06 (s, 3H), 2.12–2.02 (m, 2H), 1.99–1.89 (m, 1H), 1.74–1.64 (m, 1H), 1.64–1.54 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.8, 170.3, 170.2, 169.8, 165.6, 156.2, 152.0, 151.8, 149.4, 143.9, 143.7, 143.3, 141.4, 135.2, 133.5, 132.6, 131.1, 129.0, 128.9, 128.4, 127.9, 127.9, 127.4, 127.2, 127.2, 125.1, 123.9, 123.3, 120.1, 92.0, 87.1, 82.9, 81.7, 73.8, 72.9, 67.2, 53.6, 52.8, 47.2, 38.6, 31.1, 30.5, 28.8, 20.8, 20.5; HRMS (ESI+): calcd. for $[C_{51}H_{47}N_7O_{11}Na]^+$ 956.3226, meas. 956.3214, Δ 1.2 ppm.

(*S*)-3-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-5-(triisopropylsilyl)pent-4-yn-1-ol (S52). To a solution of **5** (5.50 g, 12.9 mmol) in MeOH (100 mL) at –78 °C was added $NaBH_4$ (588 mg, 15.5 mmol, 1.2 equiv.) portionwise. The reaction mixture was allowed to warm to 0 °C over 1 h, then was warmed to RT and stirred for 1 h. Volatiles were removed *in vacuo*. The residue was partitioned between CH_2Cl_2 (30 mL) and a NH_4Cl sat. aq. solution (25 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were dried over $MgSO_4$ and filtered over a silica plug to yield **S52** as a yellow oil (5.29 g, 12.4 mmol, 95%) which was of suitable purity to be used in the next step without further purification. While the protocol reported here was performed on purified starting material, it was found that direct treatment of the rhodium-catalyzed conjugate alkynylation reaction mixture (**5**) with $NaBH_4$ at 0 °C reliably generated alcohol **S52** in > 90% yield

over two steps (alkynylation/reduction). R_f = 0.28 (hexanes/EtOAc, 67:33); FTIR (thin-film), ν_{max} (cm^{-1}): 3424, 2936, 2891, 2864, 2163, 1463, 1377, 1372, 1347, 1272, 1242, 1211, 1193, 1161, 1104, 1092, 1057, 1019; ^1H NMR (600 MHz, $CDCl_3$): δ 4.95 (s, 1H), 4.63 (dd, J = 6.0, 1.2 Hz, 1H), 4.59 (d, J = 5.9 Hz, 1H), 4.48 (ddd, J = 10.3, 4.6, 1.2 Hz, 1H), 3.88–3.80 (m, 2H), 3.33 (s, 3H), 2.83 (dddd, J = 10.9, 9.3, 5.2, 4.0 Hz, 1H), 1.80–1.62 (m, 5H), 1.47 (s, 3H), 1.31 (s, 3H), 1.10–1.00 (m, 21H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 112.5, 110.6, 109.7, 85.6, 84.5, 83.3, 61.2, 55.2, 41.2, 38.5, 27.4, 26.7, 25.4, 18.8, 11.3; HRMS (ESI+): calcd. for $[C_{23}H_{43}O_5Si_1]^+$ 427.2874, meas. 427.2870, Δ 0.9 ppm.

(*S*)-3-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)pent-4-yn-1-ol (S53). To a solution of TIPS-alkyne **S52** (3.81 g, 8.92 mmol) in THF (15 mL) was added TBAF (1.0 M in THF, 15.0 mL, 15.0 mmol, 1.7 equiv.) at RT. The reaction mixture was stirred for 3 h and the volatiles were removed *in vacuo*. The residue was partitioned between Et_2O (40 mL) and a NH_4Cl sat. aq. solution (40 mL). The layers were separated and the aqueous phase was extracted with Et_2O (4 × 40 mL). The combined organic extracts were washed with brine (120 mL), dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 100%) to afford alkyne **S53** (2.22 g, 8.21 mmol, 92%) as a colorless oil. The pure material crystallized upon storage at –20 °C over three weeks. The crystals were of suitable quality to obtain X-ray crystallographic data which is reported in the *Supporting Information*. R_f = 0.31 (cyclohexane/EtOAc, 50:50); FTIR (thin-film), ν_{max} (cm^{-1}): 3446,

3281, 2988, 2937, 2836, 1142, 1382, 1374, 1274, 1241, 1210, 1161, 1090, 1056; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.94 (s, 1H), 4.60 (d, J = 6.0 Hz, 1H), 4.58 (dd, J = 6.0, 0.8 Hz, 1H), 4.50 (ddd, J = 11.2, 4.0, 0.8 Hz, 1H), 3.87–3.76 (m, 2H), 3.33 (s, 3H), 2.86–2.76 (m, 1H), 2.15 (d, J = 2.4 Hz, 1H), 1.79–1.66 (m, 3H), 1.62 (ddd, J = 13.3, 11.1, 4.0 Hz, 1H), 1.48 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 112.4, 110.1, 85.9, 85.7, 85.1, 84.5, 71.2, 60.8, 55.4, 40.5, 38.0, 26.6, 25.8, 25.1; HRMS (ESI+): calcd. for $[C_{14}H_{23}O_5]^+$ 271.1540, meas. 271.1547, Δ 2.6 ppm.

***N*-((*S*)-3-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)pent-4-yn-1-yl)-2-nitrobenzenesulfonamide (S54)**. To a solution of alcohol **S53** (550 mg, 2.04 mmol), DMEAD[76] (1.19 g, 5.09 mmol, 2.5 equiv.) and PPh_3 (1.39 g, 5.29 mmol, 2.6 equiv.) in a 4:1 mixture of toluene and THF (110 mL) stirred at 0 °C over activated 4 Å M.S. (1.65 g), was added a solution of 2-$NsNH_2$ (1.44 g, 7.12 mmol, 3.5 equiv.) in THF (22 mL, prepared over 1.65 g of activated 4 Å M.S.) over 5 min. The reaction mixture was allowed to warm gradually to RT and was stirred for 72 h. The media was filtered and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 100%) to afford **S54** (496 mg, 1.09 mmol, 54%) as a white amorphous solid and unreacted **S53** (81 mg, 0.30 mmol, 15%). R_f = 0.27 (hexanes/EtOAc, 50:50); FTIR (thin-film), ν_{max} (cm^{-1}): 3289, 2989, 2936, 2836, 1540, 1442, 1415, 1363, 1343, 1300, 1274, 1241, 1210, 1192, 1162, 1087, 1056; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.16–8.11 (m, 1H), 7.89–7.83 (m, 1H), 7.78–7.70 (m, 2H), 5.50 (t, J = 6.1 Hz,

1H), 4.93 (s, 1H), 4.59 (d, *J* = 5.9 Hz, 1H), 4.54 (dd, *J* = 6.0, 0.9 Hz, 1H), 4.43 (ddd, *J* = 11.1, 3.9, 0.9 Hz, 1H), 3.32 (s, 3H), 3.30–3.26 (m, 2H), 2.71 (dddd, *J* = 14.6, 8.9, 4.5, 2.9 Hz, 1H), 2.13 (d, *J* = 2.4 Hz, 1H), 1.77 (dtd, *J* = 13.2, 7.5, 4.8 Hz, 1H), 1.71–1.63 (m, 2H), 1.58 (ddd, *J* = 13.3, 11.0, 4.0 Hz, 1H), 1.47 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 148.1, 133.7, 133.6, 132.9, 131.1, 125.5, 112.4, 110.1, 85.5, 84.8, 84.7, 84.4, 71.9, 55.4, 41.9, 40.3, 35.2, 26.5, 26.5, 25.0; HRMS (ESI+): calcd. for $[C_{20}H_{26}N_2O_8S_1Na]^+$ 477.1302, meas. 477.1287, Δ 3.2 ppm.

(3*R*,4*R*,5*R*)-5-((*S*)-2-(2-((4-nitrophenyl)sulfonamido)ethyl)but-3-yn-1-yl)tetrahydrofuran-2,3,4-triyl triacetate (S55). To a solution of acetonide **S54** (210 mg, 0.462 mmol) in CH_2Cl_2(0.80 mL) at 0 °C, was added a 4:1 mixture of TFA and water (4.0 mL). The resulting mixture was stirred at RT for 15 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with benzene (2 × 1.0 mL). Crude material was used directly in the next step without further purification. To a solution of the crude triol in CH_2Cl_2(4.8 mL) at RT, were added pyridine (0.37 mL, 4.6 mmol, 10 equiv.), Ac_2O (0.44 mL, 4.6 mmol, 10 equiv.) and DMAP (11 mg, 92 μmol, 20 mol %) sequentially. The resulting mixture was stirred at RT for 1 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 15 → 75%) to afford **S55** (70 mg, 0.13 mmol, 29% over 2 steps) as a 1.8:1 mixture of diastereomers, as a colorless oil. R_f = 0.31/0.40 (hexanes/EtOAc, 40:60); FTIR (thin film), ν_{max} (cm^{-1}): 3288, 2925, 2853, 1747, 1541, 1424, 1368, 1222, 1167, 1124, 1082, 1058, 1012; ^{1}H

NMR (600 MHz, $CDCl_3$): δ 8.16–8.11 (m, 2.8H), 7.89–7.84 (m, 2.8H), 7.79–7.71 (m, 5.6H), 6.32 (d, J = 4.6 Hz, 1.0H), 6.11 (d, J = 1.0 Hz, 1.8H), 5.55 (app t, J = 6.1 Hz, 1.0H), 5.52 (app t, J = 6.1 Hz, 1.8H), 5.29 (dd, J = 4.9, 1.0 Hz, 1.8H), 5.19 (dd, J = 6.7, 4.6 Hz, 1.0H), 5.16 (dd, J = 6.9, 4.9 Hz, 1.8H), 5.02 (dd, J = 6.7, 3.7 Hz, 1.0H), 4.40 (ddd, J = 10.3, 3.7, 3.3 Hz, 1.0H), 4.34 (ddd, J = 10.2, 6.9, 3.1 Hz, 1.8H), 3.33–3.22 (m, 5.6H), 2.70–2.61 (m, 2.8H), 2.14 (d, J = 2.4 Hz, 2.8H), 2.12 (s, 5.4H), 2.11 (s, 3.0H), 2.10 (s, 3.0H), 2.08 (s, 5.4H), 2.06 (s, 5.4H), 2.06 (s, 3.0H), 1.86–1.73 (m, 5.6H), 1.71–1.60 (m, 4.6H), 1.56 (ddd, J = 14.0, 10.3, 4.2 Hz, 1.0H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2, 170.0, 169.8, 169.6, 169.5, 169.2, 148.2, 133.8, 133.7, 133.7, 132.9, 132.9, 131.2, 131.2, 125.6, 125.6, 98.4, 93.8, 84.5, 84.3, 81.2, 79.6, 74.6, 73.8, 72.5, 72.1, 72.0, 69.9, 41.9, 40.1, 39.0, 35.2, 35.0, 26.6, 26.2, 21.2, 21.2, 20.8, 20.7, 20.7, 20.4; HRMS (ESI+): calcd. for $[C_{22}H_{26}N_2O_{11}S_1Na]^+$ 549.1150, meas. 549.1138, Δ 2.1 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((*S*)-2-(2-((4-nitrophenyl)sulfonamido)ethyl)but-3-yn-1-yl)tetrahydrofuran-3,4-diyl diacetate (S56). To a suspension of N^6-benzoyladenine (93 mg, 0.39 mmol, 2.15 equiv.) in propionitrile (dried over 4 Å M.S., 4.5 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (121 μL, 0.497 mmol, 2.75 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S55** (dried by azeotropic distillation with benzene (4 ×), 95 mg, 0.18 mmol) and

2,6-di-*tert*-butyl-4-methylpyridinium triflate (13 mg, 36 μmol, 20 mol %) was added propionitrile (4.5 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 30 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (PhMe/MeCN, 0 → 50%) and lyophilized to afford nucleoside **S56** (120 mg, 0.170 mmol, 94%) as a white fluffy solid. R_f = 0.36 (EtOAc); FTIR (thin-film), ν_{max} (cm^{-1}): 3295, 3097, 2945, 1746, 1699, 1610, 1582, 1540, 1509, 1485, 1455, 1423, 1365, 1338, 1298, 1240, 1218, 1164, 1074, 1047; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.09 (s, 1H), 8.76 (s, 1H), 8.10 (dd, *J* = 7.7, 1.6 Hz, 1H), 8.07 (s, 1H), 8.02 (dt, *J* = 7.1, 1.4 Hz, 2H), 7.80 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.72 (td, *J* = 7.6, 1.6 Hz, 1H), 7.68 (td, *J* = 7.6, 1.6 Hz, 1H), 7.63–7.59 (m, 1H), 7.55–7.49 (m, 2H), 6.10 (d, *J* = 4.7 Hz, 2H), 5.55 (q, *J* = 6.2, 5.4 Hz, 2H), 4.46 (ddd, *J* = 11.0, 4.5, 2.8 Hz, 1H), 3.25 (dt, *J* = 7.5, 6.2 Hz, 2H), 2.62 (dddq, *J* = 11.2, 9.0, 4.6, 2.5 Hz, 1H), 2.17 (d, *J* = 2.4 Hz, 1H), 2.16 (s, 3H), 2.07–2.12 (m, 1H), 2.07 (s, 3H), 1.85 (ddd, *J* = 13.8, 11.2, 2.9 Hz, 1H), 1.82–1.73 (m, 2H), 1.69 (ddt, *J* = 13.5, 9.9, 6.3 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.9, 169.6, 164.8, 152.9, 151.6, 150.0, 148.1, 142.5, 133.8, 133.7, 133.6, 133.0, 133.0, 131.2, 129.0, 128.1, 125.5, 124.1, 87.2, 84.4, 80.8, 77.4, 73.7, 72.9, 72.2, 41.9, 38.3, 35.1, 26.1, 20.8, 20.6; HRMS (ESI+): calcd. for $[C_{32}H_{32}N_7O_{10}S_1]^+$ 706.1926, meas. 706.1922, Δ 0.5 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((*S*)-4-(3-carbamoylphenyl)-2-(2-((4-nitrophenyl)sulfonamido)ethyl)but-3-yn-1-yl)tetrahydrofuran-3,4-diyl diacetate

(S57). To a vial charged with **S56** (54 mg, 77 μmol) were added 3-iodobenzamide (29 mg, 0.12 mmol, 1.5 equiv.), CuI (3 mg, 0.02 mmol, 20 mol %) and $Pd(PPh_3)_4$ (4 mg, 4 μmol, 5 mol %). The vial headspace was purged with nitrogen for 10 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 3.0 mL) was added at RT. The resulting mixture was stirred at 70 °C for 4 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 15%) to afford benzamide **S57** (50 mg, 60 μmol, 78%) as a white amorphous solid. R_f = 0.16 (EtOAc/*i*-PrOH, 91:9); FTIR (thin-film), ν_{max} (cm^{-1}): 2926, 1746, 1667, 1610, 1579, 1540, 1511, 1484, 1455, 1366, 1339, 1298, 1241, 1215, 1163, 1073, 1047; ^1H NMR (500 MHz, $CDCl_3$): δ 9.13 (s, 1H), 8.76 (s, 1H), 8.11 (s, 1H), 8.10–8.07 (m, 1H), 8.05–8.00 (m, 2H), 7.87–7.86 (m, 1H), 7.80 (ddd, *J* = 7.8, 1.9, 1.2 Hz, 1H), 7.79–7.76 (m, 1H), 7.71–7.64 (m, 2H), 7.63–7.58 (m, 1H), 7.55–7.47 (m, 3H), 7.38 (td, *J* = 7.7, 0.6 Hz, 1H), 6.56 (br s, 1H), 6.17 (t, *J* = 5.5 Hz, 1H), 6.13 (d, *J* = 5.8 Hz, 1H), 5.84 (br s, 1H), 5.79 (t, *J* = 6.1 Hz, 1H), 5.64 (dd, *J* = 5.3, 4.0 Hz, 1H), 4.50 (dt, *J* = 10.3, 3.7 Hz, 1H), 3.38–3.29 (m, 1H), 3.32–3.23 (m, 1H), 2.87 (tt, *J* = 10.2, 4.2 Hz, 1H), 2.23–2.10 (m, 1H), 2.16 (s, 3H), 2.06 (s, 3H), 1.98 (ddd, *J* = 13.5, 11.1, 3.6 Hz, 1H), 1.92–1.73 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.1, 169.7, 168.6, 164.9, 152.8, 151.7, 150.0, 148.1, 142.6, 134.7, 133.8, 133.6, 133.6, 133.5, 133.0, 133.0, 131.2, 130.9, 129.0, 128.8, 128.1, 127.5, 125.5, 124.3, 123.4, 91.1, 87.1, 83.5, 81.2, 77.4, 77.2, 76.9, 73.8, 72.7,

42.1, 38.5, 35.3, 27.0, 20.8, 20.6; HRMS (ESI+): calcd. for $[C_{39}H_{37}N_8O_{11}S_1]^+$ 825.2297, meas. 825.2300, Δ 0.4 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((*S*)-4-(3-carbamoylphenyl)-2-(2-ureidoethyl)but-3-yn-1-yl)tetrahydrofuran-3,4-diyl diacetate (S58). To a solution of **S57** (112 mg, 0.136 mmol) in a 2:1 mixture of MeCN and DMF (10.5 mL) at 0 °C, were added thiophenol (56 μL, 0.54 mmol, 4.0 equiv.) and Cs_2CO_3 (354 mg, 1.09 mmol, 8.0 equiv.) sequentially. The resulting mixture was stirred at RT for 1 h and was filtered twice through a nylon syringe filter (13 mm, 0.22 μm). Volatiles were removed *in vacuo*. The crude amine was used directly in the next step without further purification. To a solution of the crude amine in a 1:1 mixture of CH_2Cl_2 and *i*-PrOH (13.5 mL) at RT, was added (trimethylsilyl)isocyanate (1.29 mL, 9.51 mmol, 70 equiv.). The resulting mixture was stirred at RT for 17 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (5% → 95% B over 40 min) to afford **S58** (24 mg, 35 μmol, 26% over two steps) as a white fluffy solid. ^{1}H NMR (600 MHz, CD_3OD): δ 8.78 (s, 1H), 8.58 (s, 1H), 8.13–8.08 (m, 2H), 7.94 (t, *J* = 1.8 Hz, 1H), 7.83 (dt, *J* = 7.9, 1.6 Hz, 1H), 7.71–7.64 (m, 1H), 7.61–7.54 (m, 3H), 7.43 (t, *J* = 7.8 Hz, 1H), 6.35 (d, *J* = 5.3 Hz, 1H), 6.20 (t, *J* = 5.5 Hz, 1H), 5.68 (t, *J* = 5.2 Hz, 1H), 4.60 (ddd, *J* = 10.5, 4.8, 3.1 Hz, 1H), 3.40 (ddd, *J* = 13.1, 7.4, 5.3 Hz, 1H), 3.28 (dt, *J* = 13.5, 7.4 Hz, 1H), 2.91 (ddt, *J* = 11.0, 9.3, 4.5 Hz, 1H), 2.28 (ddd, *J* = 14.1, 10.5, 4.0 Hz, 1H), 2.18 (s, 3H), 2.07 (s, 3H), 2.03 (ddd, *J* = 14.0, 11.2, 3.2 Hz, 1H), 1.82 (dtd, *J* = 12.7, 7.6, 5.1 Hz, 1H), 1.74 (dddd, *J* = 13.0, 9.5,

7.3, 5.4 Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ 171.6, 171.4, 171.3, 168.2, 162.1, 153.5, 153.1, 151.4, 145.2, 135.7, 135.3, 134.9, 133.9, 131.8, 129.8, 129.7, 129.5, 128.1, 125.7, 125.2, 92.8, 88.5, 83.5, 82.1, 75.2, 74.2, 39.6, 36.9, 28.0, 20.5, 20.3; HRMS (ESI+): calcd. for $[C_{34}H_{35}N_8O_8]^+$ 683.2572, meas. 683.2598, Δ 3.8 ppm.

(*S*)-4-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-6-(triisopropylsilyl)hex-5-ynenitrile (S59). To a solution of **S52** (0.54 g, 1.3 mmol) in benzene (44 mL) at RT, were added acetone cyanohydrin (0.17 mL, 1.9 mmol, 1.5 equiv.), P*n*-Bu_3 (0.47 mL, 1.9 mmol, 1.5 equiv.) and a solution of TMAD (0.33 g, 1.9 mmol, 1.5 equiv.) in benzene (20 mL). The resulting mixture, which quickly became thick and cloudy, was stirred at RT for 2 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 2 → 40%) to afford **S59** (0.49 g, 1.1 mmol, 89%) as a colorless oil. R_f = 0.44 (hexanes/EtOAc, 70:30); FTIR (thin film), ν_{max} (cm^{-1}): 2941, 2865, 2247, 2166, 1463, 1381, 1371, 1241, 1210, 1192, 1160, 1092, 1057, 1018; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.94 (s, 1H), 4.61 (dd, *J* = 6.0, 1.0 Hz, 1H), 4.58 (d, *J* = 6.0 Hz, 1H), 4.45 (ddd, *J* = 10.6, 4.5, 1.0 Hz, 1H), 3.34 (s, 3H), 2.81 (dddd, *J* = 10.6, 10.2, 4.6, 4.2 Hz, 1H), 2.59 (ddd, *J* = 16.9, 8.0, 5.6 Hz, 1H), 2.54 (ddd, *J* = 16.9, 8.0, 7.8 Hz, 1H), 1.86 (app dtd, *J* = 13.2, 8.0, 4.6 Hz, 1H), 1.74 (dddd, *J* = 13.2, 10.2, 7.8, 5.6 Hz, 1H), 1.69 (ddd, *J* = 13.2, 10.6, 4.2 Hz, 1H), 1.64 (ddd, *J* = 13.2, 10.6, 4.5 Hz, 1H), 1.46 (s, 3H), 1.30 (s, 3H), 1.07–1.03 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 119.4, 112.6, 109.9,

108.0, 85.5, 85.3, 84.8, 84.4, 55.4, 40.8, 31.6, 29.8, 26.7, 25.4, 18.8, 15.4, 11.3; HRMS (ESI+): calcd. for $[C_{24}H_{41}N_1O_4Si_1Na]^+$ 458.2697, meas. 458.2691, Δ 1.2 ppm.

Methyl (*S*)-6-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)-8-(triisopropylsilyl)oct-2-en-7-ynoate (S61). To a solution of nitrile **S59** (0.49 g, 1.1 mmol) in PhMe (22 mL) at –78 °C, was added DIBALH (1.0 M in hexanes, 1.68 mL, 1.68 mmol, 1.5 equiv.) dropwise. The resulting mixture was stirred at –78 °C for 1.3 h. The reaction was quenched by the addition of a potassium sodium tartrate sat. aq. solution (22 mL), followed by the addition of Et_2O (15 mL) and brine (15 mL). The biphasic mixture was stirred vigorously at RT for 45 min. The layers were separated and the aqueous phase was extracted with Et_2O (3 × 25 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. Crude material was filtered through a short pad of silica gel (hexanes/EtOAc, 75:25) to afford crude aldehyde **S60** which was used directly in the next step without further purification. To a suspension of NaH (95%, 33 mg, 1.3 mmol, 1.5 equiv.) in THF (1.7 mL) at 0 °C, was added a solution of phosphonate **S2** (0.39 g, 1.3 mmol, 1.5 equiv.) in THF (2.0 mL) dropwise. The resulting cloudy mixture was stirred at 0 °C for 30 min and a solution of aldehyde **S60** (0.38 g, 0.88 mmol) in THF (3.7 mL) was added in one portion. The yellow reaction mixture was stirred at 45 °C for 20 h and cooled to 0 °C, before the addition of an aqueous pH 7 buffer (5 mL) and Et_2O (5 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 10 mL). The combined organic extracts were dried over $MgSO_4$,

filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 1 → 40%) to afford olefin **S61** (0.44 g, 0.72 mmol, 82%) as a 1:1 mixture of *Z* and *E* isomers, as a colorless oil. R_f = 0.17/0.31 (hexanes/EtOAc, 80:20); FTIR (thin film), ν_{max} (cm^{-1}): 3305, 2942, 2865, 2164, 1729, 1661, 1541, 1463, 1439, 1382, 1372, 1209, 1159, 1104, 1092, 1060; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (s, 1H), 7.60 (s, 1H), 7.37 (app t, *J* = 7.6 Hz, 1H), 6.86 (app t, *J* = 7.3 Hz, 1H), 4.94 (s, 1H), 4.94 (s, 1H), 4.63 (dd, *J* = 6.0, 1.2 Hz, 1H), 4.62 (dd, *J* = 6.0, 1.2 Hz, 1H), 4.58 (d, *J* = 6.0 Hz, 1H), 4.58 (d, *J* = 6.0 Hz, 1H), 4.49–4.43 (m, 2H), 3.87 (s, 3H), 3.80 (s, 3H), 3.32 (s, 3H), 3.32 (s, 3H), 2.89–2.75 (m, 2H), 2.74–2.61 (m, 2H), 2.45–2.36 (m, 1H), 2.36–2.26 (m, 1H), 1.74–1.57 (m, 8H), 1.46 (s, 6H), 1.31 (s, 6H), 1.08–1.00 (m, 42H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 163.9, 163.8, 155.1 (q, $^2J_{C\text{-}F}$ = 38 Hz), 155.0 (q, $^2J_{C\text{-}F}$ = 38 Hz), 140.7, 135.5, 123.6, 122.9, 115.8 (q, $^1J_{C\text{-}F}$ = 288 Hz), 115.6 (q, $^1J_{C\text{-}F}$ = 288 Hz), 112.5, 112.5, 110.1, 109.8, 109.8, 109.8, 85.7, 85.6, 85.6, 84.5, 84.5, 83.4, 83.2, 55.2, 55.2, 53.1, 53.0, 41.2, 41.1, 35.6, 34.1, 30.3, 30.2, 27.2, 26.7, 25.4, 25.4, 18.8, 18.8, 11.4, 11.3; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.4, –77.2; HRMS (ESI+): calcd. for $[C_{29}H_{46}F_3N_1O_7Si_1Na]^+$ 628.2888, meas. 628.2894, Δ 1.0 ppm.

Methyl (2*S*,6*S*)-6-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)-8-(triisopropylsilyl)oct-7-ynoate (S62). In a glove box, $[Rh(nbd)_2]BF_4$ (47 mg, 0.13 mmol, 20 mol %) and (*S*,*S*)-MeBPE (36 mg, 0.14 mmol, 22 mol %) were placed in two separate vials, capped with a septum and placed under an atmosphere of nitrogen out of the glovebox. MeOH (3 mL)

was added to the vial containing (*S*,*S*)-MeBPE and the resulting colorless solution was transferred to the vial charged with $[Rh(nbd)_2]BF_4$. More MeOH (2 mL) was used for rinsing. The red solution of (*S*,*S*)-MeBPE-Rh was added to a solution of olefin **S61** (0.38 g, 0.63 mmol) in MeOH (16 mL). The flask headspace was evacuated and refilled with hydrogen (3 ×), and the reaction mixture was stirred vigorously at RT under an atmosphere of hydrogen (balloon) for 2 h. Volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 1 → 60%) to afford protected amino acid **S62** (0.35 g, 0.57 mmol, 90%) as a colorless oil. R_f = 0.40 (hexanes/EtOAc, 70:30); FTIR (thin film), ν_{max} (cm^{-1}): 3317, 2941, 2865, 2165, 1749, 1724, 1549, 1462, 1382, 1208, 1160, 1105, 1094, 1060; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.84 (d, J = 7.8 Hz, 1H), 4.94 (s, 1H), 4.65–4.60 (m, 2H), 4.58 (d, J = 5.9 Hz, 1H), 4.44 (ddd, J = 10.1, 4.7, 1.1 Hz, 1H), 3.79 (s, 3H), 3.31 (s, 3H), 2.65–2.58 (m, 1H), 1.98–1.89 (m, 1H), 1.88–1.78 (m, 1H), 1.69–1.54 (m, 3H), 1.51–1.42 (m, 3H), 1.46 (s, 3H), 1.31 (s, 3H), 1.07–1.01 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4, 156.9 (q, $^2J_{C\text{-}F}$ = 38 Hz), 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 112.5, 110.3, 109.8, 85.7, 85.6, 84.5, 82.9, 55.2, 53.1, 52.7, 41.2, 35.3, 31.9, 30.1, 26.7, 25.4, 22.7, 18.8, 11.4; ^{19}F NMR (376 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{29}H_{48}F_3N_1O_7Si_1Na]^+$ 630.3044, meas. 630.3046, Δ 0.3 ppm.

Methyl (2*S*,6*S*)-6-(((3a*R*,4*R*,6*R*,6a*R*)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)oct-7-ynoate (S63). To a solution of TIPS alkyne **S62** (381 mg, 0.627 mmol) in DMF (3.2 mL) at RT, was added a

solution of TASF (518 mg, 1.88 mmol, 3.0 equiv.) in DMF (3.2 mL). The resulting mixture was stirred at 60 °C for 2.5 h and volatiles were removed *in vacuo*. The residue was partitioned between Et_2O (15 mL) and a 1:1 mixture of brine and 5% LiCl aq. solution (15 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 2 → 50%) to afford alkyne **S63** (250 mg, 0.554 mmol, 88%) as a colorless oil. R_f = 0.48 (hexanes/EtOAc, 60:40); FTIR (thin film), ν_{max} (cm^{-1}): 3309, 2937, 2867, 1747, 1717, 1550, 1440, 1374, 1208, 1158, 1089, 1058; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.90 (d, J = 7.5 Hz, 1H), 4.93 (s, 1H), 4.63 (ddd, J = 7.5, 7.1, 5.6 Hz, 1H), 4.59 (d, J = 6.0 Hz, 1H), 4.56 (dd, J = 6.0, 0.9 Hz, 1H), 4.46 (ddd, J = 11.0, 4.1, 0.9 Hz, 1H), 3.79 (s, 3H), 3.31 (s, 3H), 2.63–2.55 (m, 1H), 2.10 (d, J = 2.4 Hz, 1H), 1.97–1.89 (m, 1H), 1.80 (dddd, J = 13.8, 10.4, 7.1, 4.6 Hz, 1H), 1.66 (ddd, J = 13.4, 11.0, 4.1 Hz, 1H), 1.60–1.52 (m, 2H), 1.47 (s, 3H), 1.51–1.39 (m, 3H), 1.30 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4, 156.9 (q, $^2J_{C\text{-}F}$ = 38 Hz), 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 112.4, 110.1, 85.6, 85.6, 85.2, 84.5, 71.1, 55.3, 53.1, 52.6, 40.5, 34.4, 31.6, 28.5, 26.6, 25.1, 22.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{20}H_{28}F_3N_1O_7Na]^+$ 474.1710, meas. 474.1721, Δ 2.3 ppm.

(3*R*,4*R*,5*R*)-5-((2*S*,6*S*)-2-ethynyl-7-methoxy-7-oxo-6-(2,2,2-trifluoroacetamido)heptyl)tetrahydrofuran-2,3,4-triyl triacetate (S64). To a solution of acetonide **S63** (125 mg, 0.277 mmol) in CH_2Cl_2 (1.1 mL) at 0 °C, was added a 4:1 mixture of TFA and water (5.5

mL). The resulting mixture was stirred at RT for 5.5 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with benzene (2 × 1.5 mL). Crude material was used directly in the next step without further purification. To a solution of the crude triol in CH_2Cl_2 (5.5 mL) at RT, were added pyridine (0.22 mL, 2.8 mmol, 10 equiv.), Ac_2O (0.26 mL, 2.8 mmol, 10 equiv.) and DMAP (24 mg, 0.19 mmol, 0.70 equiv.) sequentially. The resulting mixture was stirred at RT for 18 h and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 5 → 70%) to afford triacetate **S64** (93 mg, 0.18 mmol, 64% over 2 steps) as a 2:1 mixture of diastereomers, as a colorless oil. R_f = 0.23/0.29 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3293, 2954, 2867, 1747, 1723, 1553, 1438, 1372, 1216, 1180, 1162, 1113, 1051, 1012; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.96–6.89 (m, 3H), 6.35 (d, J = 4.6 Hz, 1H), 6.12 (d, J = 1.0 Hz, 2H), 5.30 (dd, J = 4.8, 1.0 Hz, 2H), 5.22 (dd, J = 6.8, 4.6 Hz, 1H), 5.17 (dd, J = 7.1, 4.8 Hz, 2H), 5.05 (dd, J = 6.8, 3.8 Hz, 1H), 4.65–4.60 (m, 3H), 4.45 (ddd, J = 10.3, 3.8, 3.3 Hz, 1H), 4.38 (ddd, J = 10.2, 7.1, 3.0 Hz, 2H), 3.79 (s, 6H), 3.79 (s, 3H), 2.63–2.55 (m, 3H), 2.12 (s, 6H), 2.11 (s, 3H), 2.10 (s, 3H), 2.09 (d, J = 2.4 Hz, 2H), 2.09 (d, J = 2.4 Hz, 1H), 2.07 (s, 6H), 2.07 (s, 6H), 2.05 (s, 3H), 1.99–1.89 (m, 3H), 1.85–1.73 (m, 6H), 1.66–1.53 (m, 6H), 1.53–1.37 (m, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4, 170.2, 170.1, 169.9, 169.7, 169.5, 169.3, 156.9 (q, $^2J_{C\text{-}F}$ = 38 Hz), 156.9 (q, $^2J_{C\text{-}F}$ = 38 Hz), 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 98.5, 93.8, 85.3, 85.1, 81.4, 79.8, 74.6, 73.9, 72.6, 71.2, 71.1, 69.9, 53.1, 52.6, 40.3, 39.3, 34.4, 34.4, 31.6, 31.6, 28.5, 28.1, 22.5, 22.4, 21.2, 21.2, 20.8, 20.7,

20.7, 20.4; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{22}H_{28}F_3N_1O_{10}Na]^+$ 546.1558, meas. 546.1565, Δ 1.3 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,6*S*)-2-ethynyl-7-methoxy-7-oxo-6-(2,2,2-trifluoroacetamido)heptyl)tetrahydrofuran-3,4-diyl diacetate (S65). To a suspension of N^6-benzoyladenine (302 mg, 1.26 mmol, 4.0 equiv.) in propionitrile (dried over 4 Å M.S., 6.0 mL) at RT, was added *N*,*O*-bis(trimethylsilyl)acetamide (0.39 mL, 1.6 mmol, 5.0 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of N^6-benzoyladenine was observed. To a separate flask charged with triacetate **S64** (dried by azeotropic distillation with benzene (4 ×), 165 mg, 0.315 mmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (56 mg, 0.16 mmol, 50 mol %) was added propionitrile (6.0 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N^6-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 5.5 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 30 → 100%) to afford nucleoside **S65** (209 mg, 0.297 mmol, 94%) as a colorless oil. R_f = 0.43 (hexanes/EtOAc, 20:80); FTIR (thin film), ν_{max} (cm^{-1}): 3287, 2955, 2925, 2868, 1748, 1721, 1612, 1583, 1511, 1488, 1457, 1245, 1219, 1187, 1161, 1049; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.13 (br s, 1H), 8.76 (s, 1H), 8.07 (s, 1H), 8.04–7.98 (m, 2H), 7.61–7.57 (m, 1H), 7.53–7.48 (m, 2H), 7.05 (br d, *J* = 7.9 Hz, 1H), 6.12–6.10 (m, 2H), 5.57–5.54 (m, 1H), 4.60 (ddd, *J* = 7.9, 7.4, 5.4 Hz, 1H), 4.50 (ddd, *J* = 11.0, 4.5, 2.8 Hz, 1H),

3.75 (s, 3H), 2.55–2.48 (m, 1H), 2.15 (s, 3H), 2.12 (d, J = 2.4 Hz, 1H), 2.10–2.03 (m, 1H), 2.06 (s, 3H), 1.89 (app ddt, J = 13.9, 10.8, 5.4 Hz, 1H), 1.83 (ddd, J = 13.8, 11.4, 2.8 Hz, 1H), 1.74 (dddd, J = 13.9, 10.7, 7.4, 4.8 Hz, 1H), 1.61–1.52 (m, 1H), 1.52–1.42 (m, 2H), 1.42–1.33 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4, 169.8, 169.6, 164.8, 156.9 (q, $^{2}J_{C-F}$ = 38 Hz), 152.8, 151.7, 149.9, 142.4, 133.6, 133.0, 129.0, 128.0, 124.2, 115.7 (q, $^{1}J_{C-F}$ = 288 Hz), 87.2, 85.1, 80.9, 73.7, 72.9, 71.4, 53.0, 52.5, 38.6, 34.4, 31.6, 28.1, 22.6, 20.7, 20.5; ^{19}F NMR (376 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{32}H_{34}F_3N_6O_9]^+$ 703.2334, meas. 703.2326, Δ 1.1 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,6*S*)-2-((3-carbamoylphenyl)ethynyl)-7-methoxy-7-oxo-6-(2,2,2-trifluoroacetamido)heptyl)tetrahydrofuran-3,4-diyl diacetate (S66). To a vial charged with nucleoside **S65** (89 mg, 0.13 mmol) were added 3-iodobenzamide (78 mg, 0.32 mmol, 2.5 equiv.), CuI (6 mg, 0.03 mmol, 25 mol %) and $Pd(PPh_3)_4$ (7 mg, 6 μmol, 5 mol %). The vial headspace was purged with nitrogen for 10 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 4.2 mL) was added at RT. The resulting mixture was stirred at 60 ◦C for 2 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 95:5) to afford benzamide **S66**, which was further purified by preparative HPLC (15% → 95% B' over 30 min) to afford benzamide **S66** (69 mg, 84 μmol, 66%) as a white amorphous solid. R_f = 0.51 (EtOAc/*i*-PrOH, 95:5); FTIR (thin film), ν_{max} (cm^{-1}): 3279, 3071, 2928,

1748, 1719, 1667, 1612, 1580, 1512, 1487, 1457, 1438, 1377, 1245, 1219, 1159, 1097; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.79 (s, 1H), 8.46 (s, 1H), 8.07–8.01 (m, 2H), 7.87 (app t, J = 1.8 Hz, 1H), 7.80 (ddd, J = 7.8, 1.8, 1.3 Hz, 1H), 7.64–7.60 (m, 1H), 7.55–7.49 (m, 3H), 7.39 (app t, J = 7.8 Hz, 1H), 7.18 (br d, J = 7.8 Hz, 1H), 7.12 (br s, 2H), 6.20 (d, J = 5.8 Hz, 1H), 6.12 (dd, J = 5.8, 5.4 Hz, 1H), 5.65 (dd, J = 5.4, 4.0 Hz, 1H), 4.63 (app td, J = 7.8, 5.1 Hz, 1H), 4.54 (app dt, J = 9.6, 4.0 Hz, 1H), 3.74 (s, 3H), 2.80–2.72 (m, 1H), 2.17 (s, 3H), 2.13 (ddd, J = 13.8, 9.6, 3.6 Hz, 1H), 2.06 (s, 3H), 2.05–1.92 (m, 2H), 1.85–1.76 (m, 1H), 1.71–1.45 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.5, 170.7, 170.2, 169.9, 165.9, 157.2 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 152.0, 151.2, 149.2, 143.5, 135.3, 133.7, 132.3, 132.2, 130.9, 129.1, 128.9, 128.5, 127.5, 123.9, 122.8, 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 92.0, 87.4, 82.7, 81.8, 73.8, 72.9, 53.1, 52.7, 38.8, 34.6, 31.8, 29.0, 23.1, 20.8, 20.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.8; HRMS (ESI+): calcd. for $[C_{39}H_{38}F_3N_7O_{10}Na]^+$ 844.2524, meas. 844.2532, Δ 0.8 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((4-carbamoylphenyl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S67). To a vial charged with alkyne **8** (20 mg, 29 μmol) were added 4-iodobenzamide (18 mg, 73 μmol, 2.5 equiv.), CuI (1 mg, 7 μmol, 25 mol %) and $Pd(PPh_3)_4$ (2 mg, 2 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 1.5 mL) was added at RT. The resulting mixture was stirred at 60 °C for 3 h. The

solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 95:5) to afford alkyne **S67**, which was further purified by preparative HPLC (15% → 95% B' over 30 min) to afford alkyne **S67** (19 mg, 24 μmol, 81%) as a white amorphous solid. ^{1}H NMR (500 MHz, CD_3OD): δ 8.79 (s, 1H), 8.63 (s, 1H), 8.11–8.07 (m, 2H), 7.86–7.80 (m, 2H), 7.70–7.65 (m, 1H), 7.60–7.55 (m, 2H), 7.48–7.44 (m, 2H), 6.34 (d, J = 5.0 Hz, 1H), 6.16 (dd, J = 5.7, 5.0 Hz, 1H), 5.66 (dd, J = 5.7, 5.0 Hz, 1H), 4.56 (ddd, J = 10.3, 5.0, 3.6 Hz, 1H), 4.53 (dd, J = 9.6, 4.9 Hz, 1H), 3.73 (s, 3H), 2.92–2.84 (m, 1H), 2.23 (ddd, J = 14.0, 10.3, 3.9 Hz, 1H), 2.15 (s, 3H), 2.18–2.10 (m, 1H), 2.06 (s, 3H), 2.05–2.00 (m, 2H), 1.74–1.60 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD): δ 172.5, 171.6, 171.5, 171.3, 168.7, 159.1 (q, $^{2}J_{C\text{-}F}$ = 37 Hz), 153.1, 153.0, 151.0, 145.5, 134.6, 134.2, 134.2, 132.6, 129.8, 129.6, 128.7, 128.2, 125.0, 117.4 (q, $^{1}J_{C\text{-}F}$ = 287 Hz), 94.3, 88.8, 83.9, 82.2, 75.0, 74.2, 53.8, 53.1, 39.4, 32.6, 29.9, 29.7, 20.5, 20.3; ^{19}F NMR (376 MHz, CD_3OD, BTF IStd): δ –76.5; HRMS (ESI+): calcd. for $[C_{38}H_{37}F_3N_7O_{10}]^+$ 808.2549, meas. 808.2556, Δ 0.9 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((2-carbamoylphenyl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S68). To a vial charged with alkyne **8** (20 mg, 29 μmol) were added 2-iodobenzamide (18 mg, 73 μmol, 2.5 equiv.), CuI (1 mg, 7 μmol, 25 mol %) and $Pd(PPh_3)_4$ (2 mg, 2 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for

15 min, 1.5 mL) was added at RT. The resulting mixture was stirred at 60 °C for 3 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 75:25) to afford alkyne **S68**, which was further purified by preparative HPLC (15% → 95% B' over 30 min) to afford alkyne **S68** (18 mg, 22 μmol, 77%) as a white amorphous solid. ^{1}H NMR (600 MHz, CD_3OD): δ 8.80 (s, 1H), 8.68 (s, 1H), 8.13–8.07 (m, 2H), 7.70–7.67 (m, 1H), 7.61–7.57 (m, 3H), 7.48–7.45 (m, 1H), 7.42 (app td, J = 7.5, 1.6 Hz, 1H), 7.39 (app td, J = 7.5, 1.6 Hz, 1H), 6.36 (d, J = 5.2 Hz, 1H), 6.15 (dd, J = 5.7, 5.2 Hz, 1H), 5.62 (dd, J = 5.7, 5.0 Hz, 1H), 4.62 (ddd, J = 10.4, 5.0, 3.0 Hz, 1H), 4.52 (dd, J = 9.8, 4.9 Hz, 1H), 3.73 (s, 3H), 2.92–2.86 (m, 1H), 2.22 (ddd, J = 13.7, 10.4, 4.2 Hz, 1H), 2.16 (s, 3H), 2.16–2.09 (m, 1H), 2.07 (s, 3H), 2.08–2.01 (m, 2H), 1.72–1.61 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD): δ 173.2, 172.6, 171.7, 171.3, 168.6, 159.1 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 153.1, 152.4, 150.6, 145.5, 139.2, 134.6, 134.2, 134.1, 131.2, 129.9, 129.8, 129.6, 129.2, 128.8, 122.1, 117.4 (q, $^{1}J_{C\text{-}F}$ = 287 Hz), 96.7, 88.8, 82.3, 82.2, 74.9, 74.2, 53.8, 53.1, 39.2, 32.5, 30.0, 29.7, 20.6, 20.3; ^{19}F NMR (471 MHz, CD_3OD, BTF IStd): δ –76.5; HRMS (ESI+): calcd. for $[C_{38}H_{37}F_3N_7O_{10}]^+$ 808.2549, meas. 808.2547, Δ 0.1 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-6-methoxy-6-oxo-2-((3-sulfamoylphenyl)ethynyl)-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S69). To a solution of alkyne **8** (41 mg, 59 μmol) and 3-iodobenzenesulfonamide (25 mg, 89 μmol, 1.5 equiv.) in a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt

(degassed by sparging with nitrogen for 15 min, 2.0 mL) at RT, were introduced CuI (3 mg, 0.01 mmol, 25 mol %) and $Pd(PPh_3)_4$ (3 mg, 3 μmol, 5 mol %) rapidly. The resulting mixture was stirred at 50 °C for 17 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 10%) to afford alkyne **S69** (41 mg, 49 μmol, 82%) as a colorless oil. R_f = 0.29 (EtOAc/*i*-PrOH, 95:5); FTIR (thin film), ν_{max} (cm^{-1}): 3301, 3074, 2924, 1748, 1721, 1612, 1583, 1456, 1336, 1245, 1219, 1162, 1097; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.11 (s, 1H), 8.75 (s, 1H), 8.16 (s, 1H), 8.02–7.96 (m, 2H), 7.85 (app t, *J* = 1.2 Hz, 1H), 7.77 (app dt, *J* = 7.8, 1.2 Hz, 1H), 7.62–7.57 (m, 1H), 7.53–7.48 (m, 2H), 7.47 (app dt, *J* = 7.8, 1.2 Hz, 1H), 7.37 (app t, *J* = 7.8 Hz, 1H), 7.27 (br d, *J* = 7.7 Hz, 1H), 6.15 (d, *J* = 5.7 Hz, 1H), 6.13 (dd, *J* = 5.7, 5.3 Hz, 1H), 5.63 (br s, 2H), 5.62 (dd, *J* = 5.3, 4.2 Hz, 1H), 4.65 (app td, *J* = 7.7, 5.5 Hz, 1H), 4.47 (ddd, *J* = 9.7, 4.2, 3.5 Hz, 1H), 3.77 (s, 3H), 2.86–2.81 (m, 1H), 2.21–2.14 (m, 1H), 2.16 (s, 3H), 2.10 (app ddt, *J* = 14.0, 11.1, 5.5 Hz, 1H), 2.06 (s, 3H), 2.05–1.98 (m, 2H), 1.69–1.60 (m, 1H), 1.60–1.52 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 170.1, 169.8, 165.0, 157.2 (q, $^2J_{C\text{-}F}$ = 38 Hz), 152.8, 151.8, 149.8, 142.7, 142.6, 135.2, 133.4, 133.1, 129.8, 129.1, 129.0, 128.1, 125.5, 124.4, 124.1, 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 92.7, 87.0, 82.2, 81.1, 73.6, 72.8, 53.3, 52.5, 38.1, 30.8, 29.8, 28.6, 20.8, 20.6; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.6; HRMS (ESI+): calcd. for $[C_{37}H_{37}F_3N_7O_{11}S_1]^+$ 844.2218, meas. 844.2198, Δ 2.4 ppm.

2-fluoro-5-iodobenzamide (S70). To a vial charged with 2-fluoro-5-iodobenzonitrile (0.99 g, 4.0 mmol) was added H_2SO_4 (98%, 4.0 mL). The resulting mixture was stirred at 70 °C for 2 h, cooled to 0 °C, before the addition of a 1 N NaOH aq. solution (15 mL) and a $NaHCO_3$ sat. aq. solution (5 mL). The resulting white precipitate was filtered to yield 2-fluoro-5-iodobenzamide (**S70**) as a pale orange, off-white powder (1.1 g, 4.0 mmol, quant.). R_f = 0.43 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3361, 3305, 3165, 1710, 1656, 1625, 1598, 1564, 1475, 1427, 1363, 1254, 1228; ^{1}H NMR (500 MHz, CD_3OD): δ 8.09 (1H, dd, J = 6.8, 2.4 Hz), 7.85 (1H, ddd, J = 8.7, 4.7, 2.4 Hz), 7.03 (1H, dd, J = 10.7, 8.7 Hz); ^{13}C NMR (101 MHz, CD_3OD): δ 166.9, 161.5 (d, $J_{C\text{-}F}$ = 251 Hz), 143.3 (d, $J_{C\text{-}F}$ = 8.7 Hz), 140.5 (d, $J_{C\text{-}F}$ = 2.6 Hz), 125.7 (d, $J_{C\text{-}F}$ = 15 Hz), 119.6 (d, $J_{C\text{-}F}$ = 25 Hz), 88.0 (d, $J_{C\text{-}F}$ = 3.7 Hz); ^{19}F NMR (471 MHz, CD_3OD, BTF IStd): δ –116.3; HRMS (ESI+): calcd. for $[C_7H_5F_1I_1N_1O_1Na]^+$ 287.9292, meas. 287.9283, Δ 3.1 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((3-carbamoyl-4-fluorophenyl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S71). To a vial charged with nucleoside **8** (31 mg, 46 μmol) were added 2-fluoro-5-iodobenzamide (**S70**) (20 mg, 75 μmol, 1.65 equiv.), CuI (2 mg, 9 μmol, 20 mol %) and $Pd(PPh_3)_4$ (3 mg, 2 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 3.5 mL) was added at RT. The resulting mixture was stirred at 60 °C for 1 h. More 2-fluoro-5-iodobenzamide (10 mg, 38 μmol, 0.83 equiv.) in a 5:1:1

mixture of toluene, DMF and *i*-Pr_2NEt (2.0 mL) was added and the reaction mixture was stirred at 60 °C for another 3 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 10%) to afford alkyne **S71** (34 mg, 41 μmol, 91%) as a white amorphous solid. FTIR (thin-film), ν_{max} (cm^{-1}): 3256, 3082, 3060, 3026, 2924, 2853, 1748, 1719, 1672, 1611, 1583, 1513, 1491, 1453, 1366, 1245, 1216, 1183, 1159, 1110, 1074, 1048, 1029; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.02 (s, 1H), 8.80 (s, 1H), 8.14 (dd, *J* = 7.5, 2.3 Hz, 1H), 8.12 (s, 1H), 8.03 (d, *J* = 7.4 Hz, 2H), 7.69–7.59 (m, 1H), 7.55–7.44 (m, 3H), 7.09 (dd, *J* = 11.5, 8.5 Hz, 1H), 7.05 (br d, *J* = 7.8 Hz, 1H), 6.68 (br d, *J* = 10.9 Hz, 1H), 6.14 (d, *J* = 5.4 Hz, 1H), 6.11 (t, *J* = 5.4 Hz, 1H), 5.95 (br s, 1H), 5.60 (t, *J* = 5.0 Hz, 1H), 4.65 (td, *J* = 7.7, 5.0 Hz, 1H), 4.50 (ddd, *J* = 10.7, 4.6, 3.0 Hz, 1H), 3.79 (s, 3H), 2.82 (ddt, *J* = 10.9, 9.1, 4.5 Hz, 1H), 2.17 (s, 3H), 2.18–2.08 (m, 2H), 2.08 (s, 3H), 2.04–1.92 (m, 2H), 1.64 (ddd, *J* = 18.6, 9.3, 5.4 Hz, 1H), 1.52 (dddd, *J* = 13.5, 11.0, 9.4, 4.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.3, 169.9, 169.7, 164.7, 164.1, 164.1, 161.6, 159.1, 157.3, 156.9, 156.5, 152.9, 151.7, 149.9, 142.4, 137.1, 137.0, 135.8, 135.8, 133.6, 133.0, 132.3, 132.2, 132.1, 132.1, 129.0, 128.7, 128.6, 128.0, 124.1, 120.5, 120.5, 120.4, 120.3, 117.2, 116.7, 116.4, 114.3, 91.1, 91.0, 87.2, 81.9, 80.9, 77.4, 73.7, 73.0, 53.3, 52.5, 38.6, 31.0, 30.0, 28.7, 23.6, 20.8, 20.6; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.7, –113.0; HRMS (ESI+): calcd. for $[C_{38}H_{35}F_4N_7O_{10}Na]^+$ 848.2272, meas. 848.2274, Δ 0.2 ppm.

5-iodo-2-methylbenzamide (S72).[77] To a vial charged with 5-iodo-2-methylbenzonitrile (0.12 g, 0.50 mmol) and *t*-BuOK (0.17 g, 1.5 mmol, 3.0 equiv.) was added *t*-BuOH (4.5 mL) under a nitrogen atmosphere. The resulting mixture was stirred at 60 °C for 24 h, before the addition of water (10 mL) and EtOAc (20 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (80 mL), dried over $MgSO_4$, filtered and concentrated. Crude material was dissolved in hot toluene, recrystallized and washed with hexanes to yield 5-iodo-2-methylbenzamide (**S72**) (85 mg, 0.33 mmol, 65% yield) as pale yellow crystals. R_f = 0.20 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3371, 3187, 1649, 1582, 1559, 1401, 1373; ^1H NMR (600 MHz, $CDCl_3$): δ 7.77 (d, J = 1.8 Hz, 1H), 7.65 (dd, J = 8.2, 1.8 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 5.68 (br s, 1H), 5.62 (br s, 1H), 2.43 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2, 139.3, 137.3, 136.2, 135.7, 133.2, 90.2, 19.7; HRMS (ESI+): calcd. for $[C_8H_9I_1N_1O_1]^+$ 261.9723, meas. 261.9721, Δ 0.9 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((3-carbamoyl-4-methylphenyl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S73). To a solution of alkyne **8** (50 mg, 73 μmol) and 5-iodo-2-methylbenzamide (**S72**) (47 mg, 0.18 mmol, 2.5 equiv.) in a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 3.6 mL) at RT, were introduced CuI (3 mg, 0.02 mmol, 25 mol %) and $Pd(PPh_3)_4$ (4 mg, 4 μmol, 5 mol %) rapidly. The resulting mixture was stirred at 60 °C for 2 h. The solution was allowed to

cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 95:5) to afford alkyne **S73**, which was further purified by preparative HPLC (15% → 95% B over 30 min) to afford alkyne **S73** (40 mg, 49 μmol, 67%) as a white amorphous solid. R_f = 0.34 (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3341, 1747, 1721, 1668, 1612, 1584, 1457, 1376, 1246, 1220, 1099, 1075, 1052; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.28 (s, 1H), 8.74 (s, 1H), 8.13 (s, 1H), 8.04–7.98 (m, 2H), 7.61–7.56 (m, 1H), 7.52–7.47 (m, 2H), 7.46 (d, *J* = 1.8 Hz, 1H), 7.42 (br d, *J* = 7.7 Hz, 1H), 7.28 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.11 (br d, *J* = 7.8 Hz, 1H), 6.32 (br s, 1H), 6.16 (br s, 1H), 6.13 (d, *J* = 5.6 Hz, 1H), 6.11 (dd, *J* = 5.6, 5.1 Hz, 1H), 5.61 (dd, *J* = 5.1, 4.2 Hz, 1H), 4.64 (app td, *J* = 7.7, 5.2 Hz, 1H), 4.48 (ddd, *J* = 10.0, 4.2, 3.6 Hz, 1H), 3.75 (s, 3H), 2.79 (dddd, *J* = 10.9, 9.3, 4.8, 4.0 Hz, 1H), 2.42 (s, 3H), 2.13 (s, 3H), 2.17–2.07 (m, 2H), 2.05 (s, 3H), 2.07–1.99 (m, 1H), 1.96 (ddd, *J* = 13.9, 10.9, 3.6 Hz, 1H), 1.63 (dddd, *J* = 13.4, 10.8, 5.8, 4.8 Hz, 1H), 1.53 (dddd, *J* = 13.4, 10.6, 9.3, 4.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4, 171.4, 170.0, 169.6, 165.0, 157.1 (q, $^2J_{C\text{-}F}$ = 38 Hz), 152.8, 151.8, 149.9, 142.4, 136.8, 135.2, 133.5, 133.1, 133.0, 131.3, 130.6, 128.9, 128.1, 124.2, 120.6, 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 90.5, 86.9, 83.0, 81.2, 73.7, 72.8, 53.2, 52.5, 38.5, 31.0, 29.8, 28.7, 20.8, 20.5, 20.1; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.6; HRMS (ESI+): calcd. for $[C_{39}H_{39}F_3N_7O_{10}]^+$ 822.2705, meas. 822.2718, Δ 1.6 ppm.

5-iodo-2-(trifluoromethyl)benzamide (S74). To a vial charged with 5-iodo-2-(trifluoromethyl)benzonitrile (0.30 g, 1.0 mmol) and *t*-BuOK (0.67 g, 6.0 mmol, 6.0 equiv.) was

added *t*-BuOH (5.0 mL) under a nitrogen atmosphere. The resulting mixture was stirred at 60 °C for 5 min, cooled to RT and stirred for 48 h. The reaction was quenched by the addition of water (10 mL). The resulting suspension was filtered to produce an orange solid. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 0 → 100%) to yield 5-iodo-2-(trifluoromethyl)benzamide (**S74**) as a white solid (0.13 g, 0.41 mmol, 41%). R_f = 0.46 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3369, 3183, 1649, 1588, 1569, 1400, 1378, 1311, 1287; ^{1}H NMR (500 MHz, CD_3OD): δ 8.01 (1H, ddq, J = 8.3, 1.7, 0.9 Hz), 7.95–7.92 (1H, m), 7.50 (1H, br d, J = 8.3 Hz); ^{13}C NMR (126 MHz, CD_3OD): δ 171.3, 140.3, 138.6 (q, J_{C-F} = 2.1 Hz), 138.3, 129.0 (q, J_{C-F} = 4.8 Hz), 127.7 (q, J_{C-F} = 32.6 Hz), 125.1 (q, J_{C-F} = 273 Hz), 99.4 (q, J_{C-F} = 1.4 Hz); ^{19}F NMR (471 MHz, CD_3OD, BTF IStd): δ –60.3; HRMS (ESI+): calcd. for $[C_8H_5F_3I_1N_1O_1Na]^+$ 337.9260, meas. 337.9260, Δ 0.0 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((3-carbamoyl-4-(trifluoromethyl)phenyl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S75). To a vial charged with alkyne **8** (82 mg, 0.12 mmol) were added 5-iodo-2-(trifluoromethyl)benzamide (**S74**) (62 mg, 0.20 mmol, 1.65 equiv.), CuI (5 mg, 24 μmol, 20 mol %) and $Pd(PPh_3)_4$ (7 mg, 6 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 3.5 mL) was added at RT. The resulting mixture was stirred at 70 °C for 3 h. The solution was allowed to cool to

RT and volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (EtOAc/*i*-PrOH, 0 → 5%) to afford alkyne **S75** (90 mg, 0.10 mmol, 86%) as a white amorphous solid. R_f = 0.48 (PhMe/MeCN, 50:50); FTIR (thin-film), ν_{max} (cm^{-1}): 3306, 2926, 2165, 1747, 1719, 1672, 1609, 1582, 1511, 1487, 1456, 1410, 1373, 1313, 1289, 1242, 1216, 1175, 1133, 1114, 1074, 1039; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.98 (s, 1H), 8.78 (s, 1H), 8.11 (s, 1H), 8.04–7.98 (m, 2H), 7.63–7.59 (m, 2H), 7.58 (dd, *J* = 1.6, 0.8 Hz, 1H), 7.57–7.49 (m, 2H), 7.48 (ddt, *J* = 8.2, 1.8, 0.9 Hz, 1H), 7.13 (d, *J* = 7.7 Hz, 1H), 6.20 (br s, 1H), 6.16–6.12 (m, 2H), 6.05 (br s, *J* = 2.7 Hz, 1H), 5.62 (td, *J* = 4.2, 0.8 Hz, 1H), 4.65 (td, *J* = 7.6, 5.2 Hz, 1H), 4.46 (dt, *J* = 10.3, 3.5 Hz, 1H), 2.86 (ddt, *J* = 10.6, 9.0, 4.5 Hz, 1H), 2.23–2.17 (m, 1H), 2.16 (s, 3H), 2.12 (ddd, *J* = 10.7, 8.4, 5.4 Hz, 1H), 2.07 (s, 3H), 2.04–1.96 (m, 2H), 1.66 (ddt, *J* = 13.1, 10.7, 5.4 Hz, 1H), 1.56 (ddd, *J* = 8.9, 6.3, 4.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.3, 170.0, 169.6, 169.3, 165.0, 157.2 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 152.7, 151.7, 149.9, 142.5, 135.2, 133.4, 133.0, 132.8, 132.2, 132.1, 131.7, 128.9, 128.6, 128.5, 128.1, 127.4, 126.6, 126.5, 126.3 (q, $^{2}J_{C\text{-}F}$ = 32 Hz), 124.7, 123.4 (q, $^{1}J_{C\text{-}F}$ = 274 Hz), 115.7 (q, $^{1}J_{C\text{-}F}$ = 287 Hz), 94.4, 87.0, 81.7, 81.0, 77.3, 73.6, 72.7, 53.1, 52.4, 38.1, 30.8, 29.7, 28.6, 20.7, 20.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –60.0, –76.7; HRMS (ESI+): calcd. for $[C_{39}H_{36}F_6N_7O_{10}]^+$ 876.2422, meas. 876.2426, Δ 0.4 ppm.

2-chloro-5-iodobenzamide (S76). To a vial charged with 2-chloro-5-iodobenzonitrile (1.32 g, 5.01 mmol) and *t*-BuOK (1.69 g, 15.0 mmol, 3.0 equiv.) was added *t*-BuOH (10.0 mL) under a nitrogen atmosphere. The resulting mixture was stirred at 60 °C for 5 min,

cooled to RT and stirred for 1 h. The reaction was quenched by the addition of water (10 mL). The solid amide was filtered and recrystallized from absolute ethanol to give 2-chloro-5-iodobenzamide (**S76**) as a white solid (896 mg, 3.18 mmol, 64%). R_f = 0.35 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3373, 3180, 1655, 1618, 1462, 1397, 1362; ^1H NMR (500 MHz, CD_3OD): δ 7.82 (1H, d, J = 2.2 Hz), 7.76 (1H, dd, J = 8.5, 2.2 Hz), 7.24 (1H, d, J = 8.5 Hz); ^{13}C NMR (151 MHz, CD_3OD): δ 170.5, 141.1, 139.2, 138.6, 132.9, 131.8, 92.0; HRMS (ESI+): calcd. for $[C_7H_6Cl_1I_1N_1O_1]^+$ 281.9177, meas. 281.9188, Δ 3.9 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((3-carbamoyl-4-chlorophenyl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S77). To a solution of alkyne **8** (50 mg, 73 μmol) and 2-chloro-5-iodobenzamide (**S76**) (51 mg, 0.18 mmol, 2.5 equiv.) in a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 3.6 mL) at RT, were introduced CuI (3 mg, 0.02 mmol, 25 mol %) and $Pd(PPh_3)_4$ (4 mg, 4 μmol, 5 mol %) rapidly. The resulting mixture was stirred at 70 °C for 3 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 90:10) to afford alkyne **S77**, which was further purified by preparative HPLC (15% → 95% B over 30 min) to afford alkyne **S77** (45 mg, 53 μmol, 74%) as a white amorphous solid. R_f = 0.48 (EtOAc/*i*-PrOH, 95:5); FTIR (thin film), ν_{max} (cm^{-1}): 3320, 3085, 1748, 1719, 1673, 1613, 1583, 1456, 1374, 1246, 1219, 1047; ^1H NMR (600

MHz, $CDCl_3$): δ 9.29 (s, 1H), 8.74 (s, 1H), 8.12 (s, 1H), 8.04–7.97 (m, 2H), 7.69 (d, J = 2.0 Hz, 1H), 7.60–7.56 (m, 1H), 7.50–7.46 (m, 3H), 7.31 (dd, J = 8.3, 2.0 Hz, 1H), 7.28 (d, J = 8.3 Hz, 1H), 6.65 (s, 1H), 6.48 (s, 1H), 6.16–6.08 (m, 2H), 5.58 (app t, J = 4.5 Hz, 1H), 4.63 (app td, J = 7.8, 5.1 Hz, 1H), 4.46 (ddd, J = 10.5, 4.5, 3.1 Hz, 1H), 3.75 (s, 3H), 2.80 (dddd, J = 11.1, 9.1, 5.3, 4.6 Hz, 1H), 2.14 (s, 3H), 2.19–2.11 (m, 1H), 2.12–2.06 (m, 1H), 2.05 (s, 3H), 2.03–1.96 (m, 1H), 1.94 (ddd, J = 13.8, 11.1, 3.1 Hz, 1H), 1.63 (app ddt, J = 13.0, 10.8, 5.3 Hz, 1H), 1.58–1.49 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4, 170.0, 169.7, 167.7, 165.0, 157.2 (q, $^2J_{C\text{-}F}$ = 38 Hz), 152.8, 151.7, 149.9, 142.5, 134.4, 134.1, 133.6, 133.5, 133.0, 130.5, 130.5, 128.9, 128.1, 124.1, 122.5, 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 92.5, 87.1, 81.9, 80.9, 73.6, 72.8, 53.2, 52.5, 38.3, 30.9, 29.8, 28.7, 20.8, 20.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.6; HRMS (ESI+): calcd. for $[C_{38}H_{36}Cl_1F_3N_7O_{10}]^+$ 842.2159, meas. 842.2168, Δ 1.0 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-6-methoxy-6-oxo-2-((1-oxo-1,2,3,4-tetrahydroisoquinolin-7-yl)ethynyl)-5-(2,2,2-trifluoroacetam-ido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S78). A vial was charged with alkyne **8** (40 mg, 58 μmol), 7-bromo-3,4-dihydroisoquinolin-1(*2H*)-one (33 mg, 0.15 mmol, 2.5 equiv.) and CuI (3 mg, 0.02 mmol, 25 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (degassed by sparging with nitrogen for 15 min, 1.5 mL) was added at RT. The resulting mixture was further degassed by sparging with nitrogen for 5 min and a solution of $Pd(Pt\text{-}Bu_3)_2$ (3 mg, 6 μmol,

10 mol %) in a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (1.5 mL) was added. The resulting mixture was stirred at 55 °C for 6 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 70:30) to afford alkyne **S78**, which was further purified by preparative HPLC (15% → 95% B over 30 min) to afford alkyne **S78** (29 mg, 35 μmol, 60%) as a white amorphous solid. FTIR (thin film), ν_{max} (cm^{-1}): 3262, 3082, 2938, 1749, 1720, 1681, 1612, 1579, 1513, 1482, 1456, 1373, 1243, 1218, 1097, 1048; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.16 (s, 1H), 8.78 (s, 1H), 8.78 (s, 1H), 8.13 (s, 1H), 8.05–8.00 (m, 2H), 7.62–7.58 (m, 1H), 7.54–7.49 (m, 2H), 7.24 (br d, *J* = 7.7 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 7.01 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.83 (d, *J* = 1.2 Hz, 1H), 6.14 (d, *J* = 5.6 Hz, 1H), 6.11 (dd, *J* = 5.6, 5.3 Hz, 1H), 5.59 (dd, *J* = 5.3, 4.3 Hz, 1H), 4.64 (app td, *J* = 7.7, 5.6 Hz, 1H), 4.49 (ddd, *J* = 10.4, 4.3, 3.1 Hz, 1H), 3.76 (s, 3H), 2.93 (app dd, *J* = 8.5, 6.6 Hz, 2H), 2.82–2.76 (m, 1H), 2.61 (app dd, *J* = 8.5, 6.6 Hz, 2H), 2.17–2.10 (m, 1H), 2.15 (s, 3H), 2.10–2.05 (m, 1H), 2.06 (s, 3H), 2.04–1.97 (m, 1H), 1.96 (ddd, *J* = 13.8, 11.0, 3.1 Hz, 1H), 1.64 (app ddt, *J* = 12.9, 10.6, 5.3 Hz, 1H), 1.57–1.49 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.8, 171.4, 169.9, 169.6, 164.9, 157.1 (q, $^2J_{C\text{-}F}$ = 38 Hz), 152.8, 151.8, 150.0, 142.4, 137.4, 133.5, 133.0, 129.0, 128.1, 128.0, 126.7, 124.2, 124.0, 122.5, 118.4, 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 90.4, 87.0, 83.1, 81.1, 73.8, 72.9, 53.2, 52.5, 38.7, 31.1, 30.6, 30.0, 28.8, 25.4, 20.8, 20.6; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.6; HRMS (ESI+): calcd. for $[C_{40}H_{39}F_3N_7O_{10}]^+$ 834.2705, meas. 834.2702, Δ 0.3 ppm.

7-iodobenzo[*d*][1,3]dioxole-5-carboxamide (S80). To a solution of known aldehyde 7-iodobenzo[*d*][1,3]dioxole-5-carbaldehyde (**S79**)[78-79] (500 mg, 1.81 mmol) in *t*-BuOH (20 mL) at RT, was added a solution of $NaClO_2$ (328 mg, 3.62 mmol, 2.0 equiv.) and NaH_2PO_4 (0.11 g, 0.91 mmol, 0.50 equiv.) in water (4 mL). The resulting pale yellow solution was cooled to 0 °C and 2-methyl-2-butene (4.0 mL, 38 mmol, 21 equiv.) was added dropwise. The reaction mixture was stirred at RT for 20 h, upon which more $NaClO_2$ (164 mg, 1.81 mmol, 1.0 equiv.) and NaH_2PO_4 (54 mg, 0.45 mmol, 0.25 equiv.) were added as a solution in water (2 mL). The resulting mixture was stirred at RT for 72 h and diluted with a 10% H_3PO_4 aq. solution (10 mL). Water (20 mL) and EtOAc (40 mL) were added. The layers were separated and the aqueous phase was extracted with EtOAc (2 × 40 mL). The combined organic extracts were washed with brine (80 mL), dried over Na_2SO_4, filtered and concentrated to give a white solid. Crude material was used directly in the next step without further purification. To a flask charged with the crude carboxylic acid and HATU (758 mg, 1.99 mmol, 1.1 equiv.) were added DMF (20 mL) and *i*-Pr_2NEt (0.63 mL, 3.6 mmol, 2.0 equiv.) at RT. The resulting brown mixture was stirred for 30 min and NH_4Cl (116 mg, 2.18 mmol, 1.2 equiv.) was added. The flask headspace was flushed with nitrogen and the reaction mixture was stirred for 24 h, before the addition of EtOAc (30 mL) and water (20 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with a 5% LiCl aq. solution (100 mL) and

brine (2 × 100 mL), dried over Na_2SO_4, filtered and concentrated. Crude material was purified by silica gel chromatography (hexanes/EtOAc, 5 → 100%) to afford iodobenzamide **S80** (348 mg, 1.20 mmol, 66% over 2 steps) as a white solid. R_f = 0.15 (hexanes/EtOAc, 50:50); FTIR (thin film), ν_{max} (cm^{-1}): 3355, 3187, 2924, 2854, 1658, 1592, 1479, 1424, 1254, 1044; ^1H NMR (500 MHz, CD_3OD): δ 7.77 (d, J = 1.6 Hz, 1H), 7.31 (d, J = 1.6 Hz, 1H), 6.11 (s, 2H); ^{13}C NMR (101 MHz, CD_3OD): δ 170.1, 153.9, 148.1, 132.3, 130.7, 108.7, 103.0, 70.6; HRMS (ESI+): calcd. for $[C_8H_7I_1N_1O_3]^+$ 291.9465, meas. 291.9461, Δ 1.4 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((6-carbamoylbenzo[*d*][1,3]dioxol-4-yl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S81). To a vial charged with alkyne **8** (30 mg, 44 μmol) were added iodobenzamide **S80** (32 mg, 0.11 mmol, 2.5 equiv.), CuI (2 mg, 0.01 mmol, 25 mol %) and $Pd(PPh_3)_4$ (3 mg, 2 μmol, 5 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1 mixture of toluene, DMF and *i*-Pr_2NEt (degassed by sparging with nitrogen for 15 min, 2.3 mL) was added at RT. The resulting mixture was stirred at 60 °C for 3 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 80:20) to afford alkyne **S81**, which was further purified by preparative HPLC (15% → 95% B' over 30 min) to afford alkyne **S81** (27 mg, 32 μmol, 73%) as a white amorphous solid. FTIR (thin film), ν_{max} (cm^{-1}): 3351, 3222, 3077, 2937, 1746, 1717, 1659, 1593, 1424, 1243, 1203, 1045; ^1H NMR (500 MHz, CD_3OD): δ 8.80 (s, 1H), 8.70 (s, 1H), 8.12–8.06

(m, 2H), 7.70–7.65 (m, 1H), 7.60–7.55 (m, 2H), 7.45 (d, J = 1.7 Hz, 1H), 7.28 (d, J = 1.7 Hz, 1H), 6.34 (d, J = 5.0 Hz, 1H), 6.14 (dd, J = 5.7, 5.0 Hz, 1H), 6.10 (d, J = 1.1 Hz, 1H), 6.10 (d, J = 1.1 Hz, 1H), 5.65 (dd, J = 5.7, 5.1 Hz, 1H), 4.58 (ddd, J = 10.2, 5.1, 3.2 Hz, 1H), 4.52 (dd, J = 9.6, 5.0 Hz, 1H), 3.73 (s, 3H), 2.93–2.86 (m, 1H), 2.22 (ddd, J = 14.1, 10.2, 4.1 Hz, 1H), 2.15 (s, 3H), 2.17–2.10 (m, 1H), 2.06 (s, 3H), 2.07–1.98 (m, 2H), 1.72–1.58 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD): δ 172.5, 171.6, 171.3, 170.7, 168.7, 159.1 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 153.0, 152.9, 152.2, 150.4, 149.4, 145.6, 134.4, 134.3, 129.8, 129.6, 128.9, 126.7, 124.3, 117.0 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 108.4, 105.6, 103.9, 96.7, 88.9, 82.2, 77.8, 74.9, 74.3, 53.7, 53.1, 39.3, 32.5, 29.9, 29.6, 20.5, 20.3; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.7; HRMS (ESI+): calcd. for $[C_{39}H_{37}F_3N_7O_{12}]^+$ 852.2447, meas. 852.2483, Δ 4.3 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((6-carbamoylpyridin-2-yl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S82). A vial was charged with alkyne **8** (35 mg, 51 μmol), 6-bromopyridine-2-carboxamide (26 mg, 0.13 mmol, 2.5 equiv.) and CuI (2 mg, 0.01 mmol, 25 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (degassed by sparging with nitrogen for 15 min, 1.3 mL) was added at RT. The resulting mixture was further degassed by sparging with nitrogen for 5 min and a solution of $Pd(P$*t*$\text{-}Bu_3)_2$ (3 mg, 5 μmol, 10 mol %) in a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (1.3 mL) was added. The resulting mixture was stirred at 50 °C for 5 h.

The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 95:5) to afford alkyne **S82**, which was further purified by preparative HPLC (15% → 95% B over 30 min) to afford alkyne **S82** (28 mg, 35 μmol, 68%) as a white amorphous solid. R_f = 0.43 (EtOAc/*i*-PrOH, 95:5); FTIR (thin film), ν_{max} (cm^{-1}): 3292, 2928, 1748, 1718, 1690, 1611, 1584, 1511, 1487, 1457, 1377, 1245, 1220, 1161, 1075; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.16 (s, 1H), 8.78 (s, 1H), 8.14 (s, 1H), 8.10 (dd, *J* = 7.8, 1.1 Hz, 1H), 8.05–7.99 (m, 2H), 7.95 (d, *J* = 4.0 Hz, 1H), 7.78 (app t, *J* = 7.8 Hz, 1H), 7.62–7.57 (m, 1H), 7.54–7.48 (m, 3H), 7.33 (br d, *J* = 7.7 Hz, 1H), 6.15 (d, *J* = 5.9 Hz, 1H), 6.14 (dd, *J* = 5.9, 4.8 Hz, 1H), 5.92 (d, *J* = 4.0 Hz, 1H), 5.60 (dd, *J* = 4.8, 3.9 Hz, 1H), 4.66 (app td, *J* = 7.7, 5.1 Hz, 1H), 4.51 (ddd, *J* = 10.2, 3.9, 3.4 Hz, 1H), 3.77 (s, 3H), 2.90–2.83 (m, 1H), 2.22 (ddd, *J* = 14.0, 10.2, 4.1 Hz, 1H), 2.15 (s, 3H), 2.18–2.11 (m, 1H), 2.09–2.02 (m, 2H), 2.06 (s, 3H), 1.71 (app ddt, *J* = 13.1, 10.7, 5.4 Hz, 1H), 1.65–1.57 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 169.9, 169.6, 166.3, 164.9, 157.1 (q, $^2J_{C\text{-}F}$ = 38 Hz), 152.8, 151.8, 150.0, 149.9, 142.4, 141.9, 137.6, 133.5, 133.0, 129.8, 129.0, 128.1, 124.2, 121.7, 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 91.3, 87.0, 82.9, 81.2, 73.8, 72.7, 53.2, 52.5, 38.3, 30.8, 29.8, 28.7, 20.8, 20.5; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.6; HRMS (ESI+): calcd. for $[C_{37}H_{36}F_3N_8O_{10}]^+$ 809.2501, meas. 809.2504, Δ 0.4 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((2-carbamoylpyridin-4-yl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-

3,4-diyl diacetate (S83). A vial was charged with alkyne **8** (40 mg, 58 μmol), 4-bromo-picolinamide (29 mg, 0.15 mmol, 2.5 equiv.) and CuI (3 mg, 0.02 mmol, 25 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (degassed by sparging with nitrogen for 15 min, 1.5 mL) was added at RT. The resulting mixture was further degassed by sparging with nitrogen for 5 min and a solution of $Pd(P\textit{t}\text{-}Bu_3)_2$ (3 mg, 6 μmol, 10 mol %) in a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (1.5 mL) was added. The resulting mixture was stirred at 50 °C for 5 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 70:30) to afford alkyne **S83**, which was further purified by preparative HPLC (15% → 95% B over 30 min) to afford alkyne **S83** (35 mg, 43 μmol, 75%) as a white amorphous solid. FTIR (thin film), ν_{max} (cm^{-1}): 3283, 3079, 2931, 1748, 1721, 1690, 1611, 1599, 1583, 1456, 1356, 1243, 1218, 1074; ^1H NMR (600 MHz, $CDCl_3$): δ 9.07 (s, 1H), 8.78 (s, 1H), 8.48 (dd, *J* = 5.0, 0.8 Hz, 1H), 8.12 (dd, *J* = 1.6, 0.8 Hz, 1H), 8.11 (s, 1H), 8.04–8.00 (m, 2H), 7.82 (d, *J* = 4.2 Hz, 1H), 7.62–7.58 (m, 1H), 7.54–7.49 (m, 2H), 7.38 (dd, *J* = 5.0, 1.6 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 6.15–6.11 (m, 2H), 5.93 (d, *J* = 4.2 Hz, 1H), 5.62–5.58 (m, 1H), 4.65 (app td, *J* = 7.8, 5.4 Hz, 1H), 4.47 (ddd, *J* = 10.8, 4.3, 3.0 Hz, 1H), 3.78 (s, 3H), 2.88 (app ddt, *J* = 10.8, 9.2, 4.6 Hz, 1H), 2.22–2.15 (m, 1H), 2.17 (s, 3H), 2.11 (ddt, *J* = 14.2, 11.0, 5.4 Hz, 1H), 2.08 (s, 3H), 2.03–1.95 (m, 2H), 1.67 (app ddt, *J* = 13.4, 10.8, 5.4 Hz, 1H), 1.57 (dddd, *J* = 13.4, 11.0, 9.2, 4.6 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 169.9, 169.7, 166.4,

164.8, 157.1 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 152.9, 151.7, 149.9, 149.6, 148.5, 142.5, 133.6, 133.0, 133.0, 129.0, 128.4, 128.1, 124.8, 124.2, 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 96.7, 87.3, 81.1, 80.8, 73.7, 72.9, 53.3, 52.4, 38.3, 30.8, 29.9, 28.9, 20.8, 20.6; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.6; HRMS (ESI+): calcd. for $[C_{37}H_{36}F_3N_8O_{10}]^+$ 809.2501, meas. 809.2491, Δ 1.2 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((5-carbamoylpyridin-3-yl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S84). A vial was charged with alkyne **8** (40 mg, 58 μmol), 5-bromonicotinamide (29 mg, 0.15 mmol, 2.5 equiv.) and CuI (3 mg, 0.02 mmol, 25 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (degassed by sparging with nitrogen for 15 min, 1.5 mL) was added at RT. The resulting mixture was further degassed by sparging with nitrogen for 5 min and a solution of Pd(P*t*-$Bu_3)_2$ (3 mg, 6 μmol, 10 mol %) in a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (1.5 mL) was added. The resulting mixture was stirred at 50 °C for 5 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 70:30) to afford alkyne **S84**, which was further purified by preparative HPLC (15% → 95% B over 30 min) to afford alkyne **S84** (26 mg, 32 μmol, 55%) as a white amorphous solid. FTIR (thin film), ν_{max} (cm^{-1}): 3263, 3085, 2954, 2928, 1748, 1720, 1677, 1612, 1583, 1456, 1376, 1245, 1219, 1075; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.11 (s, 1H), 8.94 (d, J = 1.4 Hz, 1H), 8.77 (s, 1H), 8.67 (d, J = 2.0 Hz, 1H), 8.16–8.11 (m, 2H), 8.01 (m, 2H), 7.63–7.58 (m, 1H), 7.55–7.48 (m, 2H),

7.46 (br d, J = 7.5 Hz, 1H), 6.83 (br s, 1H), 6.17 (dd, J = 5.7, 5.1 Hz, 1H), 6.15 (d, J = 5.7 Hz, 1H), 6.05 (br s, 1H), 5.66 (dd, J = 5.1, 4.0 Hz, 1H), 4.65 (app td, J = 7.5, 5.4 Hz, 1H), 4.48 (ddd, J = 9.8, 4.0, 3.8 Hz, 1H), 3.79 (s, 3H), 2.88–2.82 (m, 1H), 2.21–2.15 (m, 1H), 2.16 (s, 3H), 2.15–2.08 (m, 1H), 2.07 (s, 3H), 2.08–1.99 (m, 2H), 1.72–1.64 (m, 1H), 1.63–1.54 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 170.1, 169.8, 166.9, 165.0, 157.3 (q, $^{2}J_{C\text{-}F}$ = 38 Hz), 154.5, 152.8, 151.8, 149.9, 147.6, 142.6, 138.1, 133.5, 133.1, 129.0, 128.5, 128.1, 124.3, 120.2, 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 95.3, 87.1, 81.2, 79.9, 73.7, 72.7, 53.3, 52.6, 38.3, 30.9, 29.7, 28.9, 20.8, 20.6; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.6; HRMS (ESI+): calcd. for $[C_{37}H_{36}F_3N_8O_{10}]^+$ 809.2501, meas. 809.2511, Δ 1.2 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((2*S*,5*S*)-2-((4-carbamoylpyridin-2-yl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)tetrahydrofuran-3,4-diyl diacetate (S85). A vial was charged with alkyne **8** (35 mg, 51 μmol), 2-bromo-isonicotinamide (26 mg, 0.13 mmol, 2.5 equiv.) and CuI (2 mg, 0.01 mmol, 25 mol %). The vial headspace was purged with nitrogen for 5 min and a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (degassed by sparging with nitrogen for 15 min, 1.3 mL) was added at RT. The resulting mixture was further degassed by sparging with nitrogen for 5 min and a solution of $Pd(Pt\text{-}Bu_3)_2$ (3 mg, 5 μmol, 10 mol %) in a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (1.3 mL) was added. The resulting mixture was stirred at 50 °C for 5 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*-PrOH, 95:5) to afford alkyne **S85**, which

was further purified by preparative HPLC (15% → 95% B over 30 min) to afford alkyne **S85** (22 mg, 27 μmol, 54%) as a light yellow amorphous solid. R_f = 0.31 (EtOAc/*i*-PrOH, 95:5); FTIR (thin film), ν_{max} (cm^{-1}): 3289, 2925, 1747, 1719, 1681, 1612, 1583, 1549, 1457, 1414, 1376, 1245, 1220, 1162, 1047; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.18 (s, 1H), 8.77 (s, 1H), 8.64 (dd, *J* = 5.2, 0.9 Hz, 1H), 8.13 (s, 1H), 8.05–7.98 (m, 2H), 7.79 (dd, *J* = 1.7, 0.9 Hz, 1H), 7.65 (dd, *J* = 5.2, 1.7 Hz, 1H), 7.62–7.58 (m, 1H), 7.59–7.56 (m, 1H), 7.55–7.48 (m, 2H), 7.11 (br s, 1H), 6.14 (br s, 1H), 6.14 (dd, *J* = 5.4, 5.0 Hz, 1H), 6.12 (d, *J* = 5.4 Hz, 1H), 5.66 (dd, *J* = 5.0, 4.4 Hz, 1H), 4.62 (app q, *J* = 6.9 Hz, 1H), 4.53 (ddd, *J* = 10.1, 4.4, 3.6 Hz, 1H), 3.77 (s, 3H), 2.85 (app ddt, *J* = 10.9, 9.0, 4.3 Hz, 1H), 2.15 (s, 3H), 2.19–2.09 (m, 3H), 2.06 (s, 3H), 2.07–2.01 (m, 1H), 1.73–1.66 (m, 1H), 1.65–1.57 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.2, 170.1, 169.8, 166.8, 165.0, 157.4 (q, $^2J_{C\text{-}F}$ = 38 Hz), 152.8, 151.7, 150.7, 149.9, 143.9, 142.6, 141.0, 133.5, 133.1, 129.0, 128.1, 125.2, 124.2, 120.9, 115.7 (q, $^1J_{C\text{-}F}$ = 288 Hz), 92.3, 87.2, 83.1, 80.9, 73.7, 72.9, 53.3, 52.8, 38.2, 31.0, 29.6, 28.8, 20.8, 20.6; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.6; HRMS (ESI+): calcd. for $[C_{37}H_{36}F_3N_8O_{10}]^+$ 809.2501, meas. 809.2502, Δ 0.1 ppm.

(2*R*,3*R*,4*R*,5*R*)-2-((2*S*,5*S*)-2-((5-aminonaphthalen-1-yl)ethynyl)-6-methoxy-6-oxo-5-(2,2,2-trifluoroacetamido)hexyl)-5-(6-benzamido-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (S86). A vial was charged with alkyne **8** (42 mg, 61 μmol), 5-bromo-naphthalen-1-ylamine (34 mg, 0.15 mmol, 2.5 equiv.) and CuI (3 mg, 0.02 mmol, 25 mol

%). The vial headspace was purged with nitrogen for 5 min and a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (degassed by sparging with nitrogen for 15 min, 1.5 mL) was added at RT. The resulting mixture was further degassed by sparging with nitrogen for 5 min and a solution of $Pd(Pt\text{-}Bu_3)_2$ (3 mg, 6 μmol, 10 mol %) in a 5:1:1.5 mixture of toluene, DMF and *i*-Pr_2NH (1.5 mL) was added. The resulting mixture was stirred at 50 °C for 5 h. The solution was allowed to cool to RT and volatiles were removed *in vacuo*. Crude material was filtered through a short pad of silica gel (EtOAc/*i*PrOH, 70:30) to afford alkyne **S86**, which was further purified by preparative HPLC (20% → 95% B over 30 min) to afford alkyne **S86** (34 mg, 41 μmol, 67%) as a light yellow amorphous solid. R_f = 0.42 (EtOAc); FTIR (thin film), ν_{max} (cm^{-1}): 3315, 3066, 2924, 1748, 1721, 1611, 1582, 1514, 1456, 1369, 1243, 1218, 1179, 1074; ^1H NMR (600 MHz, $CDCl_3$): δ 9.02 (s, 1H), 8.80 (s, 1H), 8.12 (s, 1H), 8.03–7.99 (m, 2H), 7.80 (app dt, *J* = 8.5, 1.0 Hz, 1H), 7.70 (app dt, *J* = 8.3, 1.0 Hz, 1H), 7.62–7.58 (m, 2H), 7.53–7.50 (m, 2H), 7.37 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.34 (dd, *J* = 8.3, 7.4 Hz, 1H), 7.04 (br d, *J* = 7.7 Hz, 1H), 6.80 (dd, *J* = 7.4, 1.0 Hz, 1H), 6.15 (d, *J* = 5.3 Hz, 1H), 6.09 (dd, *J* = 5.5, 5.3 Hz, 1H), 5.61 (dd, *J* = 5.5, 4.8 Hz, 1H), 4.69 (app td, *J* = 7.7, 4.9 Hz, 1H), 4.65 (ddd, *J* = 10.8, 4.8, 2.8 Hz, 1H), 4.32 (br s, 2H), 3.74 (s, 3H), 2.97 (app ddt, *J* = 10.8, 9.1, 4.5 Hz, 1H), 2.24–2.16 (m, 2H), 2.13 (s, 3H), 2.14–2.04 (m, 2H), 2.06 (s, 3H), 1.72 (dddd, *J* = 13.3, 10.8, 6.0, 4.8 Hz, 1H), 1.61 (dddd, *J* = 13.3, 10.9, 9.4, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 169.9, 169.6, 164.7, 157.0 (q, $^2J_{C\text{-}F}$ = 38 Hz), 152.9, 151.7, 149.9, 142.7, 142.3, 134.5, 133.6, 133.0, 130.7, 129.0, 128.0, 127.3,

124.1, 124.1, 123.5, 121.5, 121.3, 117.0, 115.7 (q, $^{1}J_{C\text{-}F}$ = 288 Hz), 110.4, 94.9, 87.2, 82.4, 81.0, 73.7, 73.1, 53.2, 52.5, 38.9, 31.2, 30.3, 29.3, 20.8, 20.6; ^{19}F NMR (471 MHz, $CDCl_3$, BTF IStd): δ –76.7; HRMS (ESI+): calcd. for $[C_{41}H_{39}F_3N_7O_9]^+$ 830.2756, meas. 830.2772, Δ 1.9 ppm.

Cellular Thermal Shift Assay (CETSA)

K562 Cell Culture. The human chronic myeloid leukemia cell line K562 (ATCC CCL-243) was cultured in RPMI 1640 with Glutamax (Gibco) supplemented with 10% fetal bovine serum (Atlanta Biologicals), and 100 IU penicillin and 100 mg/mL streptomycin (Corning). Cells were cultured in T175 flasks with 50 mL of media per flask (Corning) in a 37 °C incubator with 5% CO_2 until the cells reached a density of 2 × 106 cells/mL.

Preparation of K562 Cell Lysate. K562 cells were cultured as described above. Cells were washed with PBS and then resuspended in an appropriate volume of PBS supplemented with cOmplete protease inhibitor cocktail (Roche) to obtain a final cell count of 3.0 × 10^7 to 3.6 × 10^7 cells/mL. The suspension was transferred to microcentrifuge tubes and subjected to three rounds of freeze-thaw using liquid nitrogen and a room temperature water bath. The cell lysate was clarified by centrifuging at 17,000g for 20 min at 4 °C, and the supernatant was transferred to a new microcentrifuge tube. Lysate protein concentration was determined by BCA assay and the lysate was diluted to a protein concentration of 3

mg/mL with PBS supplemented with protease inhibitor cocktail. Aliquots were snap frozen in liquid nitrogen and stored at –80 °C.

CETSA with K562 Cell Lysate. The CETSA assay was adapted from previously published protocols.[80-81] K562 lysate prepared as described above was incubated at room temperature with DMSO, 100 μM of NS1 (**10**), or 100 μM of compound **25** for 30 min. A Bio-Rad C1000 Touch Thermal Cycler was preheated to the following temperatures using the gradient setting: 37 °C, 38.8 °C, 41.6 °C, 45.2 °C, 50.1 °C, 53.7 °C, 56 °C, 57.9 °C, 60.8 °C, 64.3 °C, 69.1 °C, 72.9 °C. DMSO-treated and compound-treated lysate were aliquotted into twelve PCR tubes, and each tube was heated at one of the temperatures listed above for 3 min. The samples were cooled at room temperature for 3 min, then snap frozen in liquid nitrogen. When thawed, the samples were centrifuged at 17,000g for 20 min at 4 °C. The supernatant was transferred to a new tube, mixed with NuPAGE LDS sample buffer (Novex) and β-mercaptoethanol (2.5%). The samples were heated at 70 °C for 10 min and analyzed by SDS-PAGE and western blot using an anti-NNMT mouse antibody (Abcam, ab119758, 1:1000 dilution), an anti-actin HRP-conjugated mouse antibody (Abcam, ab49900, 1:250000 dilution), and an anti-mouse HRP-conjugated IgG (Promega, W402B, 1:5000 dilution).

Isothermal Dose Response (IDTR) CETSA with K562 Cell Lysate. K562 lysate prepared as described above was incubated at room temperature with DMSO or various concentrations of NS1 (**10**) or compound **25** for 30 min. A Bio-Rad C1000 Touch thermal cycler was

preheated to 57 °C. Samples were aliquotted into PCR tubes, and each tube was heated at 57 °C in the thermal cycler for 3 min. The samples were cooled at room temperature for 3 min and then snap frozen in liquid nitrogen. When thawed, the samples were centrifuged at 17,000g for 20 min at 4 °C. The supernatant was prepared for western blot analysis as described in the CETSA procedure above.

Cytotoxicity Assay in U2OS Cells. Cytotoxicity evaluation was performed with the Promega CellTiter-Glo Luminescent Cell Viability Assay. U2OS cells were maintained in DMEM-F12 glutamax complete growth media supplemented with 10% heat-inactivated fetal bovine serum (HI FBS). CellTiter-Glo Buffer & CellTiter-Glo Substrate were thawed at room temperature prior to use. An appropriate volume (10 mL) of CellTiter-Glo Buffer was transferred into the amber bottle containing CellTiter-Glo Substrate to reconstitute the lyophilized enzyme/substrate mixture. The contents were gently vortexed to obtain a homogeneous solution. U2OS cells were seeded at 10000 cells/150 μL/well in 96 well cell culture plate and incubated overnight at 37 °C and 5% CO_2. The next day, media was changed (150 μL), various concentrations of compounds (50 μL from 4× stock) were tested in duplicates, at eight concentrations, in semi log dilution pattern, with starting concentration of 100 μM. Final concentration of DMSO in the assay was 1 %. 10 μM, 5 μM and 3 μM of doxorubicin hydrochloride was used as positive control in the assay. The plates were incubated for 24 h and 48 h at 37 °C with 5% CO_2. After 24 h and 48 h of incubation,

CellTiter-Glo Reagent (Promega) preparation was added to the cell culture medium present in each well in a 1:1 ratio by volume (e.g., add 50 μL of reagent to 50 μL of medium containing cells). The plate was gently vortexed for 10 min on an orbital shaker to induce cell lysis. The contents of the plate were transferred to an opaque half area 96 well plate. The plate was incubated at RT for 10 min to stabilize the luminescence signal. Luminescence output was read on a Tecan Spark plate reader. The % cell viability was calculated relative to DMSO control.

MNAM Measurement in U2OS Cells by LC-MS/MS

Materials. Acetonitrile (Millipore Sigma 1000292500), D4-MNAM (BioOrganics SRK(I)-257-3226), MNAM (BioOrganics BST(I)-256-3271), DPBS (Invitrogen 14190-136), Top Seal-A Plus (Perkin Elmer 6050185), 96-well plate sterile TC treated (Eppendorf 0030730119), 96 well plate transfer plate (Costar 3364), reference compound 6-methoxy nicotinamide/(*alternate name:* JBSNF-0088) (Arbor chemical corporation Limited, P180810). U2OS cells were obtained from ATCC and maintained in DMEM-F12 growth media containing 10% HI FBS and 1% Pen-Strep (filter sterilized) in 37 °C incubator with 5% CO_2. Extraction buffer was prepared by adding internal standard (D4 MNAM, 20 ng/mL) to 100% acetonitrile.

Protocol. Cells were maintained in cell culture flasks until seeding into microwell plates. Media was removed, cells were washed with DPBS (-/-) and then detached with TrypLE

Express (4mL) into a 150 cm^2 flask (incubate at 37 °C for 5 min). The reaction was stopped by the addition of 5 mL media and cells were counted with a Vi-CELL cell counter. Cells were seeded into a 96-well microplate (100 μL/well = 10000 cells/well) and incubated for 24 h at 37 °C, 5% CO_2, 95% humidity with the lid on. Media was replaced by addition of 100 μL of media/compound mixture and cells were incubated for 24 h at 37 °C, 5% CO_2, 95% humidity with the lid on. The media/compound mixture was removed and cells were washed two times with 150 μL of DPBS(-/-). The DPBS was removed and 100 μL of extraction buffer was added. The plate was incubated for 20 min at RT with mild shaking. 100 μL autoclaved water was added and the plate was mixed gently. The plate was centrifuged at 5000g for 10 min and then 150 μL of the extraction buffer/water mixture (final D4 MNAM concentration of 10 ng/mL) was transferred into a 96-well microplate. MNAM levels were quantified by LC-MS/MS according to methods previously reported by Kannt et al.[15]

ASSOCIATED CONTENT

Supporting Information

The *Supporting Information* (SI) is available free of charge on the ACS Publications website.

Supplementary figures and tables, synthetic schemes, and experimental protocols not listed in the main manuscript, including: molecular docking, protein expression and purification, biochemical assays, bioinformatic analyses, and protein crystallography (SI: Part 1); NMR spectra (SI: Part 2); Enzyme Kinetics (SI: Part 3); Small molecule X-ray crystallography data for NS1 (**10**), **S30**, **S53** (CIF files); Molecular formula strings for compounds **10**-**41** (CSV).

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The authors declare no competing financial interests. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

NNMT, nicotinamide N-methyltransferase; SAM, S-adenosylmethionine; NAM, nicotinamide; MNAM, 1-methylnicotinamide; SAH, S-adenosylhomocysteine; COMT, catechol O-methyltransferase; BPE, 1,2-bis(phospholano)ethane; INMT, indolethylamine N-methyltransferase; PNMT, phenylethanolamine N-methyltransferase; TPMT, thiopurine S-methyltransferase; IStd, internal standard; rbf, round-bottom flask; PMHS, polymethylhydrosiloxane.

Accession Codes

The coordinates and structure factors of the hNNMT-NS1 (**10**) co-crystal structure have been deposited with the RCSB Protein Data Bank under accession code 6ORR. The authors will release the atomic coordinates upon article publication.

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