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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval/a samostatně a že jsem uvedl/a všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 14.09.2017

Podpis

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Abstract

As they make up DNA and RNA, nucleosides are considered the key to life. Synthetic nucleosides also constitute many drugs that treat viral infections and cancer. As a result, more efficient methods to access these crucial molecules would have implications that extend beyond a synthetic chemist's benchtop and into medicinal chemistry and medical research. One of the most challenging steps in the synthesis of nucleosides is the glycosylation step between the acceptor heterocycle (nucleobase) and the saccharide-based donor. Often to obtain satisfactory yield of this step with good regio- and stereochemical control the extensive use of protecting groups must be employed to squelch reactivity at unwanted reactive groups. Consequently, this process of protection–glycosylation–deprotection is laborious, inefficient, and often requires the use of toxic reagents. It would be, therefore, highly welcomed if new methodology to effect this glycosylation step was designed that reduces or removes the need to use protecting groups, but would still provide nucleosides in good yield, regio- and stereoselectively. Herein, this thesis presents my efforts into achieving this end. By employing modified Mitsunobu conditions, I determined that it is possible to directly glycosylate a nucleobase with D-ribose to afford stereoselectively β-ribopyranosyl nucleosides in the complete absence of protecting groups. By then employing a 5-*O*-monoprotected ribosyl unit, I could use a two-step one-pot process to provide the more medicinally and physiologically relevant β-ribofuranosyl nucleosides, but with some shortcomings to be discussed. In our second study, I improved the reaction conditions and elucidated a plausible mechanism that proceeds through an *in situ*-formed α-1,2-anhydrosugar (termed "anhydrose") that is then opened nucleophilically by the nucleobase stereoselectively at gram scale. This key anhydrose intermediate is stable indefinitely *in situ* and can be formed using other *C*5-modified ribosyl monomers as well. It can also be opened by other non-nucleobase-based substrates still perfectly stereoselectively for the β-anomer. We demonstrate that this anhydrose is a powerful electrophilic intermediate that can glycosylate a wide range of nucleobases and other nucleophiles to provide a host of β-ribosyl glycosides. This research provides the foundation for a new stereoselective reaction for medicinal chemists to add to their toolbox of reactions to aid in the design of novel drugs and therapeutics.

Abstrakt

Nukleosidy jsou jako stěžejní složka DNA a RNA považovány za základ života. Na bázi nukleosidů je také založeno množství protinádorových a protivirových léčiv. Efektivnější metody pro získání těchto důležitých molekul by proto měly dopady, které přesahují rámec práce syntetického chemika a zasahují až do medicinální chemie a lékařského výzkumu. Jedním z nejnáročnějších kroků syntézy nukleosidů je glykosylační krok mezi heterocyklickým akceptorem (nukleobáze) a donorem na bázi sacharidu. Pro zabránění nežádoucích reakcí dalších reaktivních skupin a získání uspokojivých výtěžků s dobrými regio- a stereochemickými vlastnostmi je často nutné použít chránící skupiny. Tento proces protekce–glykosylace–deprotekce je pracný, neefektivní a často vyžaduje použití toxických činidel. Nová metoda, která by snižovala nebo odstraňovala potřebu použití protektivních skupin při glykosylaci, a přesto regio- a stereoselektivně poskytovala dostatečný výtěžek nukleosidů, by proto byla nanejvýš vítána. Tato disertační práce představuje moji snahu dosáhnout tohoto cíle. Bylo zjištěno, že za použití modifikovaných podmínek Mitsunobuovi rekce je možné bez potřeby chránění ostatních skupin přímo a stereoselektivně glykosylovat nukleobázi D-ribosou za vzniku β-ribopyranosyl nukleosidů. Použití 5-*O*-monochráněné ribosylové jednotky bylo možné postupovat tzv. „dvoustupňovém, one-pot“ procesem, který poskytoval medicínsky a fyziologicky relevantní ribofuranosyl nukleosidy. Tento proces má však určité nedostatky, které budou dále diskutovány. V naší druhé studii jsem optimalizoval reakční podmínky a objasnil pravděpodobný mechanismus reakce. Ten probíhá přes *in situ* připravený α-1,2-anhydrocukr (tzv. anhydrosa), který se nukleofilním působením nukleobáze stereoselektivně. Reakci je možné provést rovněž v gramových navážkách s velmi dobrým výtěžkem. Tento klíčový intermediát v podobě anhydrosy je stabilní *in situ* a může být vytvořen i za použití dalších *C*5-modifikovaných sacharidových jednotek. Při zachování stereoselektivity reakce pro β-anomer mohou otevření cyklického intermediátu zajišťovat i další substráty, které nejsou založeny na nukleobázích. Zde demonstrujeme, že tato anhydrosa je silný elektrofilní intermediát, schopný glykosylovat širokou škálu nukleobází a dalších nukleofilů za zisku řady β-ribosyl glykosidů. Tento výzkum poskytuje základ pro novou stereoselektivní reakci, kterou mohou medicinální chemici využít při návrhu nových léků a terapeutik.

List of publications relevant to this thesis

1. Downey, A. M.; Hocek, M., *Beilstein J. Org. Chem.* **2017**, *13*, 1239–1279.
2. Downey, A. M.; Richter, C.; Pohl, R.; Mahrwald, R.; Hocek, M., *Org. Lett.* **2015**, *17*, 4604–4607.
3. Downey, A. M.; Pohl, R.; Roithová, J.; Hocek, M., *Chem. Eur. J.* **2017**, *23*, 3910–3917.

List of Abbreviations

Ac	acetyl
ADDP	1,1′-(azodicarbonyl)dipiperdine
Ara*f*	arabinofuranosyl
$BF_3{\cdot}Et_2O$	boron trifluoride etherate
Bn	benzyl
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
Bz	benzoyl
CuAAC	copper(I)-catalyzed azide–alkyne cycloaddition (click reaction)
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMC	2-chloro-1,3-dimethylimidazolium chloride
DMDO	dimethyldioxorane
DMF	dimethylformamide
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
DMT	4,4'-dimethoxytrityl
DNA	deoxyribonucleic acid
equiv.	equivalents
Gal*f*	galactofuranosyl

Gal*p*	galactopyranosyl
GSH	glycosyl sulfonohydrazines
GT	glycosyl transferase
HMDS	hexamethyldisilazane
IM	imidazole
IOCB	Institute of Organic Chemistry and Biochemistry
IUPAC	International Union of Pure and Applied Chemists
m.p.	melting point
MeCN	acetonitrile
MMTr	4-methyoxytrityl
MOP	3-methoxypyridyl
NBS	*N*-bromosuccinimide
NDP	nucleotide diphosphate
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
PDB	Protein Data Bank
PNP	purine nucleoside phosphorylase
r.t.	room temperature
RNA	ribonucleic acid
ROESY	rotational frame nuclear Overhauser effect spectroscopy
TBAF	tetrabutylammonium fluoride
TFA	trifluoroacetic acid
THF	tetrahydrofuran

TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TP	thymidine phosphorylase
Tr	trityl (triphenylmethyl)
TsCl	*p*-toluenesulfonyl chloride
TsOH	*p*-toluenesulfonic acid
UDP	uridine diphosphate
UP	uridine phosphorylase
WHO	World Health Organization

Table of Contents

1. Introduction

1.1 Nucleosides

Nucleosides are crucial to life as they constitute the fundamental components of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in nature. Natural nucleosides consist of a ribose-based sugar component and a heterocycle, specifically a purinyl- or pyrimidinyl-based heterocycle. In DNA, the sugar component is 2-deoxy-D-ribose and in RNA the sugar component is D-ribose. There are four canonical nucleosides of which DNA is constructed. Two of the nucleosides contain a purine-based heterocycle, 2′-deoxyadenosine and 2′-deoxyguanosine, and two contain a pyrimidine-based heterocycle, thymidine and 2′-deoxycytidine (Figure 1.1). In RNA there are also four. The purine-based nucleosides are adenosine and guanosine and the pyrimidine-based ones are called uridine and cytidine. The numbering of the positions on the heterocycle is classical and does not abide by the nomenclature rules described by the International Union of Pure and Applied Chemists (IUPAC).

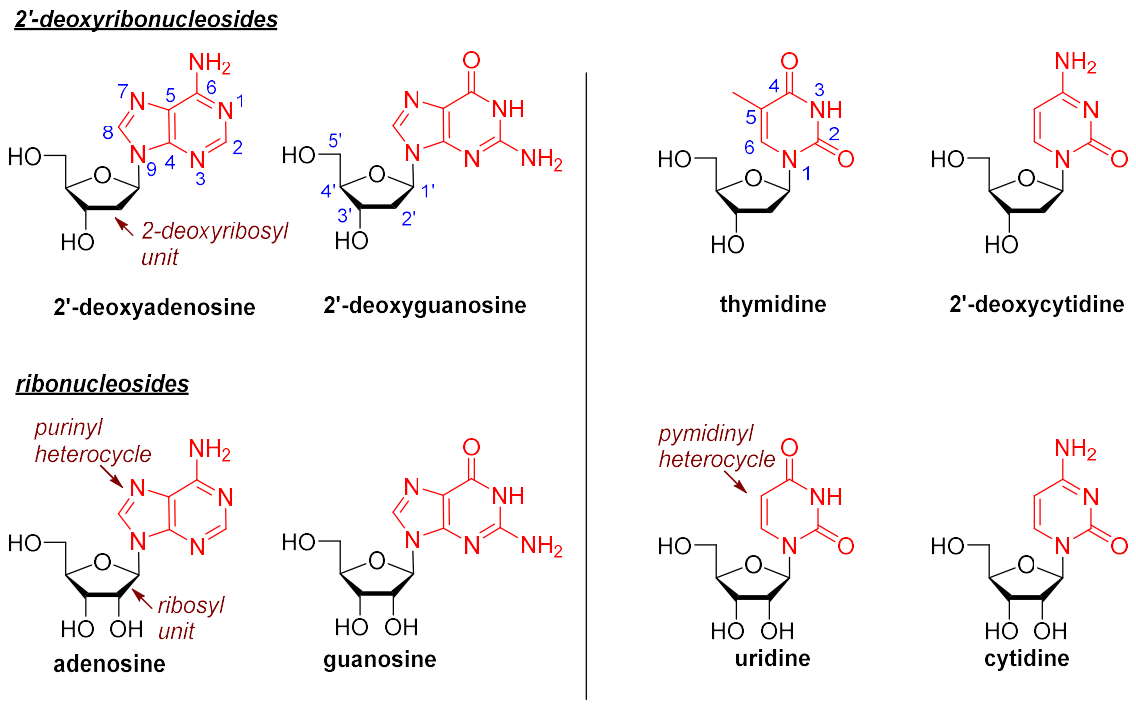


Figure 1.1: The eight canonical nucleosides that make up DNA and RNA in nature.

Synthetic nucleosides (those not found in nature) are also extremely valuable as they are a component of many medicines, especially anticancer and antiviral agents.[1-3] Shown in Figure

1.2 is a list of some of the important antiviral and anticancer medicines currently on the market or undergoing clinical trials.[1] In each case the nucleobase is highlighted in red and the saccharide component in black. It would be of tremendous value to the medical community if the saccharide component and the nucleobase component could be coupled directly, potentially dramatically expediting access to these precious compounds.

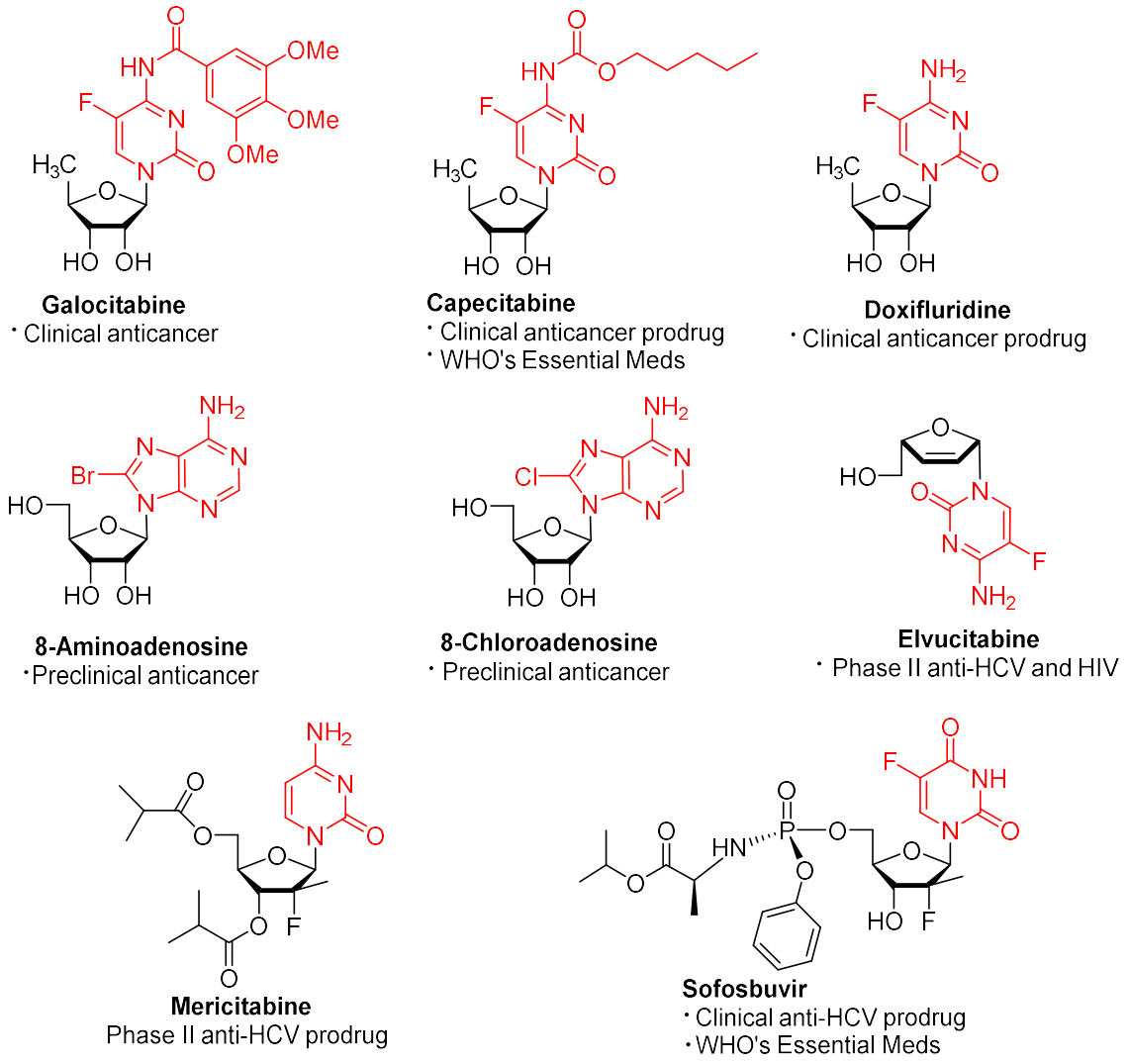


Figure 1.2: The structures of some important nucleoside-based anticancer and antiviral drugs.

1.2 Glycosylation

Glycosylation is one of the most important chemical transformations known in nature as it is thought to be the most prevalent post-translational modification process.[4] Glycosylation is a coupling reaction that ligates the anomeric centre (*C*1-OH) of a carbohydrate, called the donor,

to a second molecule, called the acceptor to afford a glycoside (the product). In the chemical synthesis of glycosides the donor molecule is electrophilic. This means that typically the other potentially nucleophilic hydroxyl groups on the saccharide must be protected to prevent the donor from reacting with itself. The acceptor molecule serves as the nucleophile and very often the other reactive groups on the molecule must be blocked to prevent glycosylation at unwanted molecular sites. This results in what is very often a laborious, multistep process that can require the employment of toxic reagents and organic solvents.[5-8]

Natural acceptor molecules are other saccharides to form oligosaccharides, amino acid side chain chains to produce glycoproteins or proteoglycan, lipids to form glycolipids, and nucleobases to yield nucleosides. Figure 1.3 contains a common example of each class of glycoside. Oligosaccharides, glycoproteins, glycolipids, and nucleosides are crucial to life, and are, therefore, implicated in a plethora of biological pathways, but also diseases. As a result, more streamlined approaches to effect glycosylation to produce glycosides is a highly attractive area of research for synthetic chemists as these methods could find utility in applications that extend well beyond a chemical laboratory, including medicine. This thesis will first provide an overview of classical protecting group-employed glycosylation strategies for the synthesis of *O*-glycosides and nucleosides (*N*-glycosides) before discussing novel protecting group-free glycosylation methods and their mechanism for success.

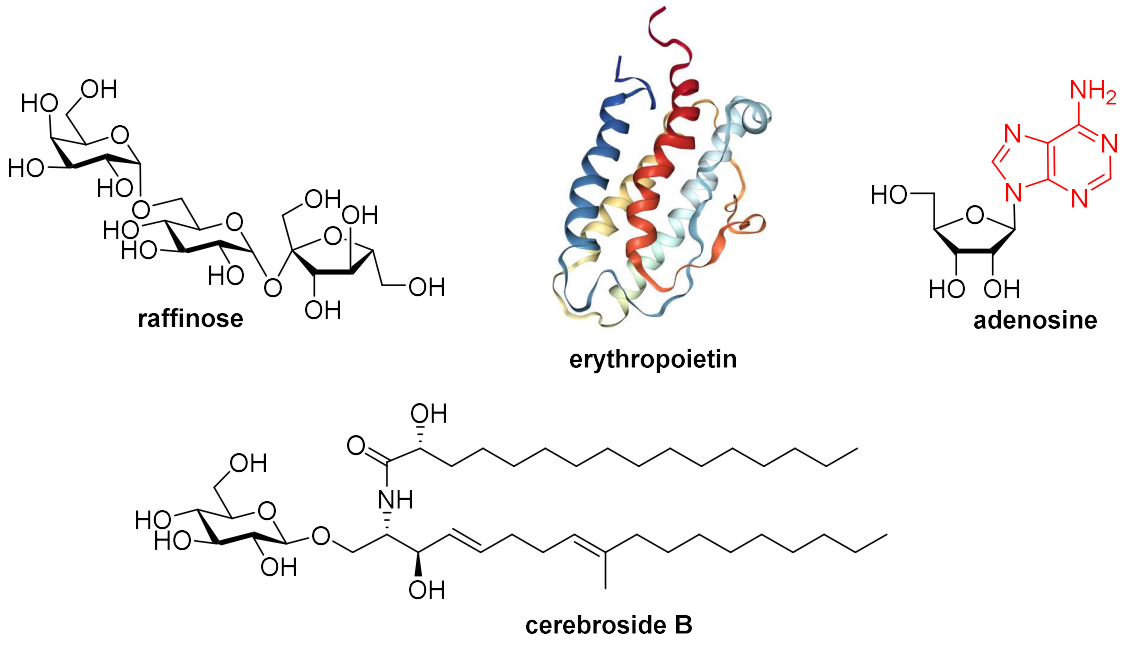


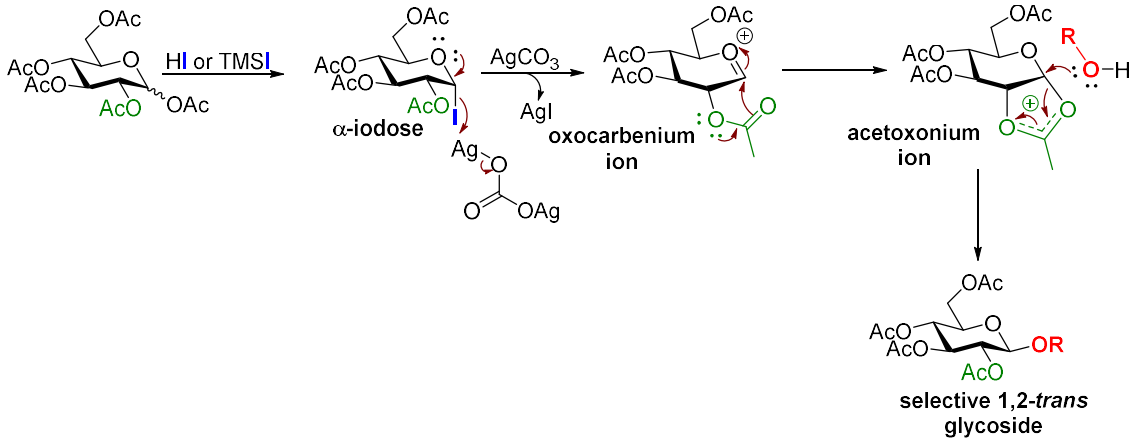
Figure 1.3: Structures of a common oligosaccharide, glycoprotein, glycolipide, and nucleoside. Image of human erythropoietin taken from the Protein Data Bank (PDB), code 1BUY, actual glycans have been omitted.[9]

1.2.1 Classic protecting group-employed glycosylation approaches

1.2.1.1 O-glycosylation

1.2.1.1 Koenigs-Knorr reaction

In the classical approaches to the synthesis of *O*-glycosides several methods have been described over the last 100 years. One of the most common and oldest methods to effect *O*-glycosylation is the Koenigs-Knorr reaction[10-11] which was discovered over 100 years ago. The reaction utilizes a (usually) *in situ*-formed glycosyl halide derived from the parent *per*-acetylated sugar (representative example shown Scheme 1.1). Typical reagents to provide the iodide are HI or now more commonly trimethylsilyl iodide (TMSI). Initially the β-iodose is formed before quickly equilibrating to the α-anomer.[12] The donor is then treated with an alcohol acceptor and the promoter $AgCO_3$. A putative oxocarbenium ion is formed as shown in Scheme 1.1 that then reacts with the acetyl group at the *C*2-OH to produce an acetoxonium ion in a process known as neighboring group participation or anchimeric assistance. The acetoxonium ion blocks the α-face of the molecule so the acceptor can only attack from the β-side to provide the 1,2-*trans* product with good diastereoselectivity.[13-14] When mercury salts are used in lieu of silver salts, the reaction is referred to as the Helferich method.[15-16] Major drawbacks of this method are controlling the formation and reactivity of the glycosyl halide and the necessity to employ highly toxic reagents.[17-18]

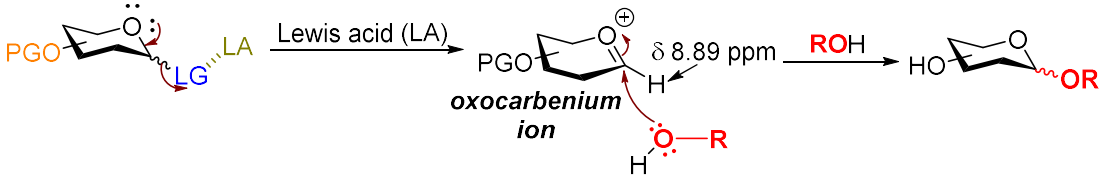


Scheme 1.1: Koenigs-Knorr glycosylation.

1.2.1.2 Lewis acids

Lewis acid-catalyzed (or mediated) activation of the anomeric centre is another classical heavily utilized glycosylation strategy. Typical Lewis acids employed include trimethylsilyl trifluoromethanesulfonate (TMSOTf) and boron trifluoride etherate ($BF_3{\cdot}Et_2O$). Generally a

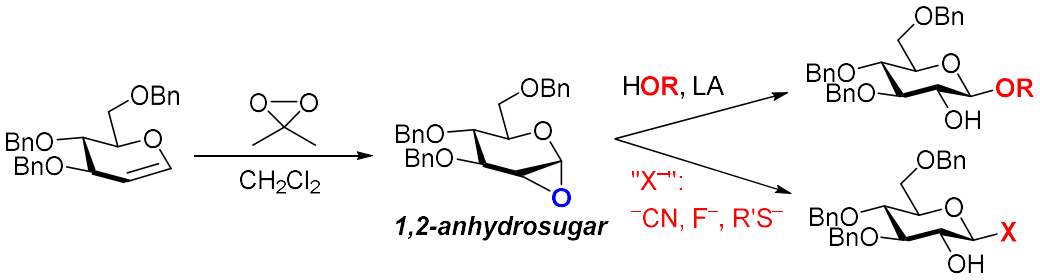
leaving group is installed on the *C*1-hydroxyl that is primed for displacement by the Lewis acid (Scheme 1.2). The reaction then proceeds through an oxocarbenium ion that was very recently observed using low-temperature (–40 ºC) NMR stabilized by the HF/SbF_5 superacid.[19] The highly electrophilic carbon (as can be inferred by the extreme downfield shift in the ^{1}H NMR of *C*1-H) adjacent to the oxocarbenium ion is subsequently reacted with the nucleophilic acceptor in either and S_N1- or S_N2-like mechanism depending on the chemical stability of the glycosyl cation.[20] The stereochemistry of the product is generally directed either by neighboring group participation of the protecting group on the *C*2-OH or by the anomeric effect when the protecting group is incompatible with neighboring group participation.[21-22]



Scheme 1.2: Classic Lewis acid-catalyzed glycosyation.

1.2.1.3 1,2-anhydro sugars

Danishefsky and collaborators,[23-24] have famously studied another approach to synthesize pyranosyl *O*-glycosides stereoselectively. The method operates via a fully protected intermediate 1,2-anhydrosugar that can be carefully isolated after treatment of the parent glycal with dimethyldioxorane (DMDO). Protected disaccharides can then be generated via epoxide ring opening in the presence of many Lewis acids (commonly $ZnCl_2$) or other pyrano-glycosides can be synthesized by nucleophilic ring opening to afford (for example) fluoro, thiol, and cyano pyranosides (Scheme 3) with good stereoselectively to furnish 1,2-*cis* glycosides.[25]



Scheme 1.3: Diastereoselective glycosylation via epoxide ring opening.

One major benefit of this pathway is it is inherently stereoselective as generally the 1,2-*cis* glycoside is formed in solely or in large excess. The glycosylation is also effected under mild

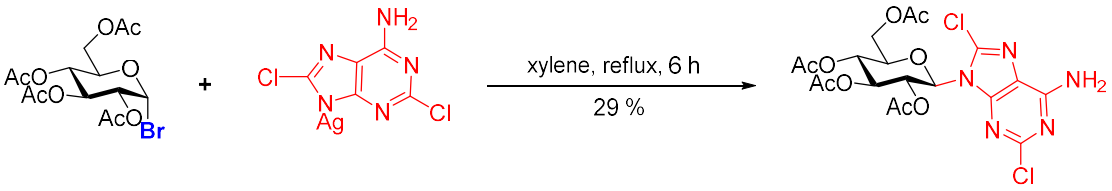
conditions. However, the synthesis of the parent glycal is laborious and is accessible only after numerous steps.

1.2.2 N-glycosylation (nucleosidation)

A glycosylation reaction between a heterocycle (nucleobase) and a saccharide to produce a nucleoside is termed *N*-glycosylation (nucleosidation) and is also very often troublesome as both the donor (the sugar) and the acceptor (the nucleobase) can be reactive at several positions. This means in the chemical laboratory both regio- and stereochemical outcomes must be considered, and very often protecting groups must be employed on both the carbohydrate and the heterocycle to prevent unwanted reactivity.[26]

1.2.2.1 Fischer Method

Historically, three classical methods for the synthesis of nucleosides and nucleoside analogues are most commonly employed. The oldest is known as the Fischer method (or metal salt method) and was first described by Emil Fischer in 1914.[27] In the most general terms, the metal salt of a nucleobase is reacted with an α-halogenose to form a β-nucleoside. Fischer's original paper employed the silver salt of the nucleobase, but other early examples also used the mercury salt.[15, 28] Modern versions use the sodium salt[29] which is likely less toxic. Scheme 1.4 highlights one example from Fischer's seminal work to synthesize (presumably) a β-glucosyl nucleoside from the silver salt of 6-amino-2,8-dichloropurine and bromoacetoglucose. The reaction should be stereoselective and the mechanism is thought to be similar to the one described in Scheme 1.1 above with anchimeric assistance resulting from the *C*2-OAc group.

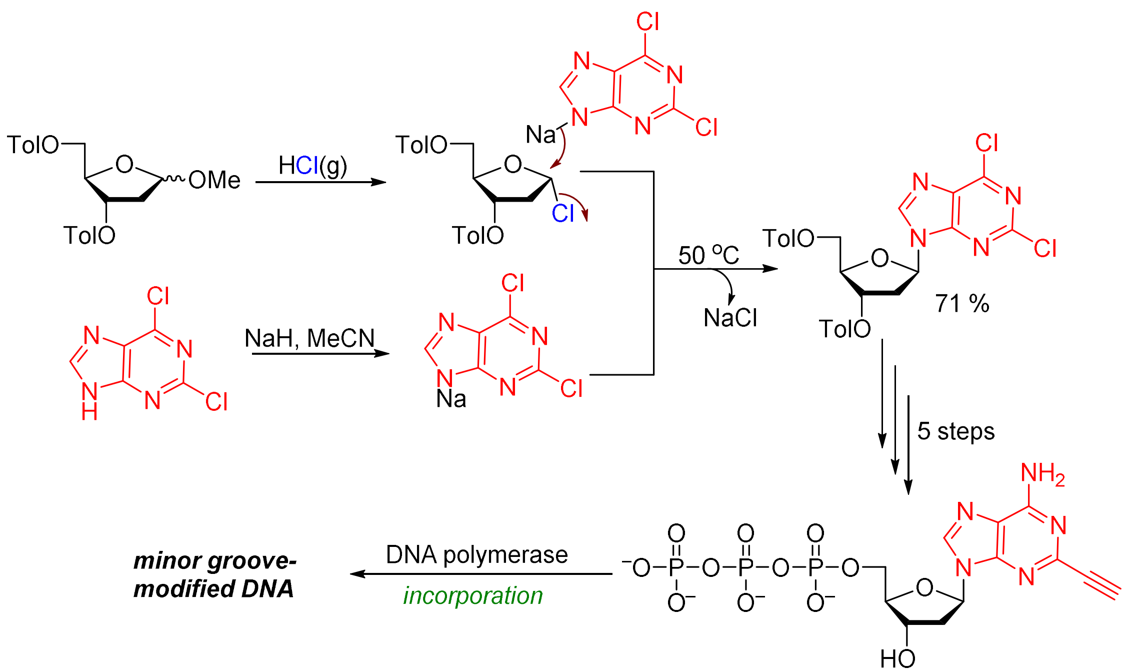


Scheme 1.4: Fischer's original synthesis of a glucosyl nucleoside by displacement of a glycosyl halide.

1.2.2.2 Metal salt method

The metal salt method is utilized for the synthesis of 2′-deoxy nucleosides as well. In this case neighboring group participation is obviously not possible, however, under the right conditions, the l-chloro-2-deoxy-α-*erythro*-ribofuranose can be formed selectively[30-32] then attack by the sodium salt of the nucleobase will furnish the desired β-2′-ribofuranosyl nucleoside in a

stereospecific S_N2 process. A very early example of this from Robins and colleagues[29] is depicted in Scheme 1.5. The sodium salt of 2,6-dichloropurine is generated *in situ* and then reacted with the α-halogenose with mild heating to afford the β-nucleoside in good yield. Recently our group then utilized this intermediate as starting material in the synthesis of nucleosides for minor groove modification of DNA.[33] Once incorporated, a fluorescent label can be attached via the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC, "click reaction").[34]

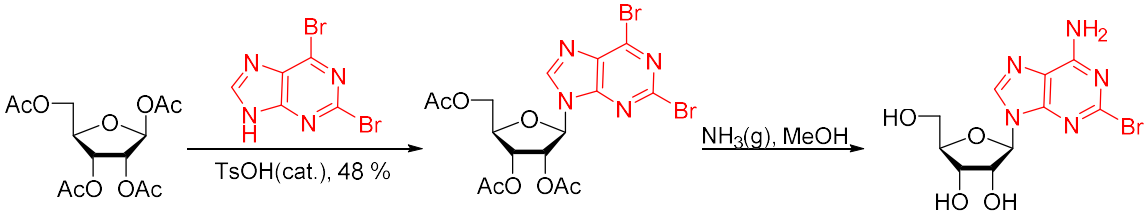


Scheme 1.5: The metal salt method applied to the synthesis of 2′-deoxyribofuranosyl nucleosides.

1.2.2.3 Fusion method

The second example, first described in 1961 by the Sato group,[35] utilizes the direct fusion of a *per*-acylated saccharide with a nucleobase in the absence of solvent at elevated temperature in the presence of an acid catalyst to provide the protected nucleoside. One example is highlighted in Scheme 1.6 for the synthesis of 2-bromoadenosine.[36] The fusion reaction is apparently stereoselective for the β-anomer, albeit in moderate yield. The dibromonucleoside could then very simply converted to 2-bromoadenosine by treatment with methanolic NH_3. This method is also amenable to the synthesis of purine-based 2′-deoxy-[37] and 2′,3′-dideoxynucleosides[38] as well, however, the reaction suffers from a complete lack of stereochemical control.

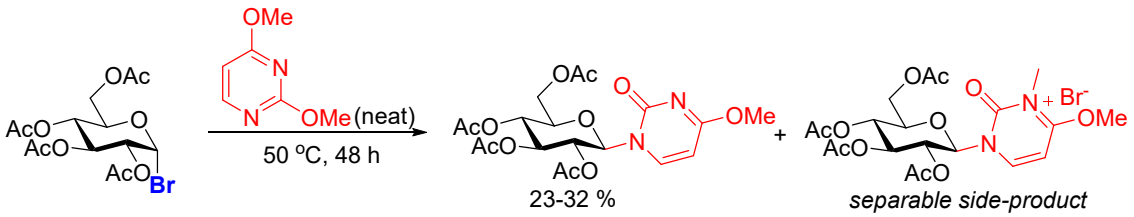
The definite benefit of this procedure is its operational simplicity and the fact that no manipulation is required on the nucleobase. However, it is limited in scope to purines and is hampered by low yields.[39]



Scheme 1.6: Direct fusion method to access purine nucleosides.

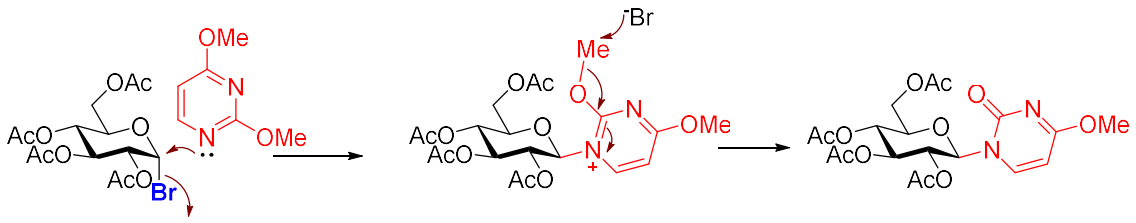
1.2.2.4 Vorbrüggen reaction

The third, but by-far the most widely utilized chemical glycosylation strategy for the synthesis of nucleosides, is the Vorbrüggen variation[40-41] of the Hilbert–Johnson reaction.[41-42] The reaction, originally described in a communication by G.E. Hilbert and T.B. Johnson in *Science* in 1929[42] and a full paper in the *Journal of the American Chemical Society* in 1930,[43] is similar to the Fischer method,[27] however, it describes the coupling between a 2,4-dialkoxypyrimidines and an acylated glycosyl halide to afford pyrimidine nucleosides. In their seminal work, they describe the synthesis of a glucosyl uridine analog from 2,4-dimethoxyuracil and 2,3,4,6-tetraacetylribofuranosyl bromide in very modest yield (Scheme 1.7).[43] The undesired *N*-methylated side-product can be inferred by reaction of the nucleophilic nitrogen with MeBr.



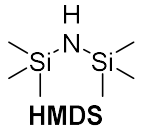
Scheme 1.7: Original Hilbert-Johnson reaction.

It is believed that a full mechanistic investigation has never been conducted, but a proposed mechanism[44] is shown in Scheme 1.8 below. It can also be assumed the β-anomer was formed in pure form due to the narrow melting range of the product reported by the authors (220–221 °C), however, this is impossible to confirm.

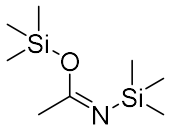


Scheme 1.8: Plausible mechanism for the Hilbert–Johnson reaction.

This reaction was dramatically improved upon by Vorbrüggen and colleagues over the course of many years, but was first described in 1970.[40] The Vorbrüggen reaction (or the *silyl*-Hilbert-Johnson reaction) in the simplest terms describes the glycosylation of a silylated nucleobase by a *per*-acylated sugar in the presence of a Lewis acid to provide a protected nucleoside in an analogous manner to the Friedel–Crafts reaction.[41] Typical nucleobase silyating agents include bis(trimethylsilyl)amine (hexamethyldisilazane, HMDS) and *N*,*O*-bis(trimethylsilyl)acetamide (BSA).

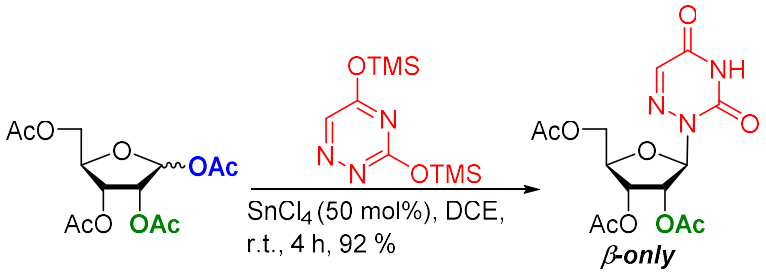


HMDS



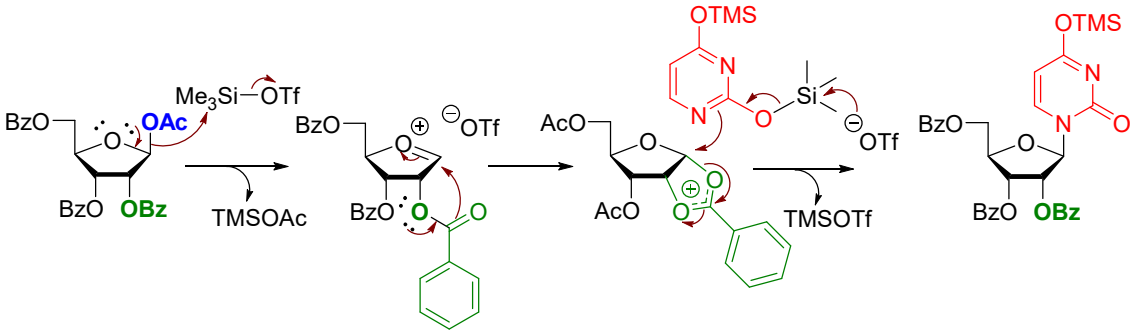
BSA

The most common Lewis acids to effect the transformation are TMSOTf or $SnCl_4$, and were actually first applied in 1963 to the Hilbert–Johnson reaction, prior to adoption by Vorbrüggen.[45] Other Lewis acids have been employed, as well, including recently published Au(I)-[46] and Tf_2O-catalysed[47] conditions. MeCN is the most popular solvent to employ for the reaction,[41] however, DCE can be employed when $SnCl_4$ is used.[41, 48] Highlighted in Scheme 1.9 is Vorbrüggen's original synthesis of protected 6-azauridine. Their seminal work utilized DCE as solvent and $SnCl_4$ as the acid catalyst. It was wholly stereoselective for the β-anomer and formed in very good yield at room temperature.



Scheme 1.9: Vorbrüggen's original synthesis of pyrimidine nucleosides in the presence of the Lewis acid, $SnCl_4$.

It can be noted that this reaction is robust and works for a host of pyrimidines and purine nucleobases further adding to its appeal. As a result, the mechanism for this reaction has been well studied. When the acceptor molecule is a pyrimidine, the mechanism is rather straightforward (Scheme 1.10). The mechanism shown uses TMSOTf as this Lewis acid catalyst as it considered to be milder, easier to handle, and has better regiochemical control than its most common counterpart, $SnCl_4$.[49-51] The rationale for the perfect or near perfect stereoselectivity comes from neighboring group participation of the *C*2-OH acyl group. In the first step of the reaction there is Lewis acid-promoted oxocarbenium ion formation via displacement of the anomeric acetyl group. The *C*2-OBz group then attacks the oxocarbenium ion from the α-face of the molecule to produce a benzoxonium ion that obstructs further nucleophilic attack from the bottom face. The TMSOTf is then regenerated by attack of the triflate on the *O*5-silyl group on the uracil derivative which nucleophilically opens the transient benzoxonium ion ring to furnish the protected β-uridine analog stereoselectively.

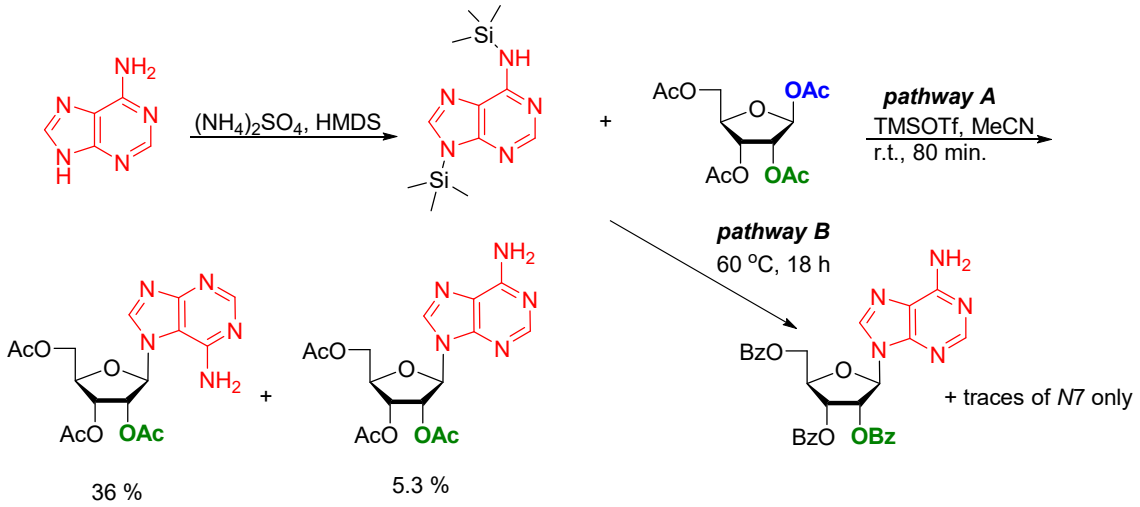


Scheme 1.10: Proposed mechanism for Vorbrüggen glycosylation.

The reason for the widespread use of this procedure is obvious: the troubling and toxic synthesis of the parent halogenose is circumvented. Furthermore, when the sugar contains an acylated *C*2-OH group, anchimeric assistance takes place resulting in a highly stereoselective reaction for the 1,2-*trans* product.

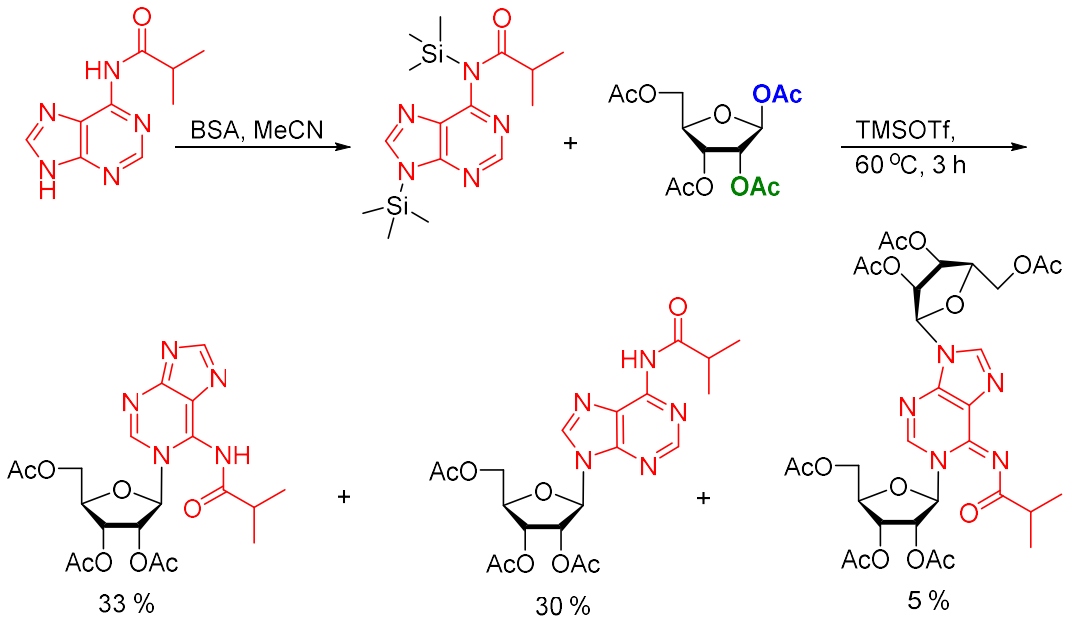
Although the reaction is highly utilized for the synthesis of purine nucleosides as well, depending on the nucleobase, some regiochemical hurdles may present.[51-53] One particularly thorough study on the glycosylation of adenine and its analogs, was published in 2006 by Framski et al to highlight this regioselectivity issue and to shed mechanistic insight on the matter. They showed that when adenine is used as the glycosyl acceptor, the Vorbrüggen reaction first generates the kinetic N^7 product in large excess (Scheme 1.11, pathway A), that

rearranges to the thermodynamically-favored (and usually desired) N^9-adenosine analog under prolonged reaction time and heating (Scheme 1.11, pathway B).[52]



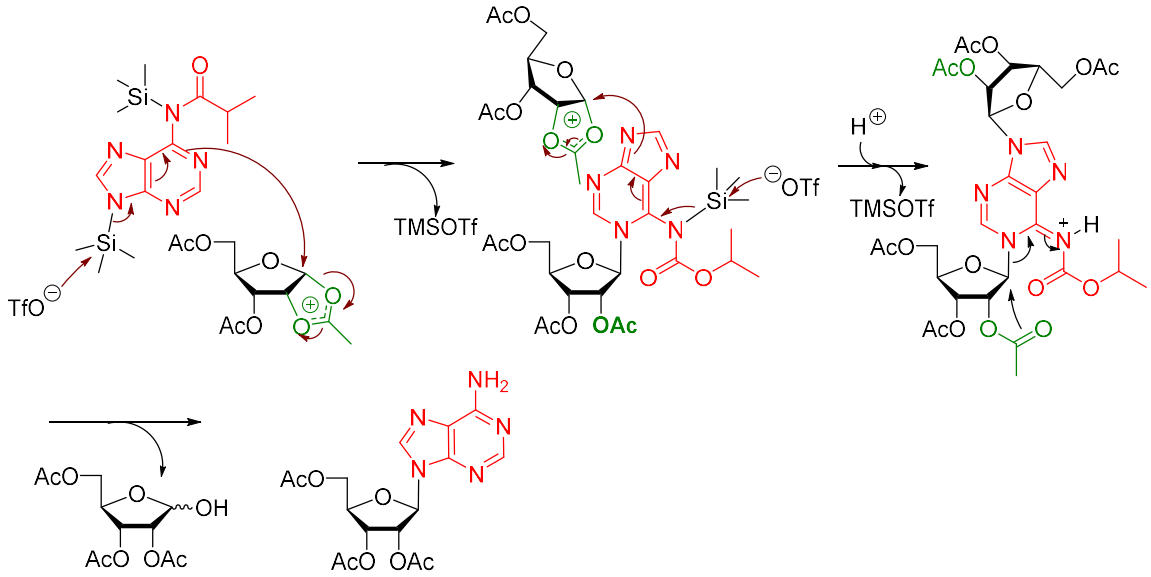
Scheme 1.11: Vorbrüggen glycosylation of adenine under both kinetic (pathway A) and thermodynamic control (pathway B).

Interestingly, when partially protected N^6-isobutyryladenine (or N^6-benzoyl) is employed a different kinetic product is observed (N^1 regioisomer) initially, before rearrangement to the thermodynamically-favoured adenosine analog (Scheme 1.12).[52] They also noticed that if careful chromatographic separation was employed, they could isolate the N^1,N^9-bisribosyl derivative as well.



Scheme 1.12: Vorbrüggen glycosylation of N^6-isobutyryladenine by 1,2,3,5-tetraacetly-β-D-ribofuranose.

As a result of these findings, the authors could posit a very plausible mechanism for the isomerization of the reaction from N^1 to N^9, which was first proposed in 1981 by Vorbrüggen and Hofle, but could not be confirmed[49] (Scheme 1.13). Initially the bis-silyated purine reacts with the acyloxonium ion of the tetraacetyl sugar generated under the Lewis acid conditions. The desired glycosylation position, N^9, is in fact blocked by a TMS group, hence, it can be presumed that intially ribosylation would take place more expediently at an alternative nitrogen. The N^1-glycosylated adenine analog then undergoes intermolecular transglycosylation to provide the thermodynamically favoured aromatic system, which is the N^9-H tautomer. With the N^9 no longer blocked by the silyl group, a second glycosylation takes place at N^9 to provide a bis-ribosylated adenine, which upon protonation at the N^6 position, results in liberation of the saccharide at N^1 to provide the thermodynamic monoglycosylated N^9 regioisomeric product. Noteworthy, is this pathway is analogous to a mechanism proposed by Vorbrüggen himself and his colleagues for the isomerization of the kinetic N^3 analog to the thermodynamically-favored N^1 analog in when glycosylating pyrimidines.[51]



Scheme 1.13. Plausible mechanism for the conversion of the kinetic N^1 product to the thermodynamic N^9 product.

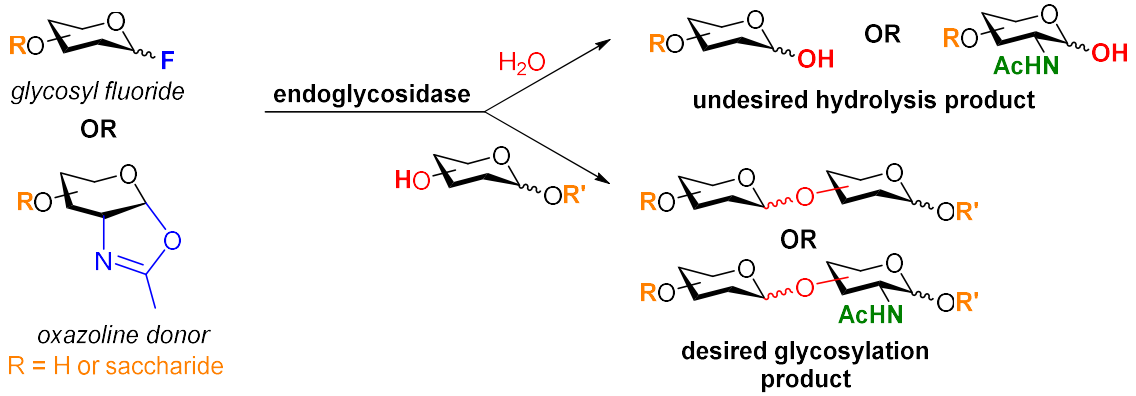
As seen in this section, chemical glycosylation and nucleosidation is of extreme importance to synthetic chemists and numerous methods are now available to effect these transformations.

Another very useful tool that must be discussed as well is the enzymatic synthesis of glycosides and nucleosides which also offers many advantages when constructing molecules. This thesis briefly discusses three important methods in the following section.

1.2.3 Enzymatic glycosylation

1.2.3.1 Glycosynthases

For the synthesis of glycosides, two classes of enzymes dominate, glycosynthases and glycosyltransferases. Glycosynthases are synthetic enzymes that catalyze the *formation* of a glycosidic bond between two sugar molecules. They were evolved over the course of many years from naturally-occurring glycosidase enzymes, which, in fact, catalyze the *hydrolysis* (i.e. the reverse process) of glycosyl bonds.[54] Traditional approaches in the transglycosylation of saccharides to form longer oligosaccharide chains utilize an endoglycosidase that couples a donor and an acceptor *in situ* to provide the lengthened oligosaccharide.[55-56] The primary challenge in transglycosylation is the competing hydrolysis reaction, which is thermodynamically favored. However, due to many incredible years of research in the field this problem can be largely circumvented with appropriate mutant selection through directed evolution[54] and donor design advancement.[56] The two most successful saccharide donors to date have been 1-fluoroglycosides or oxazolines derivatives (Scheme 1.14).

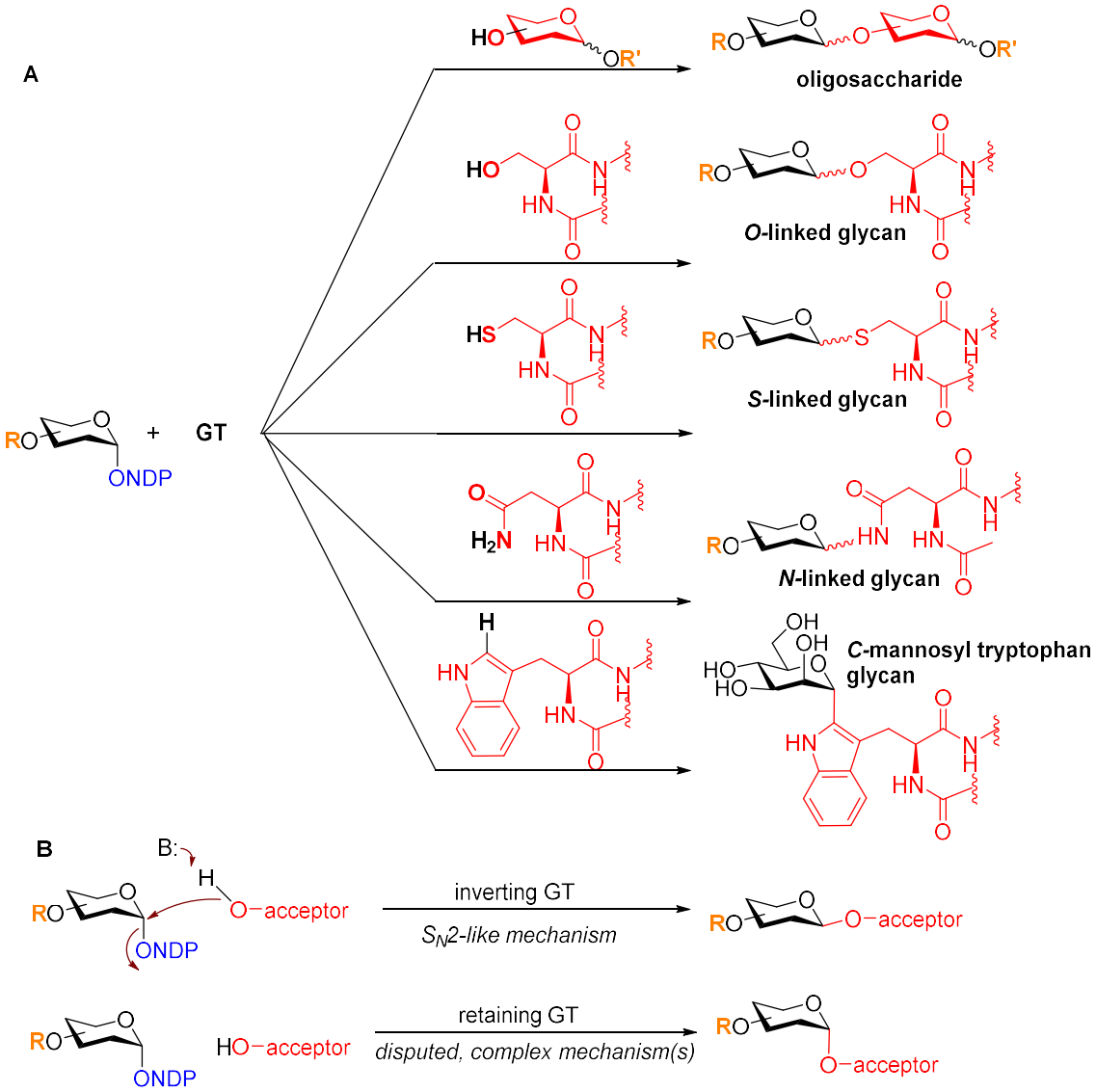


Scheme 1.14: Enzymatic synthesis of oligosaccharides.

1.2.3.2 Glycosyltransferases

Glycosyltransferases (GTs) catalyze the transfer of a carbohydrate from an activated nucleotide saccharide donor, typically a nucleotide diphosphate (NDP), to a nucleophilic glycosyl acceptor to provide *O*-, *N*-, *S*-[57] and even *C*-linked[58] glycosides (Scheme 1.15A). GTs are generally split into two classes, inverting and retaining, characterized by their mechanism of

action.[59] Inverting GTs furnish glycosides where the acceptor is glycosylated with the opposite stereochemistry at the anomeric position to the donor. The glycosylation goes through an S_N2-like pathway that is reasonably well understood. Retaining GTs create glycosides with the same stereochemistry at the anomeric position as the donor in a mechanism(s) that is less clear and is still a topic of much debate (Scheme 1.15B).[60] The power of these enzymes is well established and extends beyond glycobiology and into natural product synthesis as the aglycone of a natural product glycoside can be ligated to the carbohydrate moiety using natural or engineered GTs.[61]

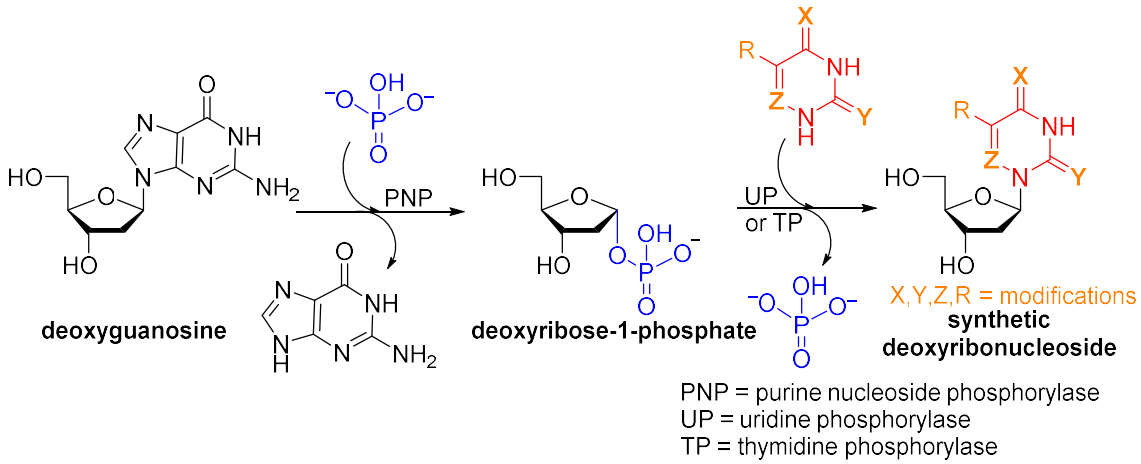


Scheme 1.15: A) Highlighting GTs for the synthesis of oligosaccharides and other biologically active glycosides. **B)** Inverting and retaining GTs. NDP = Nucleotide diphosphate

The power of glycosyl transferases is not limited to carbohydrate chemistry either. It is a powerful tool for the synthesis of nucleotides as well. These enzymes can be implemented in drug design, hence offering value to the medicinal chemistry community, too. Furthermore, these enzymes are usually regioselective for the N^9 position of purines and the N^1 position of pyrmidines, which, as discussed above is a persisting challenge in the chemical synthesis of nucleosides and nucleoside analogs.[26, 62]

1.2.3.3 Nucleoside synthesis

In the enzymatic synthesis of nucleosides, two enzymes are typically used in a tandem process. In the first step, a nucleoside phosphorylase in the presence of inorganic phosphate converts a natural nucleoside into (2-deoxy)ribose-1-phosphate that then serves as the donor substrate for a deoxyribosyltransferase to forge the connection between the saccharide and the synthetic heterocycle acceptor.[63] In Scheme 1.16, a recent elegant example from Seela and colleagues is highlighted for the synthesis of biologically active pyrimidine nucleosides using *E. Coli* NPs.[64]



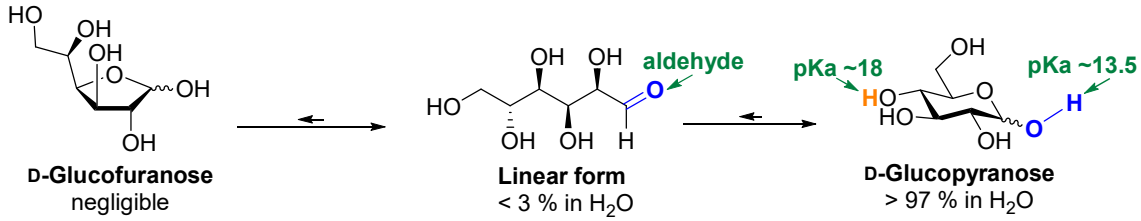
Scheme 1.16: Enzymatic synthesis of nucleosides.

As demonstrated in this section, the power of utilizing enzymes in the synthesis of glycosides and nucleosides is obvious. Perhaps most importantly because they usually offer high fidelity and chemo-, regio-, and stereoselective products. However, it should be noted that usually the acceptor moiety (and less often the donor saccharide) is a complex molecule that is laborious to make and extensive use of protecting groups is required.

1.2.2 Protecting group-free glycosylation strategies

Obviously, an idealized glycosylation scenario would be a strategy that is still chemo-, regio-, and stereoselective even when protecting groups are not employed under mild conditions. Superficially, this seems exceedingly difficult, however, thanks to incredible efforts of many

scientists several protecting group-free glycosylation strategies do exist. Also, in the last 10 years a large increase in the volume of literature available on this topic has been published, demonstrating that this is a rapidly evolving field. The cornerstone of protecting group-free glycosylation is exploiting the differential reactivity of the anomeric center. Two key features of the anomeric center provide this possibility. Firstly, the anomeric position of all unprotected monosaccharides is a reducing end (i.e. in equilibrium as an aldehyde or ketone) making this center more electrophilic (Scheme 1.17).[65] Secondly, the pKa of the anomeric OH group (in glucose pKa ~12.5[66] or 14[67]) is several orders of magnitude lower than the other hydroxyl groups (pKa ~16–18)[66-67] so by making a judicious selection of base should allow for selective deprotonation of this hydroxyl group over the others, hence creating selectively a better nucleophile than the other protonated ones, in what is essentially an umpolung process.



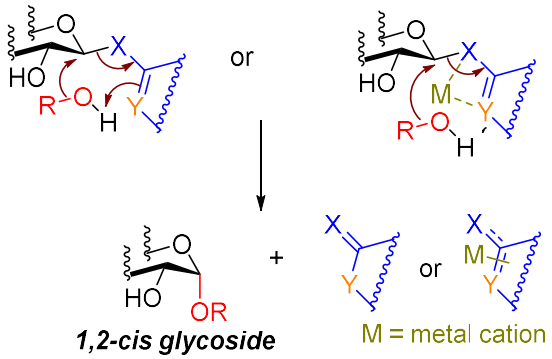
Scheme 1.17: Solution-state conformations of D-glucose.

This thesis next provides an overview on three classes of compounds that are known to effect glycosylation in the absence of protecting groups based on their method of activation at the anomeric centre. Firstly, this thesis will highlight some strategies that can be termed an indirect method to activate the anomeric center. In these strategies, the actual glycosylation coupling takes place in the absence of protecting groups, however, the donor in the reaction did require the use of protecting groups to access. Despite this is very major drawback, many of the methods are very innovative and will be discussed briefly. Incredibly, the other two classes of molecules to carry out the glycosylation, do so in the complete absence of protecting groups. The first of these are termed direct anomeric activating agents and the second class are *in situ* activators of the anomeric centre. The mechanism by which these molecules produce glycosides in the absence of protecting groups will be discussed when known. A more in depth version of the following sections can be found in a review by Downey & Hocek, *Beilstein J. Org. Chem.* **2017**, *13,* 1239–1279.[68]

1.2.2.1 Remote intramolecular activation

The first concept discussed is that of remote intramolecular activation which has been primarily studied and developed by Hanessian and colleagues over the course of 30 years. In 2000 his

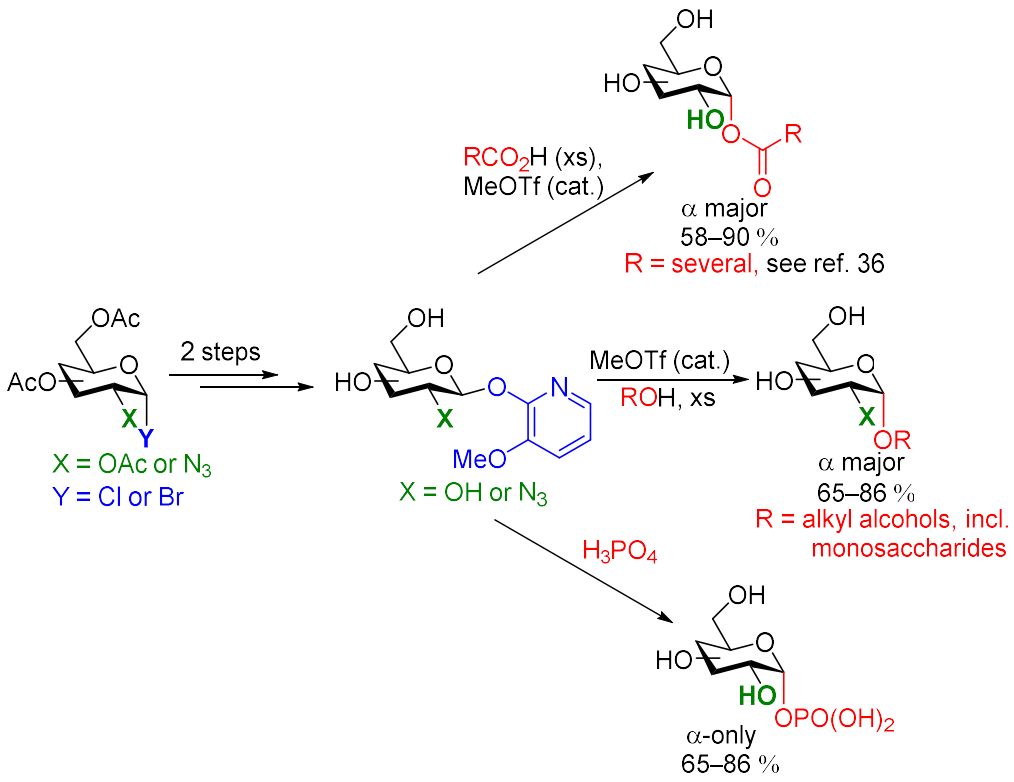
group published a thorough review on this method[69] so this thesis will focus on the more recent applications of this chemistry. The basis of this chemistry is that the activation of the *C*1-OH group of the donor molecule is effected by an interaction between a promotor and an atom not directly attached to the anomeric position (Scheme 1.18). The anomeric activating group contains two heteroatoms, X and Y, that can be activated at the remote atom (Y) by an electrophilic species (H of an alcohol) or a metal cation resulting in a reactive intermediate. This complex could then undergo an S_N2-like attack of a hydroxyl group to furnish the glycoside with inversion of stereochemistry at the anomeric position.



Scheme 1.18: The basis of remote activation adapted from ref. 68.

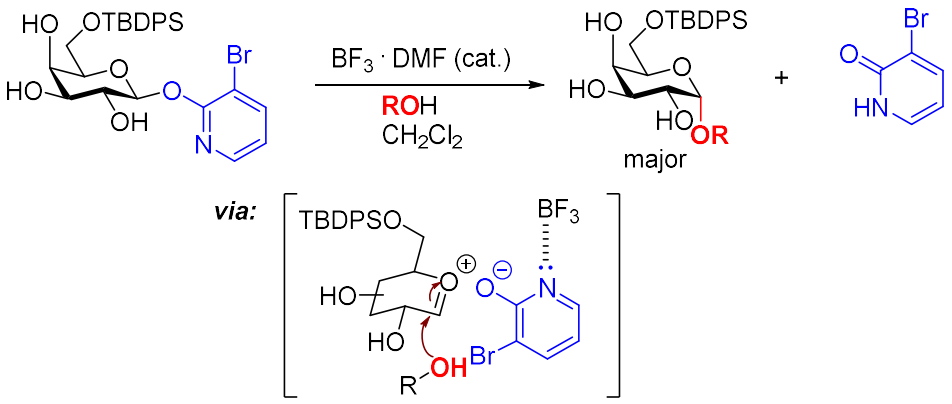
1.2.2.1.1 Pyridyl donors

The bulk of their early work employed a 3-methyoxypyridyl (MOP) activating agent at the anomeric position which could provide under the right conditions regioselectively and stereoselectively 1,2-*cis* glycosides of a wide variety. The substrate scope is broad and even includes glycosyl phosphates and esters in addition to many alcohol acceptors (Scheme 1.19). Certainly the most important application of this chemistry is its ability to provide 1,2-*cis* glycosides with good stereoselectivity, as these glycosides still remain among the most challenging stereoisomers to synthesize as *C*2 neighboring group participation is not possible.[70] In fact, access to 1,2-*cis* glycosides is considered a major impetus for progress in synthetic carbohydrate chemistry.[71] Also noteworthy is the fact that this chemistry is tolerant of many functional groups at *C*2, since their mechanistic proposal does not include a role for this carbon. Finally, it must still be noted the obvious drawback of this procedure is the need to use extensive protecting groups to synthesize the parent donors (minimum four steps from the unprotected, commercially available saccharides) to access the deprotected MOP-donor for the glycosylation.



Scheme 1.19: Classic remote activation employing a MOP donor to access α-anomeric alcohols, carboxylates, and phosphates.

Much more recently in 2016, the Hanessian group published a new activating group for the synthesis of monoprotected 1,2-*cis* galactopyranosides in good yield.[72] The conditions were also shown to be amenable to solid phase synthesis. By employing BF_3·DMF complex, 1,2-*cis* monoprotected galactopyranosides could be accessed in excellent yield and good diastereoselectively in a short time (usually 30 min). The conditions were compliant not only with simple aliphatic and phenolic alcohols but also amino acid and steroid alcohols as well (Scheme 1.19). The authors posit that the stereoselectivity can be rationalized by an oxycarbenium/BF_3-coordinated 3-bromo-2-pyridyloxy ion-pair intermediate that is displaced by the alcohol in an S_N2-like reaction (Scheme 1.20).



Scheme 1.20: Lewis acid-catalyzed synthesis of monoprotected glycosides from a (3-bromo-2-pyridyloxy) β-D-glycopyranosyl donor.

The most unfortunate drawbacks of the procedure include the need for an excessive use of the alcohol acceptor and the multistep synthesis of the galactosyl donor (three steps from *per*-acetylated galactose.

1.2.2.1.2-Thioimidoyl donors

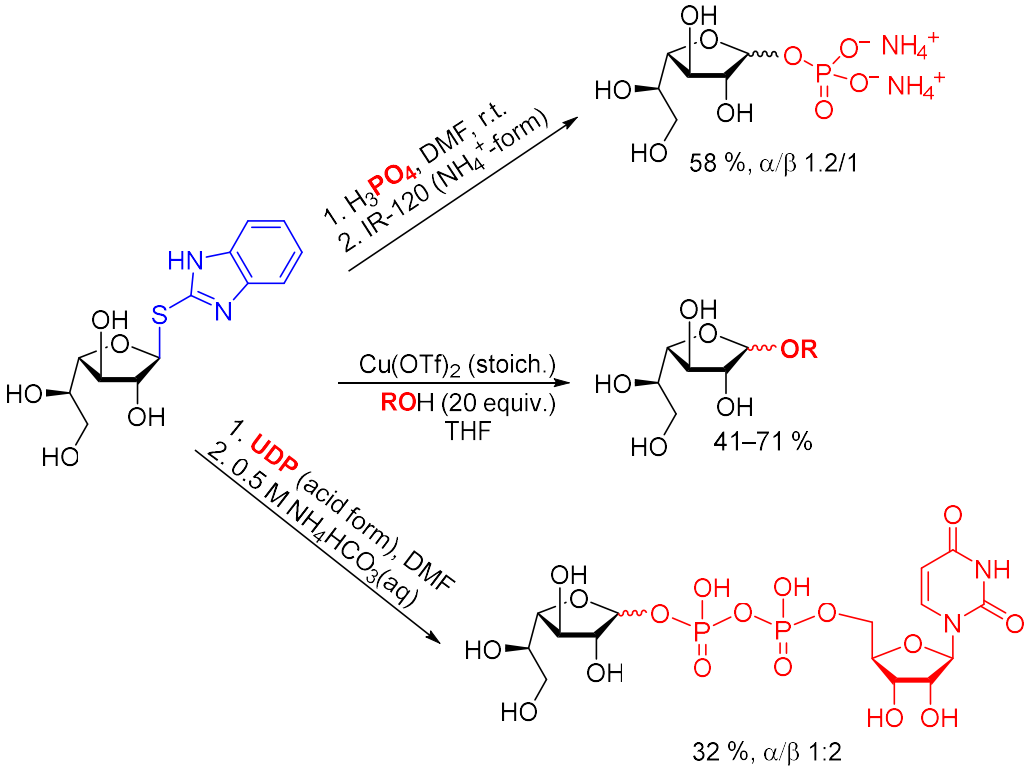
This concept of remote activation has now been adopted by other research groups as well and is highlighted briefly below. Plusquellec, Ferrières, and coworkers[73] built upon the concept of remote activation for the construction of furanosyl hexose saccharides using a 1-thioimidoyl donor. Interest in hexofuranoses stems from the arabinogalactan-rich membrane of *Mycobacterium tuberculosis* and other harmful microorganisms which consists of primarily Ara*f* and Gal*f* subunits.[74] The chemistry to access this thioimidoyl donor (5 steps from Gal*p*, not shown) is straightforward and described in three papers.[75-77] The donor is available in good yield and five steps. Although not ideal, the 1-thioimidoyl donor has numerous applications and can still be accessed through straightforward chemistry.

Initially the direct synthesis of 1-*O*-phosphofuranosyl hexoses from the corresponding 1-thioimidoyl donor was described. Subsequent treatment of the donors with phosphoric acid in DMF at room temperature provided 1-*O*-phosphofuranosyl hexoses in good to excellent yield (Scheme 1.21, top).[73] The conditions provided only very modest stereoselectivity; however, the α anomer was slightly favored regardless of the stereochemistry of the sugar at *C*2. Most importantly, very little or no ring expansion to the pyranose was observed in any instance.

In a 2007 follow-up study, Plusquellec and colleagues optimized a Lewis acid-directed glycosylation approach in the presence of divalent cations to synthesize galactosyl furanosides using the same thioimidoyl activating group. The conditions (Scheme 1.21, middle) allowed

for the synthesis of not only simple alcohols, but also disaccharides if the accepting hydroxyl group is primary, however there was little to no stereochemical control.[78]

In their most recent study, the same activating group was employed for the synthesis of UDP-furanoses which could aid in the study and discovery of other UDP-pyranose mutases. Excitingly, their remarkably simple procedure provides the UDP-furanose analogs in moderate yield, however, the reaction is not appreciably stereoselective for the desired α anomer (Scheme 1.21, bottom).[79]

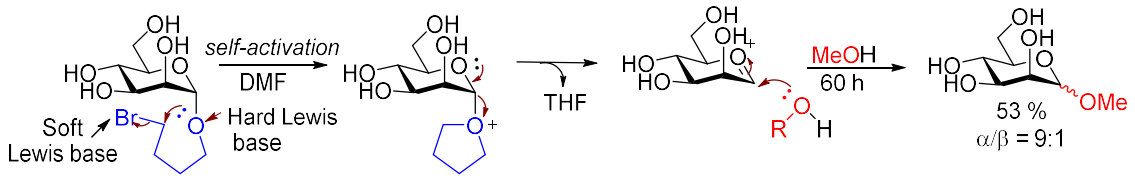


Scheme 1.21: Protecting group-free synthesis of hexofuranosyl 1-phosphates, phosphates and UDP-furanoses from hexofuranosyl 2-thiobenzimidazole donors using remote activation.

1.2.2.2 Self-activating donors

One final example showcases the concept of self-activation. Davis and colleagues[80] developed a glycosylation strategy that uses the 4-bromobutanyl group as a self-activating aglycone on a mannose monomer (Scheme 1.22) even without a Lewis acid activator.

In the proposed mechanism (Scheme 1.22), the anomeric oxygen self-displaces the bromide (hard Lewis base, soft Lewis base pairing) at the 4-position to form a THF ring. The ring oxygen then displaces THF hence forming an oxocarbenium ion. Subsequent nucleophilic attack by the alcohol provides the 1,2-*trans* glycoside with good diastereoselectivity and moderate yield.



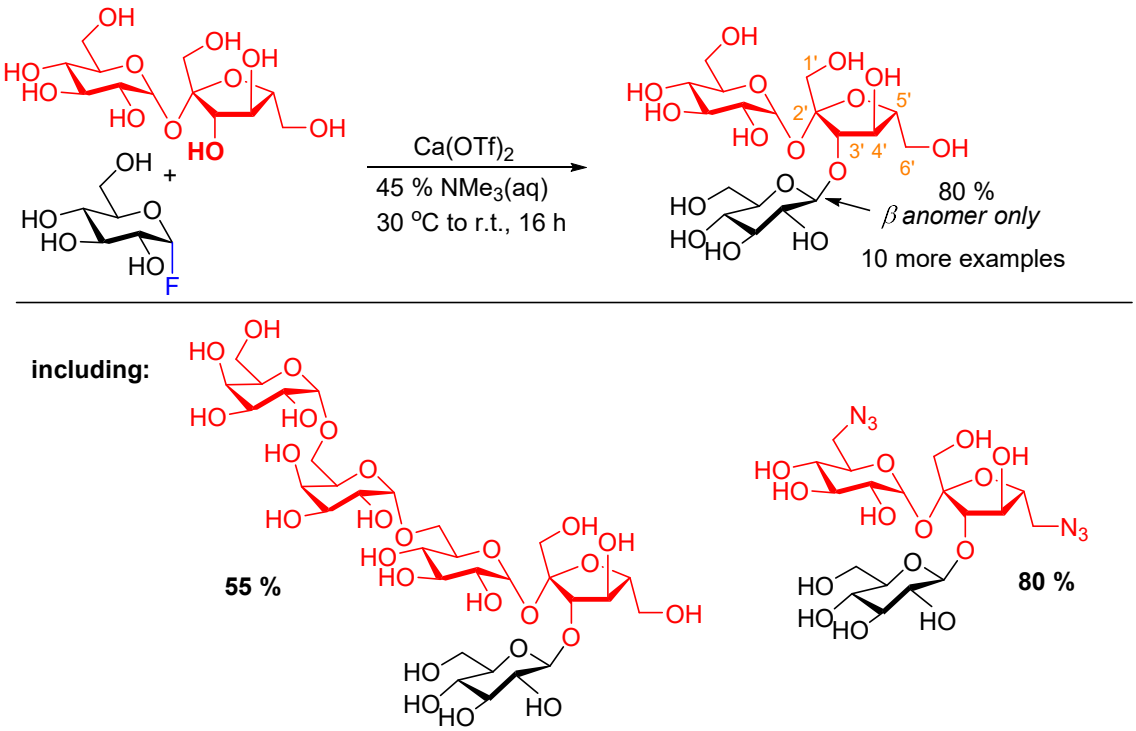
Scheme 1.22: Proposed mechanism for the glycosylation of MeOH using a self-activating donor in the absence of an external activator.

This methodology holds tremendous potential if the alcohol acceptor is a saccharide moiety as the diastereoselectivity of the reaction is significant, the mechanism unique, and the synthesis of the donor is reasonably easy and inexpensive (four steps). However, to date no follow-up study has been published.

1.2.2.3 Lewis acid-mediated

1.2.2.3.1 Divalent cation

Another remarkably simple protocol that provides *C*3′-regioselective glycosylation of unprotected sucrose under aqueous conditions was described by Schepartz, Miller and colleagues.[81] They postulate that since most glycosyl transferase enzymes operate in a divalent metal cation-dependent fashion,[82-83] that by using the correct divalent cation and suitable Lewis acid/Lewis base pairing, the necessary transition-state organization to favor glycosylation of a glycosyl fluoride would outcompete hydrolysis in an aqueous medium. This would lead to a simple enzymomimetic glycosylation procedure. The authors could obtain the regioselective *C*3′-glycosylated sucrose analog in very good yield with complete inversion of stereochemistry at the anomeric position (Scheme 1.23) by using $Ca(OTf)_2$ and NMe_3(aq).



Scheme 1.23: Protecting group-free *C*3′-regioselective glycosylation of sucrose with α-glucosyl fluoride.

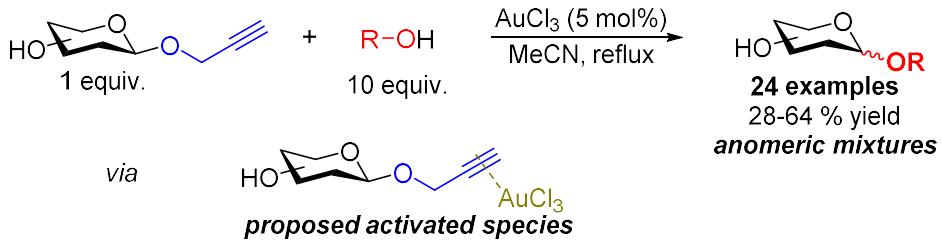
The procedure proved robust but two very interesting substrates that were still regioselectively glycosylated were stachyose which contains 14 hydroxyl groups (left), all of which could serve as acceptor sites, and the azide analog (right) which can serve as a potential precursor in the synthesis of aminoglycosides, well known antibiotics.[84] Through detailed NMR experiments the authors determined that it is the complex hydrogen bonding network present in sucrose that played the most important role in determining the reactivity and selectivity of the reaction.[81] Although the donor α-fluoroglycoside does require the use of protecting group to synthesize is available in only two steps from *per*-acetylated glucose.

1.2.2.3.2 Metal-catalyzed

Metal catalysed glycosylation, although well established, is still an area of rapid development due to the appeal of greener methods and reduction of chemical waste that using a reagent stoichiometrically causes.[85] This thesis examines two very creative ways that utilize Au and Pd to provide aliphatic and aromatic glycosides, respectively.

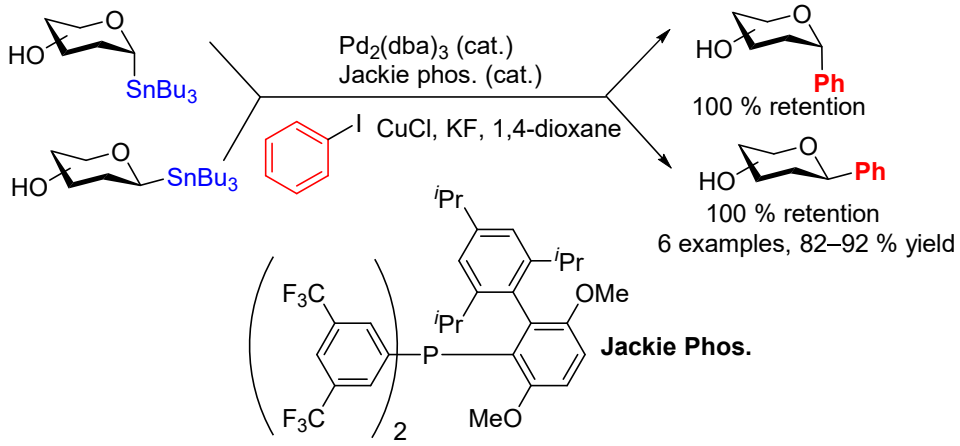
Finn and colleagues designed a method to synthesize aliphatic or disaccharides under Au(III)-catalyzed conditions with a propargylated monosaccharide as the donor.[86] They attribute their success to the fact that Au(III) is not tremendously oxophilic and is also viable

in organic solvents. They believed that the reaction proceeded through the formation of a π-complex with the alkyne and Au(III).[87] They synthesized a total 24 glycosides in moderate to good yield, albeit with very poor regiochemical control (Scheme 1.24).[86] One other unfortunate fact is the multistep synthesis of the propargylic donors.



Scheme 1.24: Protecting group-free glycosyl donors and acceptors used in the Au(III)-catalyzed glycosylation

In 2016, Pd-catalyzed conditions were described by the Walczak group for the synthesis of aromatic glycosides from anomeric stannes.[88] They claim that the reaction is perfectly stereospecific with retention of stereochemistry at the anomeric position. The majority of the study focused on benzyl protected sugars (not shown), but to demonstrate the amenability of the conditions to unprotected carbohydrates a series of deprotected stannyl glycosides (through incredibly laborious methods) were prepared. In all cases the reaction proved stereospecific and in very good yield (Scheme 1.25).



Scheme 1.25: Pd-catalyzed stereoretentive glycosylation of arenes using anomeric stannane donors

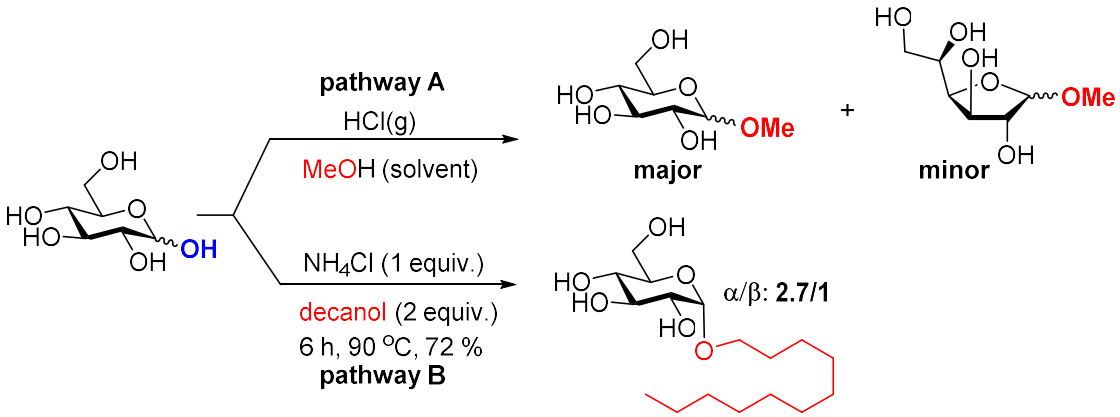
Recent remote activation and self-activation methods offer tremendous potential as viable synthetic options to access difficult hexose furanosides as enzyme substrates and possibly as inhibitors is an area of ongoing research due to the implications in several bacterial diseases, as well as in solid phase oligosaccharide synthesis as shown. The Lewis acid, and metal-

catalyzed methods demonstrate that the reagents can still be chemoselective for the anomeric position in the presence of other free hydroxyl groups due to the activating group installed there. This is incredibly powerful and will certainly be explored more in the future. However, the biggest and obvious drawback will always remain the multistep synthesis of these anomeric activating donors. Excitingly, some extremely creative methods exist to accomplish glycosylation in the complete absence of protecting groups and this thesis discusses these efforts in the next section.

1.2.2.4 Direct anomeric activation strategies

1.2.2.4.1 Fischer glycosylation

Classical totally synthetic protecting group-free (pre-2000) strategies date back to well over 100 years with the discovery of the Fischer glycosylation (Scheme 1.26).[89-90] In Fischer's seminal work methanol was glycosylated by D-glucose in the presence of HCl(g) to provide the methyl glycoside (pathway A). The reaction does proceed chemoselectively at the anomeric position, but there is little regio- or stereocontrol on the donor. Lewis acids[91-96] or microwave irradiation[97-98] to accelerate the reaction techniques are now more commonly used, but shortcomings still include the need to use stoichiometric or excessive quantities of the often toxic acid as well as long reaction times, high temperature, and almost a complete lack of stereochemical control.[69, 99] Highlighted is one recent interesting example from 2013 where ammonium chloride was effective in mediating the formation of a decanyl glucoside under reasonably mild conditions in good yield, however, the stereochemical preference for the α-anomer was quite poor (pathway B).[100]



Scheme 1.26: Fischer glycosylation strategies.

1.2.2.2 Chemoselective anomeric activating agents

Remarkably, through exploitation of the differential reactivity of the anomeric position, activating groups can be installed directly without the need for protecting groups first. Three main classes of activating groups currently dominate the literature (Figure 1.4). Strikingly, many of the reactions also take place under aqueous conditions which is an incredible advantage. Superficially, it appears that these methods must always be superior to the methods discussed in the previous section, however, typically the substrate scope is not as general so improvements are necessary but are most certainly underway.

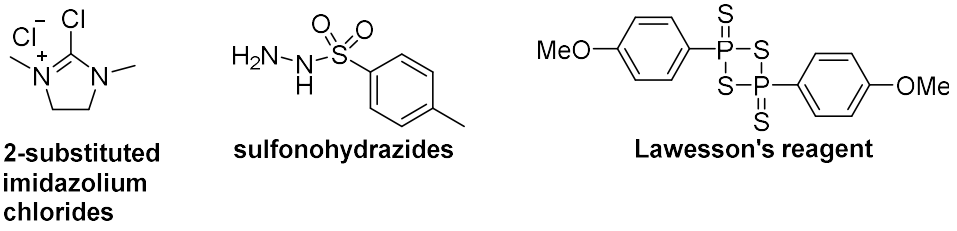
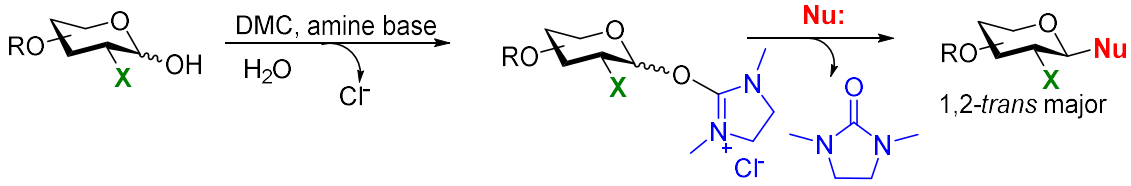


Figure 1.4: Three classes of activating agents specific for the anomeric position of unprotected saccharides.

1.2.2.2.1 2-chloro-1,3-dimethylimidazolium chloride (DMC)

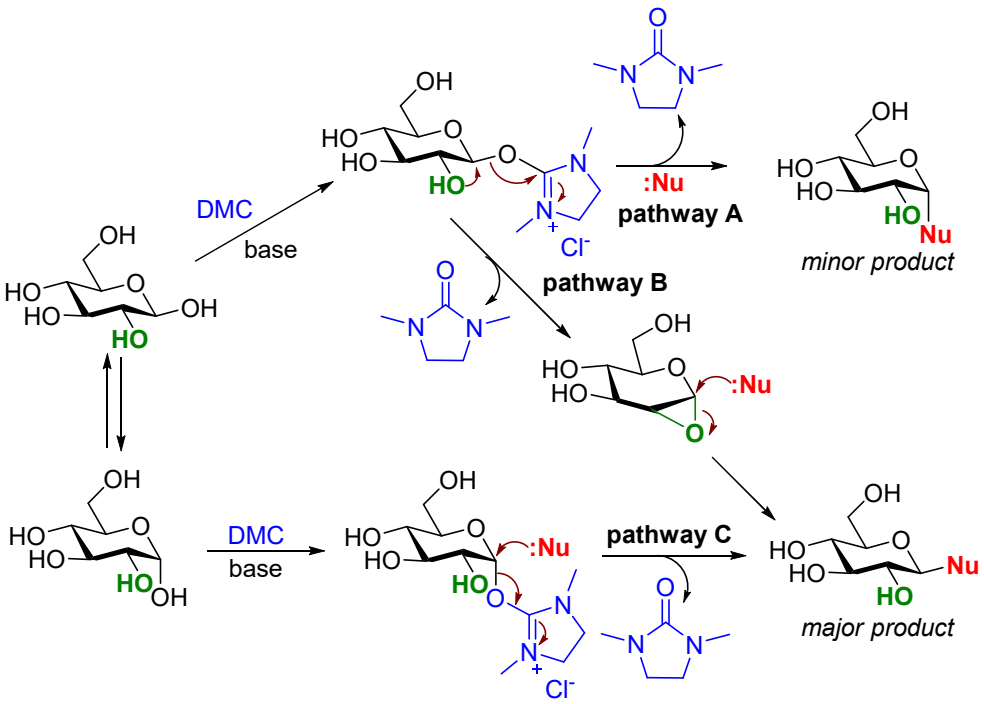
Of the selective anomeric activating agents, 2-chloro-1,3-dimethylimidazolium chloride (DMC) has been most commonly employed and is a good reagent to obtain 1,2-*trans* glycosides. In the presence of an excess of amine base (usually NEt_3) DMC reacts selectively with the anomeric position of many unprotected saccharides in aqueous or partially aqueous media creating a strong electrophile at the anomeric position that can be displaced by a nucleophile in an S_N2 like fashion (Scheme xx).



Scheme 1.27: Conceptual representation of anomeric activation by DMC.

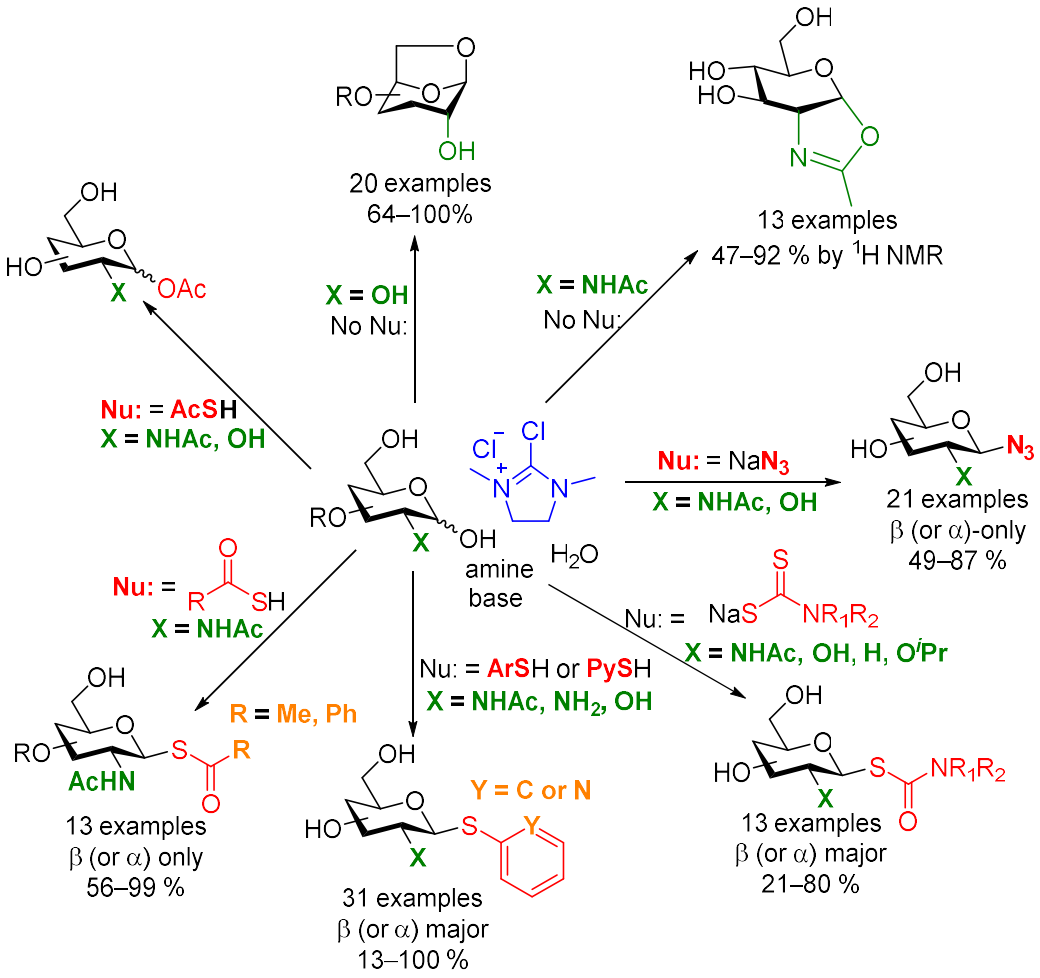
The Shoda group,[101] one of the pioneers of this chemistry, propose that the selective reactivity for the anomeric position can be supposed by the lower pKa of the *C*1-OH group (Scheme 1.28, glucose shown for convenience). They propose that either the α- or β-anomer of an unprotected sugar can react and that there is amine base promoted nucleophilic attack at position 2 of DMC creating the good electrophile at the anomeric position. Once a nucleophile

is then added to the reaction mixture, two pathways dominate to form the major 1,2-*trans* product (pathways B and C), either by epoxide ring opening due to anchimeric assistance at position 2 (pathway B) or by double inversion at the anomeric centre (pathway C). The minor or not observed 1,2-*cis* product results only from the double inversion proposed in pathway A.



Scheme 1.28: Plausible reaction mechanism.

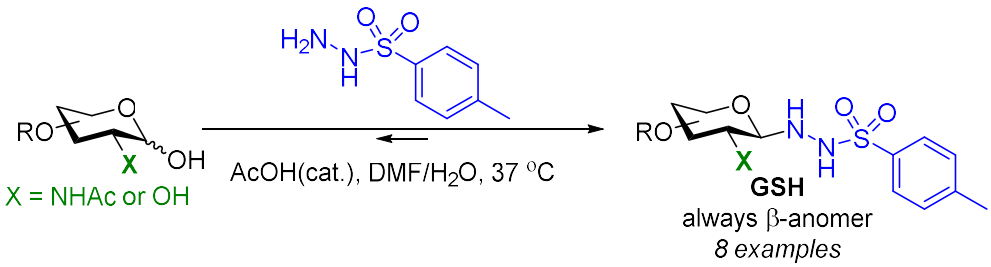
This chemistry has proven to be tremendously robust and a large number of nucleophiles have been demonstrated to be glycosylated in an aqueous environment this way (Scheme 1.29). To date, DMC has been used as an activating agent to access 1,6-anhydrosugars,[102] saccharide oxazolines,[103-105] glycosyl azides,[106-107] dithiocarbamates,[108] aryl thiols,[101, 109] 2-*N*-acetyl glycosyl thiols,[110-111] and glycosyl acetates.[112] Interestingly, with the exception of the glycosyl acetates, the 1,2-*trans* product dominated in all instances, demonstrating that DMC can be used to provide glycosides stereoselectively.



Scheme 1.29: DMC as a robust anomeric activating agent for the synthesis of many classes of organic molecules.

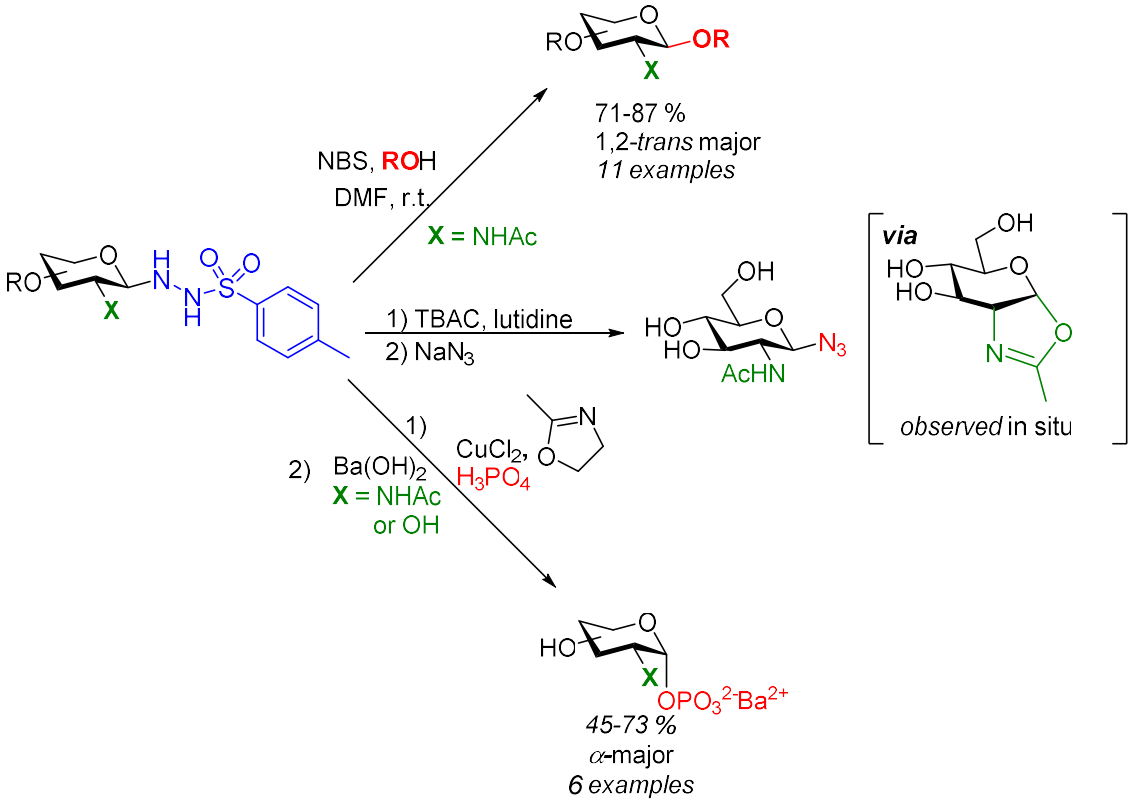
1.2.2.2.2 Glycosyl sulfonohydrazines (GSH)

The use of sulfonohydrazines for the protecting group-free synthesis of glycosides has been primarily spearheaded by the Nitz group. In a series of three studies the authors have demonstrated that access to glycosyl azides, phosphates, and alcohols is possible completely devoid of any protecting group using their method. This thesis examines these three methods briefly below. The reaction between the free sugar and a *p*-toluoylsulfonohydrazide is a condensation reaction that occurs under catalytic acidic conditions, implying an equilibrium process, that could be potentially problematic. However, the authors discovered that if an excess of the hydrazide or concentrated conditions are employed that this issue is largely circumvented and they could isolate exclusively β-glycosyl sulfonohydrazide (GSH) donors in very high yield (including 2-deoxy-2-NHAc glycosides as well) (Scheme 1.30).



Scheme 1.30: Accessing GSH donors under very mild conditions.

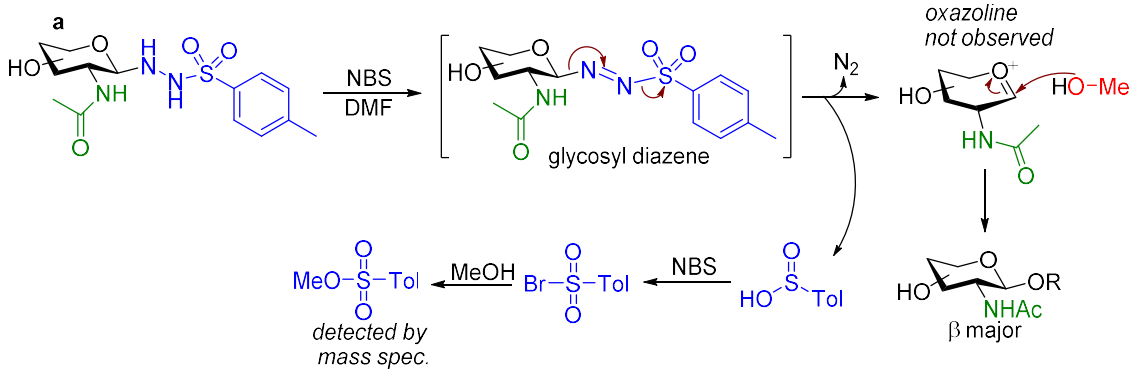
Their first work focused on the diastereoselective glycosylation of simple alcohols using 2-NHAc GSH as donors.[113-114] A nice series of glycosides that included disaccharides were amenable to the conditions and the reaction showed good to excellent diastereoselectivity for 1,2-*trans* product in good yield (Scheme 1.31, top).



Scheme 1.31: Employing GSHs for the synthesis of aliphatic glycosides, glycosyl azides, and glycosyl phosphates.

The mechanism is thought to go via a glycosyl diazene intermediate that has been reported during the oxidation of *N'*-alkylsulfonohydrazides.[115] The glycosyl diazene then decomposes to generate N_2 gas and sulfinic acid that could catalyze the formation of an oxocarbenium ion to be trapped by the alcohol acceptor (Scheme 1.32). The authors tried many alcohol acceptors

and none proved to be incompatible with the optimized conditions, however, 20 equivalents was required for the yields provided suggesting this method is not yet suitable for nucleoside or oligosaccharide synthesis.[113]

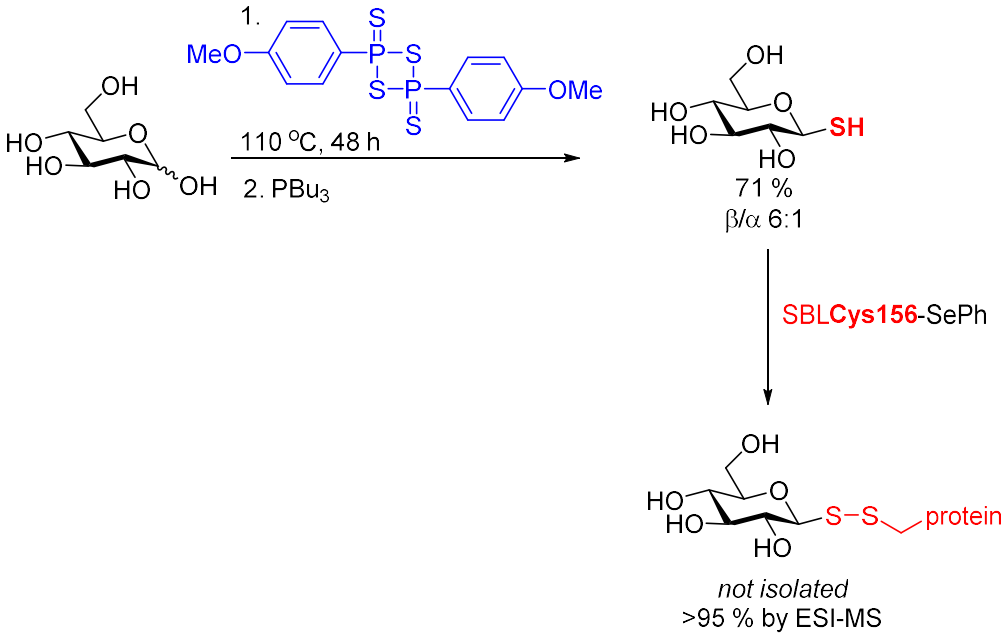


Scheme 1.32: Mechanistic rationale for the formation of 1,2-*trans* glycosides using GSH donors.

One very nice application of this chemistry is its ability to create 1,2-*trans* glycosyl azides stereoselectively (Scheme 1.31, middle) and its ability to produce reasonably stereoselectively α-glycosyl 1-phosphates (regardless of the *C*2 stereochemistry) when using $CuCl_2$ as an oxidant and 2-methyl-2-oxazoline as an additive in the presence of phosphoric acid (Scheme 1.31, bottom). No stereochemical rationale could be provided, however.[116]

1.2.2.2.3 Lawesson's reagent

One final reagent that also reacts chemoselectively at the anomeric centre of unprotected carbohydrates is Lawesson's reagent. The Davis group employed this reagent for the synthesis of both protected and unprotected glycosyl thiols.[117] On protected sugars the study proved to be robust for 1,2-*trans* thiol glycosides and most common protecting groups were compatible (not shown). When they tested the amenability of their conditions to unprotected carbohydrates they discovered that disulphide bridges were forming between the glycosyl thiols providing difficult mixtures to purify. They alleviated this problem by adding to PBu_3 to the reaction mixture to cause reduction of these bonds. With these conditions in hand, they were able to ligate a selenylsulfide-activated single-cysteine mutant protein (subtilisin Bacillus lentus, SBLS156C[118]) quantitatively as determined by ESI-MS (Scheme 1.33). This demonstrates the ability to create glycoproteins without the use of protecting groups if the correct reagents are selected.



Scheme 1.33: Using Lawesson's reagent to activate unprotected carbohydrates for the downstream ligation to protein.

1.2.3.3 In situ *activating reagents*

The last class of compounds that can effect protecting group-free glycosylation are termed *in situ* activating agents. These agents function by first activating the anomeric hydroxyl group in the flask followed by immediate glycosylation of the acceptor in a one-pot process under neutral conditions.

1.2.3.3.1 Mitsunobu reaction

The Mitsunobu reaction is one of the key reactions used widely in organic synthesis developed within the last 50 years.[119] It has such high utility in organic synthesis that it or partial variants of the procedure are now being employed in glycosylation reactions of unprotected and unactivated saccharides. One of the most attractive features of this procedure is its ability to operate in a non-pH dependent manner and the reagents are generally considered to have low toxicity. Definite drawbacks of this procedure, as will be discussed in detail in the next sections of this thesis, are the difficult to remove by-products of the reaction and the need to exclude water to a large extent.

The first example employing the Mitsunobu reaction for glycosylation using free saccharide donors was described in 1979 to synthesize aryl glycosides.[120-121] In this seminal work a small series of both pentoses and hexoses were used as donors to furnish phenolic glycosides in moderate to good yield with the 1,2-*trans* diastereomer presenting in large excess

or exclusively (Scheme 1.34). In the case of pentose donors the glycoside formed was always the thermodynamic pyranoside, with no furanoside presenting. Interestingly, though, in the case of 2-deoxyglucose solely the α-anomeric product was observed, but, unfortunately, no explanation for this variance could be established.[120]

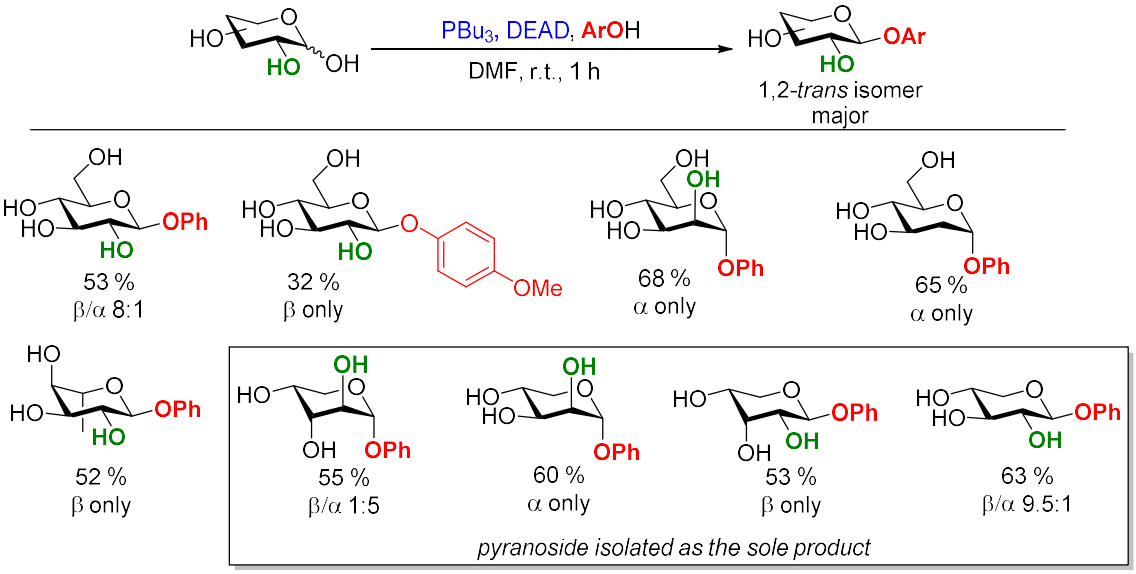


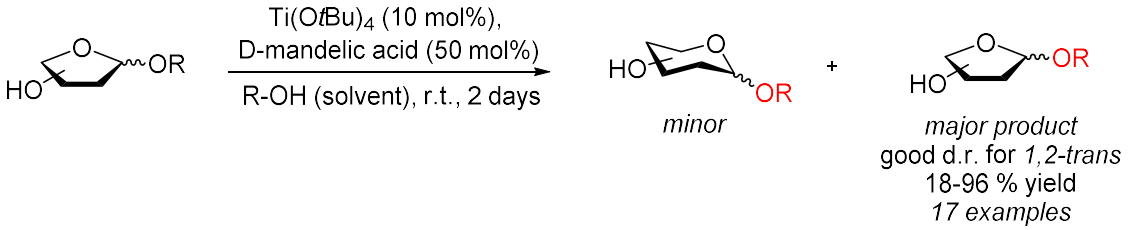
Figure 1.34: Protecting group-free availability of phenolic glycosides under Mitsunobu conditions. DEAD = diethyl azodicarboxylate

Although the paper did not provide any concrete mechanistic insight, the author did shrewdly suggest that perhaps there was a directing effect or neighboring group participation from the *C*2 hydroxyl group which could account for the stereochemistry. It is believed that this technique went largely unutilized until the this decade when slight variations of the technique were employed to synthesize α-mannopyranosyl hydroxyazobenzenes with application to photoswitchable labels[122] and in a protecting group-free synthesis of two ellagitannins.[123]

1.2.3.3.2 Catalytic conditions

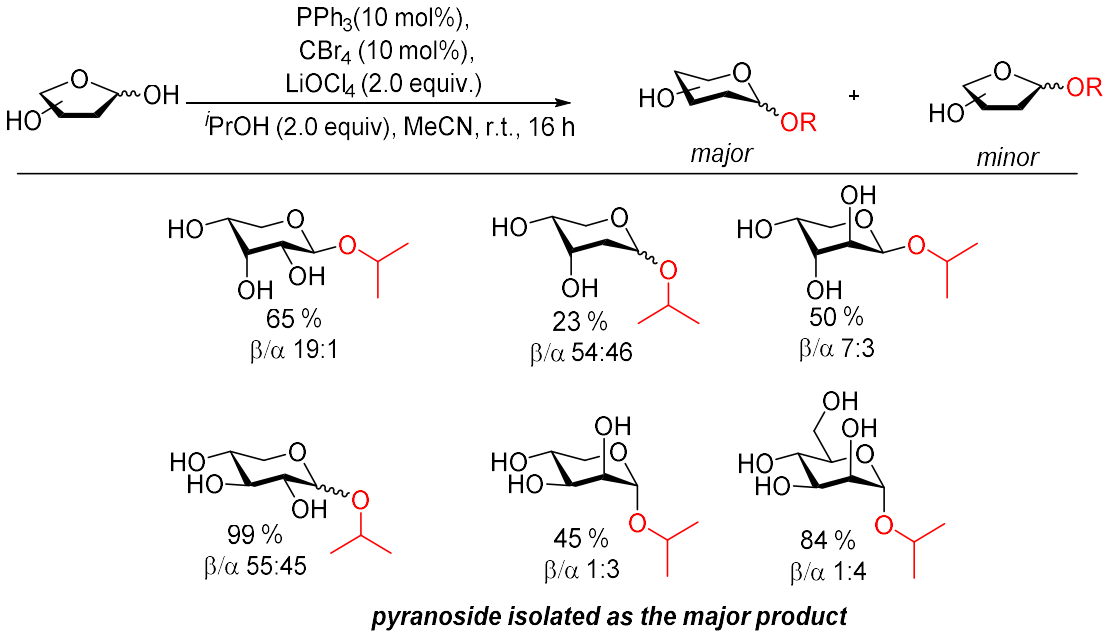
Very interestingly, catalytic conditions have now been described to glycosylate aliphatic alcohols with unprotected, unactivated pentose donors. In the first study[124] Mahrwald and coworkers utilized Ti(II)-catalyzed conditions with D-mandelic acid to synthesize furanosyl aliphatic glycosides from a pentose and an aliphatic alcohol (as solvent) with reasonably good stereoselectivity for the 1,2-*trans* product and high regioselectivity for the more biologically relevant furanosyl product (Scheme 1.35). However, it must be noted that the alcohol had to

be used as solvent, limiting the possibility of this reaction to be used to form higher di- or oligosaccharieds and the reaction time was two days. Also, no mechanistic insight has been provided yet.



Scheme 1.35: Ti(II)-catalyzed conditions of aliphatic alcohols using unprotected donors.

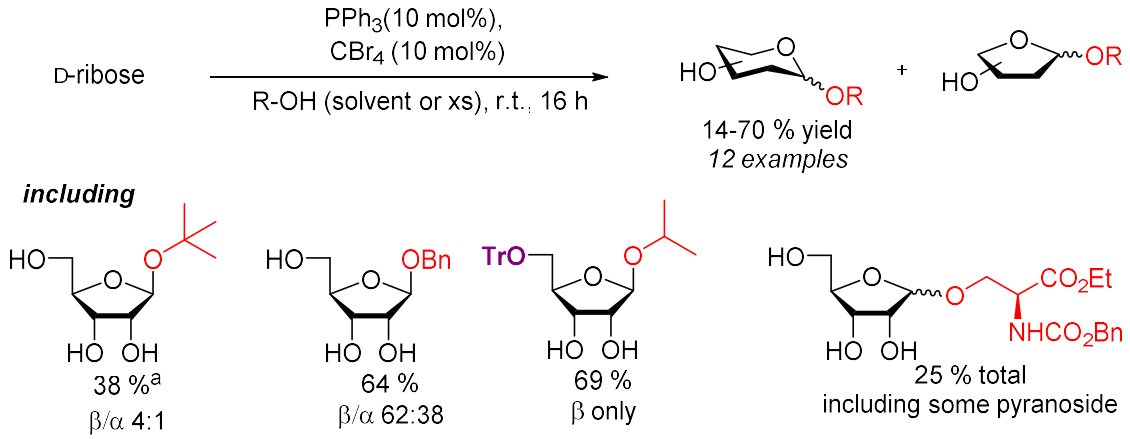
In a 2013 follow-up publication,[125] the Mahrwald group developed an even further simplified catalytic procedure with a broader substrate scope and shorter reaction time. In the first half of the study they glycosylated isopropanol with pentosyl donors in the presence of PPh_3 and CBr_4 (each 10 mol%) in the presence of $Li(OCl)_4$, as Li^+ salts are known to promote glycosylation. In this case the pyranosyl regioisomer was observed in excess over the furanoside and there was good to moderate diastereoselectivity for the 1,2-*trans* product (Scheme 1.36).



Scheme 1.36. Isopropyl glycosides under catalytic PPh_3 and CBr_4 in the presence of $Li(OCl)_4$.

In the second half of the study an expansive series of aliphatic and benzylic acceptors were used in the presence of ribose to demonstrate the operational simplicity and appreciable

substrate scope of their work. They showed that the reaction takes place under neutral conditions and is not driven by the formation of triphenylphosphine oxide either. They even demonstrated they could forge an *O*-linked glycosyl bond as a mixture of regio- and stereoisomers using a protected serine moiety. The acceptor still had to be used in excess or as solvent in each example and a mechanistic investigation was not undertaken, however, the fact that protecting group-free glycosylation can take place at all under catalytic conditions is an incredible breakthrough.



Scheme 1.37: Ribofuranosyl glycosides synthesized using catalytic conditions.

2. Specific aims of the thesis

1) To develop and optimize the conditions for and to synthesize nucleosides under totally protecting group-free conditions.
2) To devise conditions to obtain the biologically relevant furanosyl regioisomer.
3) To elucidate a plausible reaction mechanism by either observing key intermediates *in situ* via NMR techniques or by direct isolation of the intermediates.
4) To expand of substrate scope to include more difficult-to-access nucleobase acceptors and non-nucleobase aglycones.
5) To demonstrate the amenability of the method to synthesize medicinally relevant compounds and potential building blocks for solid phase oligonucleotide chemistry.

2.1 Rationale for the specific aims

As discussed in the previous section, the glycosylation step in the synthesis of glycosides, including the glycosylation of nucleobases and heterocycles, is very challenging and the success almost invariably depends on the correct selection of protecting groups. Protecting groups are laborious to install and can employ toxic reagents. The aim of this thesis was to elucidate a method(s) to synthesize nucleosides in a mild manner in the complete absence of protecting groups. The implications of this research, if successful, could extend beyond basic organic chemistry and into medicinal chemistry as many antiviral and anticancer drugs are nucleosides or nucleoside analogs.[1-3] More expedient procedures to synthesize these invaluable classes of drugs would surely be welcomed.

This was likely to be a difficult task and one that carried no precedence in our research group. In fact, the protecting group-free synthesis of nucleosides has only been described a handful of times in the study of prebiosis and to the best of my knowledge the yields were exceedingly low with remarkably poor stereo- and regiochemical control.[126-128] Therefore, it was a task armed with a high degree of difficulty, novelty, and potential utility which was certainly attractive.

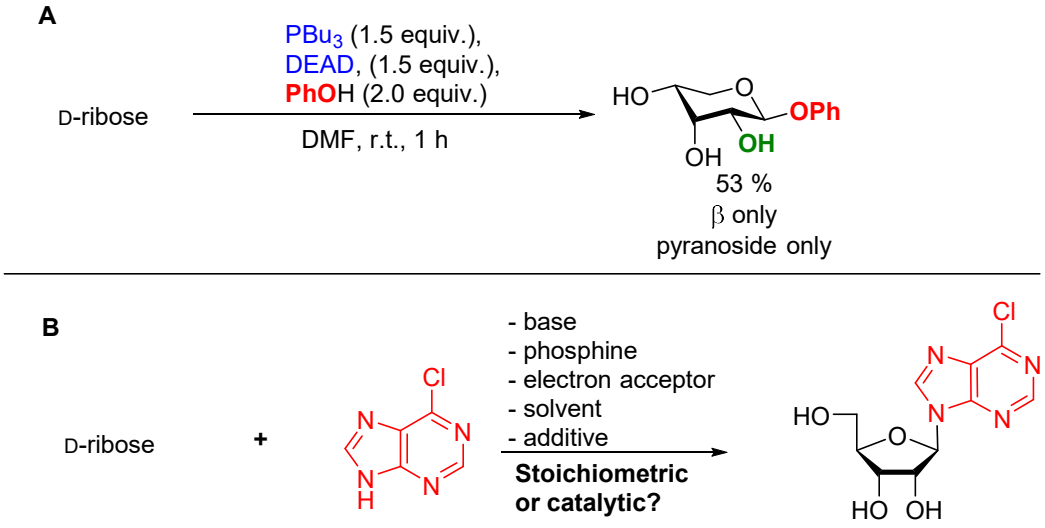
From the outset, I expected the most important considerations to be regioisomeric control of ribose and how to selectively exploit the differential activity of the anomeric position to allow glycosylation to take place with other free hydroxyl groups present in the molecule. If these issues were resolved, a plausible mechanism for the reaction and a completely general procedure to include numerous substrates would be necessitated. Finally, I wanted to demonstrate that the research could be valuable to more aspects of science by synthesizing medicinally active compounds and precursors for solid phase oligonucleotide synthesis.

3. Results and discussion

3.1 Development of a method for the protecting group-free synthesis of nucleosides

3.1.1 Optimization of Mitsunobu reaction conditions for glycosylation

The primary goal of this work was to design a completely protecting group-free method for the synthesis of nucleosides by glycosylating nucleobases with totally unprotected and unactivated D-ribose. My initial inspiration to reach this end came from the seminal work of G. Grynkiewicz in his 1979 publication in the *Polish Journal of Chemistry*.[120] As discussed in section 1.2.3.3.1, under optimized Mitsunobu reaction conditions, he was able to synthesize phenolic glycosides simply from an aryl alcohol and an unprotected pentose or hexose in moderate to good yield and notable stereoselectivity for the 1,2-*trans* isomeric product. Shown in Scheme 3.1 is his original glycosylation procedure for the glycosylation of phenol with D-ribose as the donor. In the case of ribose the reaction was perfectly stereoselective for the β-anomer and the pyranoside was the exclusive glycoside formed (Scheme 3.1A).

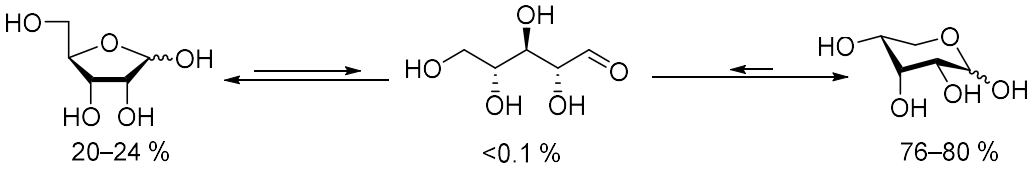


Scheme 3.1. A) Original protecting group-free glycosylation of phenol with ribose described by Grynkiewicz. **B**) Idealized nucleosidation scenario.

I envisioned that perhaps these conditions could be amenable to the synthesis of nucleosides if a nucleobase was substituted for the phenolic alcohol. Furthermore, there is precedence for the synthesis of acyclic nucleosides or non-carbohydrate carbocycles using the Mitsunobu reaction, however, this has only been described when there is but one free hydroxyl

group.[129-130] For the optimization study, I initially thought to use adenine as the model nucleobase, but due to its poor solubility in most organic solvents, I very quickly changed to 6-chloropurine, a more soluble, but still reasonably inexpensive alternative and is still a very common precursor in nucleoside synthesis. I supposed that the five most important variables to consider in the optimization of the glycosylation procedure would be the appropriate selection of a base to deprotonate the nucleobase acceptor, the phosphine, the electron acceptor, the solvent, and whether any glycosylation promotor would be useful. I also thought to test any catalytic possibilities, especially those described by the Mahrwald group.[124-125] The idealized glycosylation outcome is shown in Scheme 1.1B. Finally, I wanted to prove that these conditions would be amenable to at least a 1 mmol scale, so as to demonstrate that these conditions could be useful for the preparation of nucleosides in a real medicinal chemistry project, not just a methodological study.

In an aqueous medium, D-ribose exists in the more stable chair-like pyranose conformation between 76[131]–80[132] % of the time and the furanose conformation only 20–24 % of the time with the β-pyranose being overall the most abundant form (Scheme 3.2).[132] Also noteworthy is the fact that in the solid state ribose exists exclusively in the pyranose form.[133] Due to these data and the results obtained by Grynkiewicz,[120] I expected from the onset to obtain the less biologically relevant pyranosyl nucleoside if the glycosylation could work at all, however, it would demonstrate a totally protecting group-free glycosylation strategy for the synthesis of nucleosides.



Scheme 3.2. Regioisomeric conformational abundances of D-ribose in aqueous solution.

My first experiment duplicated the conditions described by Grynkiewicz and encouragingly I could isolate the β-nucleoside pyranoside in 22 % overall yield, albeit with heavy contamination by tributylphosphine oxide (Table 3.1, entry 1). This confirmed, even if unfortunately, that the thermodynamic pyranoside product would be the sole or major regioisomer formed in the reaction, but I was encouraged that the reaction was perfectly stereoselective for the desired β-anomer. With these results in hand, I sought to optimize the synthesis of the β-pyranosyl ribonucleoside. One important goal was to ensure that the potentially precious nucleobase was used in equimolar ratio with ribose or as the limiting

reagent. Early on I examined the feasibility of using the much more stable and easier to handle phosphine, PPh_3, (entries 3–4), however, it was very clear that the more reactive P(*n*Bu)$_3$ (all other entries) was necessary to obtain appreciable yield. Electron acceptors tested in the study included CCl_4 (entries 2, 5–6, 8–9), CBr_4 (entries 3–4), 1,1′-(azodicarbonyl)dipiperdine (ADDP), and diisopropyl azodicarboxylate (DIAD). I also tested $AgCO_3$ (entry 8) as a glycosylation promotor like in the Koenigs-Knorr reaction.[14, 134-135] I obtained a combined yield of 23 % with the desired β-furanosyl nucleoside presenting in ~12 % overall yield, however, due to the low combined yield I decided not to pursue this end further. I also attempted the catalytic conditions of the Mahrwald group (entry 4),[125] however, no reaction took place, confirming that stoichiometric proportions of reagents were required for the reaction to take place.

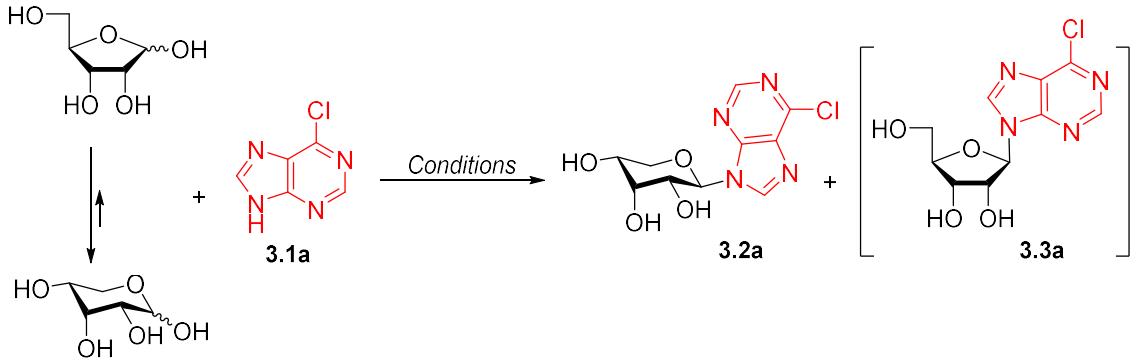
As I progressed through the survey, I sought to explore solvents other than DMF due to the difficulty in its removal, especially in the presence of so many polar, unprotected hydroxyl groups. Due to the low solubility of 6-chloropurine in THF, I found this solvent to be non-viable (not shown). I also tested DMSO as a possibility but its removal proved impossible. Fortuitously, MeCN proved to be a very good alternative. 6-chloropurine was still reactive and its removal on a rotory evaporator could be carried out quickly under mild temperatures without successive time consuming coevaporations with toluene or water.

From the onset of the optimization study it was clear that the yield was being dramatically enhanced by the utilization of a base to deprotonate the nucleobase at N^9. The bases screened were NaH (entries 2, 4–5, 7–10) and $CsCO_3$ (entry 6), but the yield improved two-fold when I used 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (entry 11). Not only does DBU provide the ability to deprotonate the N^9 position hence increasing the nucleophilicity of the nucleobase, it also offered a solubilizing effect on the nucleobase resulting in a homogenous mixture. I discovered that if the more valuable nucleobase was used as the limiting reagent and ribose used in slight excess (2 molar equivalents) then the yield was enhanced, as well.

I also determined that the order of addition of the reagents was tremendously important for reproducibility of the results. Once DBU was determined to be the best base I optimized the order of addition of the reagents and found that it was important to first deprotonate the nucleobase at room temperature, then after 15 minutes to cool the reaction to 0 ^oC in an ice bath, then add DIAD followed by P(*n*Bu)$_3$ dropwise over 5 minutes. Finally, ribose was added at the end and the reaction allowed to warm to room temperature over 12 hours.

With the order of addition of reagents optimal, one last key yield enhancement strategy was discovered. I noted that if I triturated the crude reaction mixture (dissolved in a minimal volume of MeOH) into petroleum ether–Et_2O to remove a large amount of the tributylphosphine oxide, the pure nucleoside could be isolated after only one iteration of normal-phase column chromatographic separation. As I had the correct conditions in hand, I could isolate pure pyranosyl nucleoside **2a** in good yield (76 %) with a minor amount of the furanoside (3.4 %) being isolated as well. As a final effort, logically, I thought we could further augment the yield by increasing the equivalencies of Mitsunobu reagents employed, but the tremendous amount of phosphine oxide generated made purification of pure nucleoside impossible. As a last note, I was encouraged that throughout the entire course of optimization the α-anomer was never detected by ^1H NMR analysis of the crude reaction mixture.

Table 3.1: Optimization study for the formation of nucleosides with a total lack of protecting groups.



Entry	Base	Conditions	Isolated yield
1	none	**1a** (1.5 equiv.), D-ribose (1.0 equiv.), P(*n*Bu)$_3$ (1.5 equiv.), DEAD (1.5 equiv.), DMF, 1 h, r.t.	22 %[a]
2	NaH	**1a** (1.0 equiv.), D-ribose (1.0 equiv.), P(*n*Bu)$_3$ (1.05 equiv.), CCl_4 (10.0 equiv.), THF, 1 h, r.t.	24 %[a]
3	none	**1a** (1.0 equiv.), D-ribose (1.0 equiv.), PPh_3, (1.6 equiv.) CBr_4 (2 equiv.), ribose (1.5 equiv.), DMF, 1 h, r.t.	27 %[a]
4	NaH	**1a** (1.0 equiv.), D-ribose (1.5 equiv.), PPh_3, (0.1 equiv.) CBr_4 (0.1 equiv.), DMF, 1 h, r.t.	No reaction
5	NaH	**1a** (1.0 equiv.), D-ribose (1.0 equiv.), P(*n*Bu)$_3$ (1.2 equiv.), CCl_4 (2 equiv.), DMF, 12 h, r.t.	26 %[a]

6	$CsCO_3$	**1a** (1.0 equiv.), D-ribose (1.0 equiv.), P(*n*Bu)$_3$ (1.2 equiv.), CCl_4 (2 equiv.), DMF, 12 h, r.t.	trace
7	NaH	**1a** (1.0 equiv.), D-ribose (1.0 equiv.), P(*n*Bu)$_3$ (1.2 equiv.), DIAD (2 equiv.), DMF, 12 h, r.t.	12
8	NaH	**1a** (1.0 equiv.), D-ribose (1.0 equiv.), P(*n*Bu)$_3$ (1.2 equiv.), CCl_4 (2 equiv.), MeCN, 12 h, r.t.	33
9	NaH	**1a** (1.0 equiv.), D-ribose (1.0 equiv.), P(*n*Bu)$_3$ (1.2 equiv.), CCl_4 (2 equiv.), MeCN, $AgCO_3$ (1 equiv.), 12 h, r.t.	23 %[b]
10	NaH	**1a** (1.0 equiv.), D-ribose (2 equiv.), P(*n*Bu)$_3$ (1.2 equiv.), ADDP (2 equiv.), MeCN, 12 h, 0 °C to r.t.	trace
11	DBU	**1a** (1.0 equiv.), D-ribose (2 equiv.), P(*n*Bu)$_3$ (2 equiv.), DIAD (2.1 equiv.), MeCN, 12 h, 0 °C to r.t.	52 %[c] (76 %)[d]
12	DBU	**1a** (1.0 equiv.), D-ribose (2 equiv.), P(*n*Bu)$_3$ (3 equiv.), DIAD (3.1 equiv.), MeCN, 12 h, 0 °C to r.t.	29 %[a]

(a) Product contaminated with phosphine oxide. (b) Furanoside **3a** was also isolated in 12%. (c) Furanoside **3a** was also isolated in 3%. (d) Yield after triturating crude reaction through Et_2O–pet. ether prior to purification.

3.1.2 Synthesis of β-ribopyranosyl nucleosides

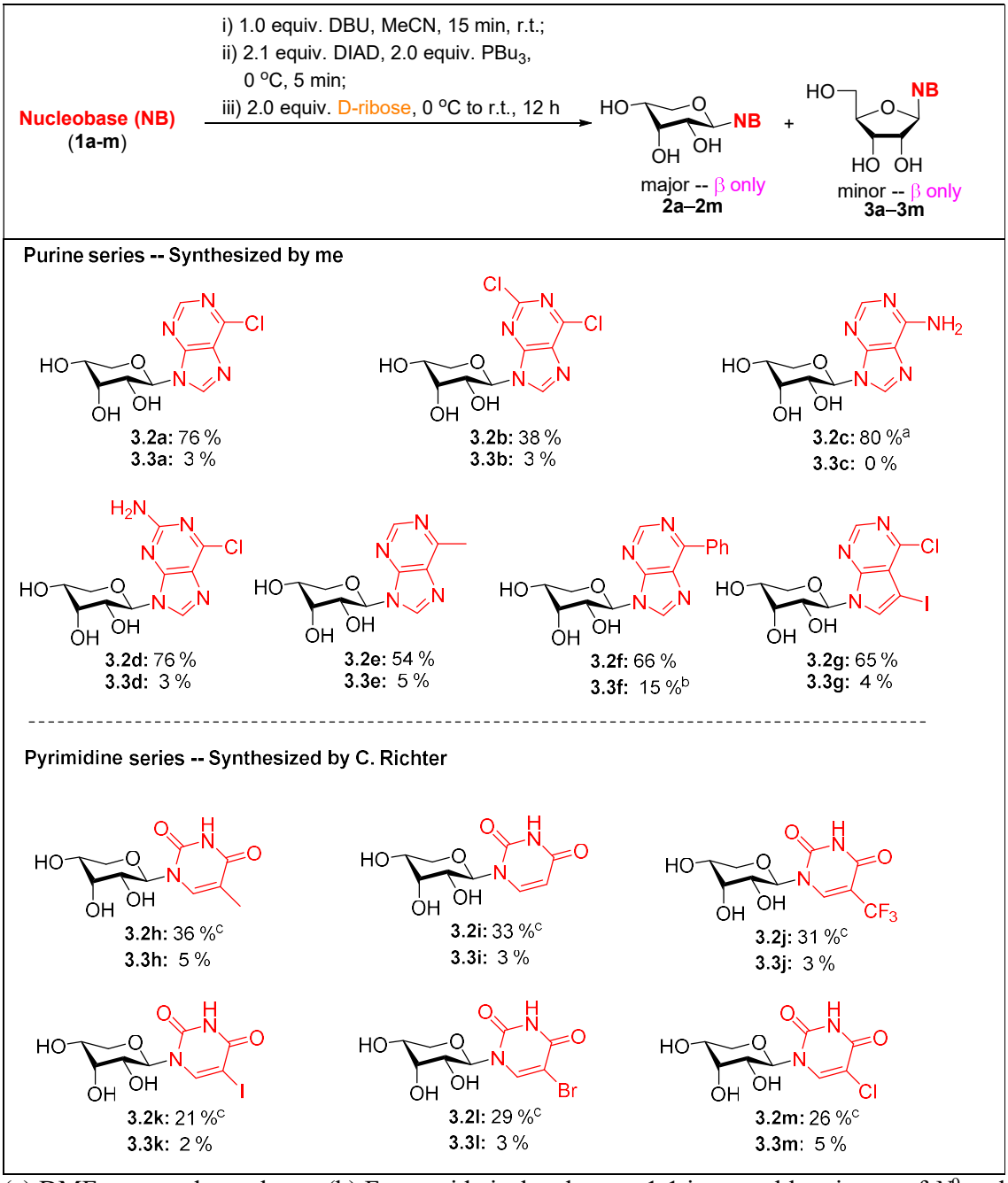
With the conditions optimized conditions in hand I was primed to examine whether these conditions could provide a series of nucleosides containing heterocyles with common synthetic precursor functional groups, alkyl or aromatic substituents, and natural occurring nucleobases at a 1 mmol scale. I must acknowledge, once again, the collaboration with C. Richter from the Mahrwald group (Humbolt University in Berlin) for his contributions to this series. He synthesized all of the pyrimidinyl nucleosides shown in Table 3.1. We synthesized a total of 13 nucleosides bearing these functional groups at this semi-preparative scale (Table 3.2). In all cases solely the β-anomer was observed as determined by ^{1}H NMR analysis of the crude reaction mixture and as a general trend the reactivity of the purines was greater than that of the pyrimidines.

In the purine series, I was pleased to observe that in addition to **3.1a**, synthetic precursor nucleobases **3.1b** and **3.1d** were also compatible with the optimized conditions, albeit dichloro-substituted **3.1b** in only very moderate yield. I was also pleased to observe that 6-methylpurine

3.1e[87] and 6-phenylpurine **3.1f**[136] could also be glycosylated both in good yield (53 % and 66 %, respectively). Interestingly, in the case of 6-phenylpurine riboside the minor furanoside **3.3f** was isolated in a non-trivial amount (15 % yield), but as a 1:1 mixture of the desired N^9 glycosylated product and the less desired N^3 product. I also discovered that adenine (**3.1c**) was glycosylated very efficiently (80 %) if the less desired DMF was employed as solvent, however, due to the lack of any solubility of the nucleobase in MeCN this could not be circumvented. Finally, I was pleased to observe that deazapurine **3.1g** was also compatible and in good yield (65 %). In all instances in the purinyl series, the major pyranoside product was separable from the minor furanosyl nucleoside.

The pyrimidine series, was unfortunately, not as high yielding and the major pyranoside product was not separable from the minor furanoside nucleoside when purification was carried out on a column of silica gel. On the other hand, we were still able to glycosylate in moderate yield thymine (**3.1h**) and uracil (**3.1i**) to produce pyranosyl 5-methyluridine (**3.2h**, 36 %) and uridine (**3.2i**, 33 %) analogs and they were, in fact, the highest yielding in the pyrimidine series. In modest yield, 5-trifluoromethyluridine analog **3.2j** could also be synthesized (31 %). Perhaps most encouraging was that 5-halogenateduridine cross-coupling precursors **3.2k** (21 %), **3.2l** (29 %), and **3.2m** (26 %) could all be afforded under the conditions, although often in very moderate yield. I postulated that the reason for the poorer reactivity in the purine series was due to poorer solubility of the substrates in MeCN and decreased solubility of the nucleoside product in organic solvents. The pyrimidine nucleosides were also much more polar than the purine nucleosides (R_f values can be found in the experimental section) so the possibility of incomplete elution of the product from the normal phase silica gel column could not be ruled out and could certainly be responsible for the inseparable nature of the pyranosyl and furanosyl products.

Table 3.2: Synthesis of β-ribopyranosyl nucleosides under the optimized conditions.



(a) DMF was used as solvent. (b) Furanoside isolated was a 1:1 inseparable mixture of N^9 and N^3 glycosylated products. (c) As determined by ^{1}H NMR. The products were inseparable when purified by chromatography on silica gel.

3.1.2.1 Attempts at isomerization

This initial study proved to be a good proof-of-concept demonstrating that nucleosides can be synthesized in the complete absence of protecting groups under the right conditions using mild

reagents. Furthermore, some biological application for pyranosyl nucleosides has even been described,[137-138] however, for the vast majority of biological studies the furanosyl nucleoside is required. As a result, I attempted to isomerize the pyranoside to the furanoside using acid-catalyzed or mediated conditions. I attempted a number of Lewis acid catalyzed conditions including *p*-toluenesulfonic acid (TsOH), $SnCl_4$,[139] TMSOTf,[140] and Ti(O^iPr)[124] but all attempts were unsuccessful: no reaction (TsOH) or decomposition (all others) took place.

3.1.3 Synthesis of biologically relevant β-ribofuranosyl nucleosides

3.1.3.1 Selecting an appropriate protecting group

Armed with suitable stereoselective glycosylation conditions, I next strived for the best method to synthesize β-ribofuranosyl nucleosides as a reliable technique to do so would be most likely to have the biggest impact outside of a synthetic laboratory. I postulated that the easiest way to provide the furanosyl product would be to first select a suitable protecting group that is bulky enough to react exclusively or with very great preference for primary alcohols. Hence, when such group is reacted with ribose, only the furanosyl regioisomer will react with the protecting group and, thus, lock ribose in the furanose configuration. I also wanted a protecting group that was sufficiently acid-labile to be removed *in situ* by a mild acid after the glycosylation took place, but would not compromise the integrity of the anomeric position. This idealized scenario would provide access to β-furanosyl nucleosides in two-step one-pot process. Three major protecting groups that could be suitable for such a transformation are the trityl (triphenylmethyl), 4-methoxytrityl (MMTr), and the 4,4′-dimethoxytrityl group (DMT) (Figure 3.1). I was concerned that the DMT group would be too unstable to be produced and stored for extended periods of time. I was also interested in a group that would remain intact should isolation of the monoprotected nucleoside be desired. The acidity of silica gel, was, therefore, of some concern. The trityl group, I feared woud have had the opposite effect, and could have been too stable to be cleaved selectively under acidic conditions without causing partial or complete decomposition of the nucleoside. Therefore, I selected the MMTr group for the next study.

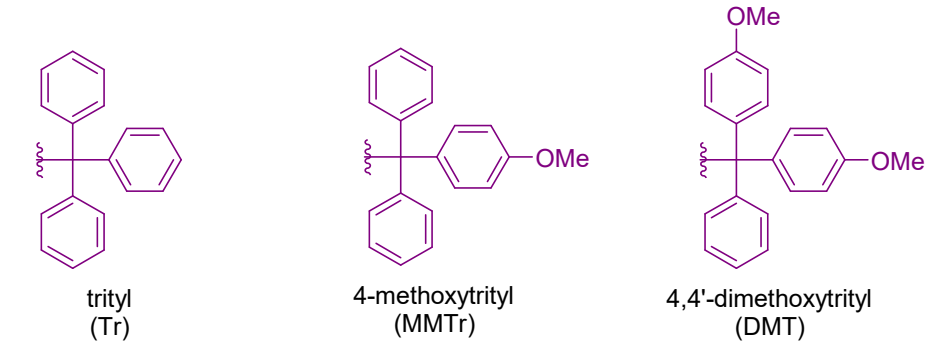
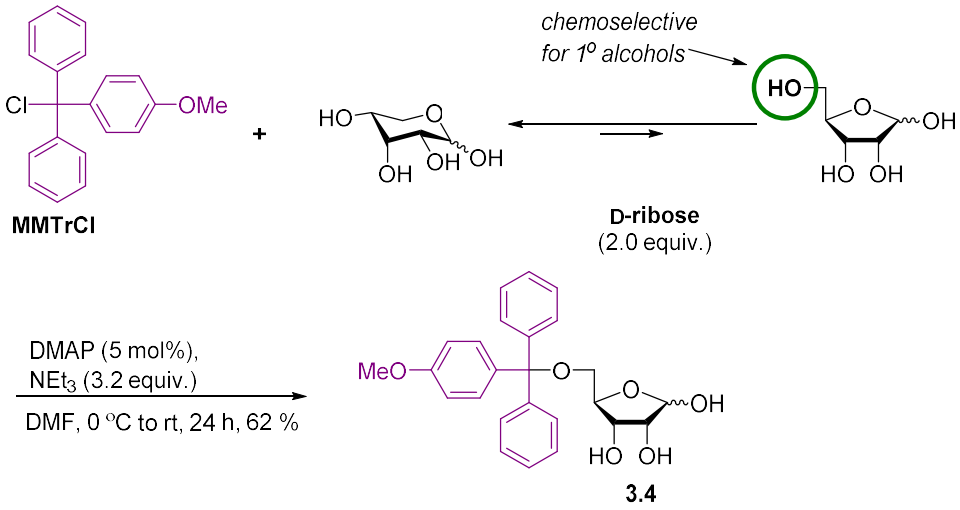


Figure 3.1: The structures of three bulky, acid labile protecting groups considered in this work.

Much to my delight, the synthesis of 5-*O*-monomethoxytrityl-D-ribose proved trivial and could be isolated in good yield (62 %) after very minor optimization (Scheme 3.3). With donor **3.4** in hand I was ready to test the pyranosyl conditions glycosylation conditions to see if the same series synthesized in Section 3.2 could be accessed with the monoprotected donor.



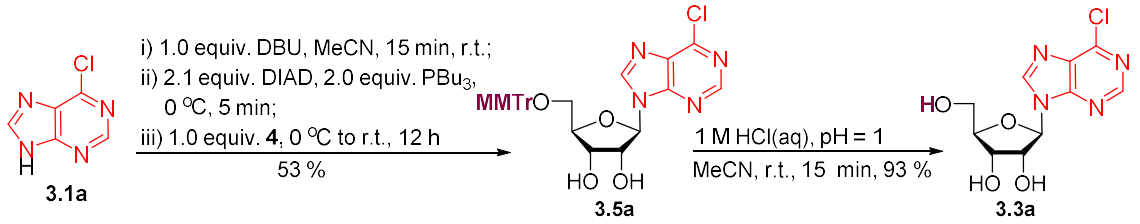
Scheme 3.3: Synthesis of 5-*O*-MMTr-D-ribose (**3.4**).

3.1.3.2 Synthesis of β-furanosyl nucleosides as a two-step process

I wanted to first analyze the ability of my conditions to produce an isolable monoprotected nucleoside that could be characterized and the yield determined. I would then find appropriate acidic conditions to cleave the MMTr group in a separate step to arrive at the biologically relevant β-ribofuranosyl nucleoside. To do it this way would allow me to determine if there was any difference in yield when forming the nucleoside pyranoside vs the furanoside and, if so, if it resulted from the glycosylation or the deprotection step.

I opted to use the nucleobase 6-chloropurine (**3.1a**) from the pyranoside series to carry out this procedure as I did in the pyranosyl series. In the first step, I utilized the same conditions

and order of addition of reagents as in the pyranoside series, except to decrease the molar equivalency of the sugar donor to 1.0 equivalents to improve the atom economy of the reaction. I was pleased to observe that the glycosylation took place in 53 % overall yield after purification on normal phase silica gel on the first attempt and was stereoselective for the β-anomer (Scheme 3.4). NMR analysis of the crude product indicated, once again, that the α-anomer was not present to any extent and that unreacted starting materials made up the remainder of the yield. I attempted to heat the reaction mixture to improve the yield, but no increase in yield of the desired N^9-glycosylated nucleobase was observed, just degradation of the starting sugar or an increase in glycosylation products at the other ring nitrogens. In the second step, I was pleased to observe that the MMTr group was cleaved simply after treatment of compound **3.5a** in acidic MeCN (1 M HCl(aq), pH = 1) after only 15 minutes (as monitored by TLC). After purification by column chromatography on silica gel the desired nucleoside furanoside was isolated in 93 % yield. This meant that the cleavage of the MMTr proceeds essentially quantitatively and that the glycosylation step is where the yield is lost.



Scheme 3.4: Two-step synthesis of furanosyl nucleoside **3a.**

3.1.3.3 Synthesis of β-ribofuranosyl nucleosides in a two-step one-pot process

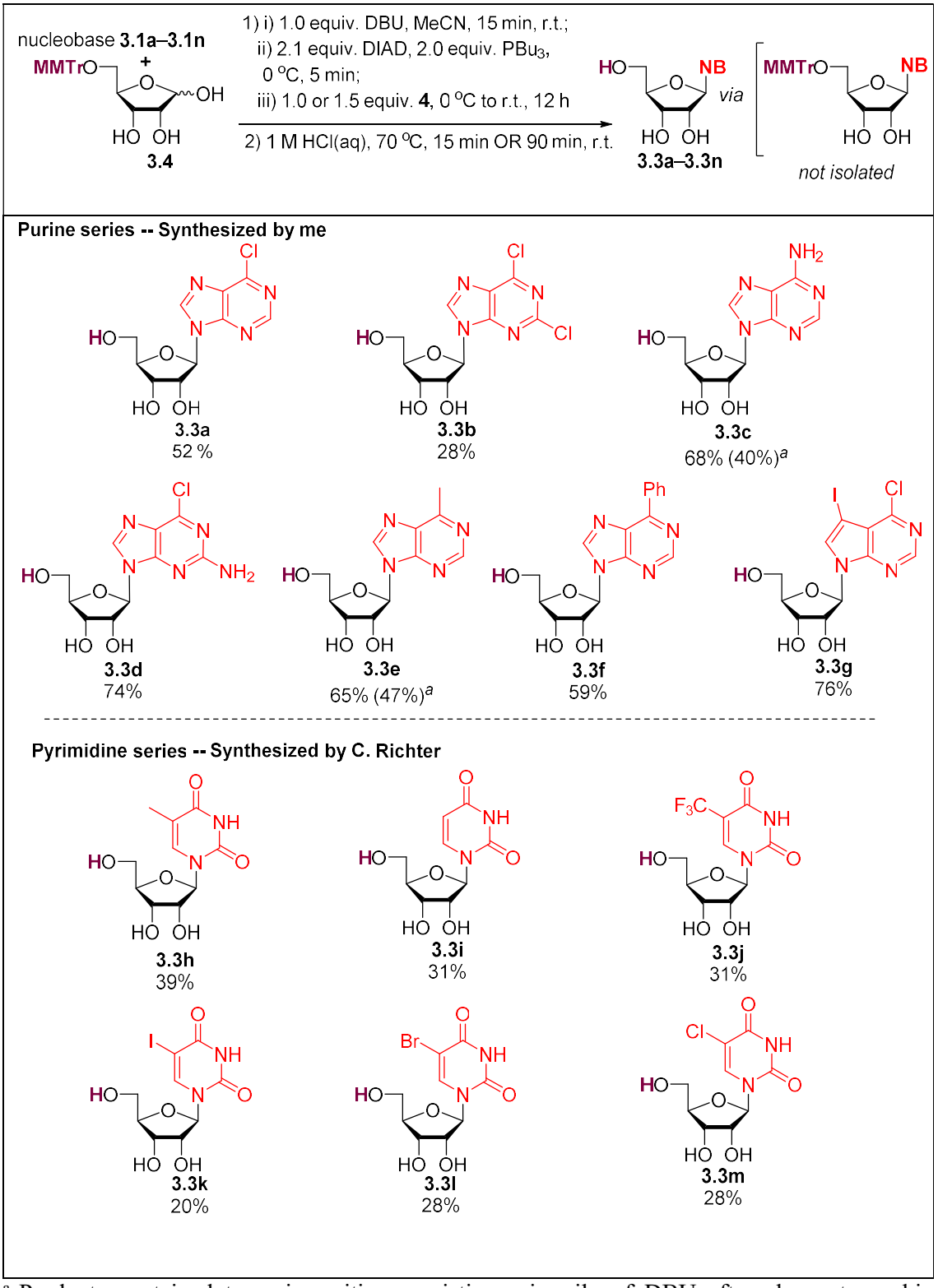
With the appropriate conditions in hand, I, with the collaborative effort of C. Richter, pursued the glycosylation of the same series of nucleobases as in the pyranoside series in a one-pot process. The only change from the conditions seen in Table 3.2 above is that slightly more acid and longer reaction time (pyrimidines) or heating to 60 °C (purines) was required to cleave the MMTr group, almost certainly due to the presence of the reduced DIAD hydrazine and the phosphine oxide buffering the Mitsunobu reaction. Once again, the general trend of the purines being more reactive than the purines held true, and fortuitously, the same series was accessible with little to no complications. In most instances the yields were comparable to the those of the pyranoside series, indicating once again, that the MMTr cleavage proceeded quantitatively without compromising the anomeric position at all (Table 3.3).

In the purine series, the nucleobase and monoprotected sugar **3.4** were reacted in a 1:1 molar ratio. Pleasing, synthetic precursors **3.3a** (53 %), **3.3b** (28 %), and **3.3d** (74 %) were still

viable, with once again, **3.3b** only being available in very moderate yield for unknown reasons. It was also encouraging to see that cytostatic[141-142] agents **3.3e** and **3.3f** could be synthesized under these conditions as well as substituted 7-deazapurine analog **3.3h**. Adenosine could also be isolated in good yield (68 %, 40 % after recrystallization).

In the pyrimidine series, the reaction was once again lower yielding, however, by increasing the amount of 5-*O*-MMTr ribose to 1.5 equivalents the furanoside products could be isolated in nearly identical yield as in the pyranoside series. 5-methyluridine (**3.3h**) as well as cross-coupling precursors 5-halo derivatives **3.3k**–**3.3m** were all available albeit in very moderate yield, but essentially the same yield as in the pyranosyl nucleosides. Finally, naturally occurring RNA component uridine **3.3i** was also provided in moderate yield (31 %). I also note that the pyranoside product did not present at any time in the syntheses and that by ^{1}H NMR analysis the β-anomer was the sole product formed in every case.

Table 3.3: Synthesis of nucleosides containing the naturally-occurring furanosyl conformation of ribose using a two-step one-pot approach.

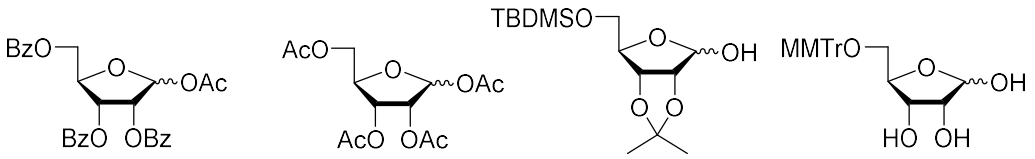


[a] Products contained trace impurities consisting primarily of DBU after chromatographic purification. Yield in parentheses is after crystallization from MeOH.

3.1.3.4 Comparison of the new procedure to precedence.

As mentioned, uridine (**3.3i**, 31 %) and adenosine (**3.3c**, 68 %) could be accessed using the new procedure. This is particularly important, as to the best of my knowledge, this stands as the most efficient (shortest) non-enzymatic synthesis of adenosine known to date apart from very low yielding (maximum 20 %, non-stereoselective) theoretical prebiotic syntheses.[126-128]

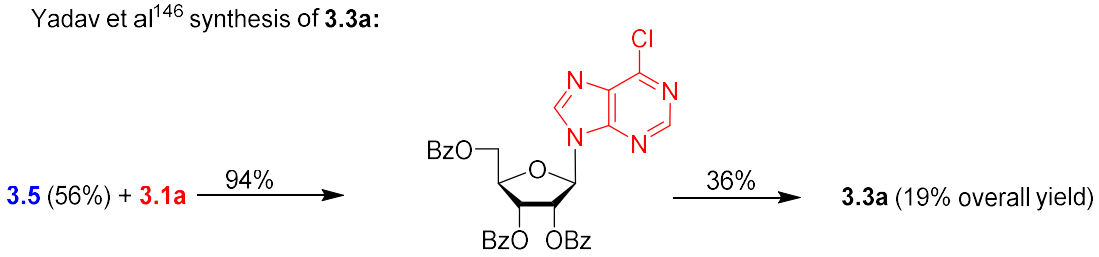
To further demonstrate the power of this novel procedure, I also discovered that many of the furanosides synthesized in this series were available in better overall yield using the optimized procedure than ever described previously using the typical protection–Vorbrüggen glycosylation–deprotection strategy when the total yield is calculated from ribose and the nucleobase. The newly developed procedure always started from ribose donor **3.4**. The previous syntheses always started from either **3.5**,[143] **3.6**[144] or **3.7**.[145] The yields of **3.5** and **3.6** were obtained from classical, highly cited papers. In all instances, I sought to find modern procedures (i.e. published within the last 15 years) that accessed these molecules. In all the β-ribofuranosyl purines studied in which yields of the syntheses were available, my procedure was superior. I provide a comparison of the known syntheses to my syntheses in Scheme 3.5. The nucleosides where the procedure proved to be superior than ever published before were nucleosides **3.3a** (32 % vs 19 %,[146] Scheme 3.5A), **3.3c** (42 % vs 25 %,[147] Scheme 3.5B), **3.3d** (46 % vs 7.3 %,[147] Scheme 3.5C), **3.3e** (40 % vs 14 %,[148] Scheme 3.5D), **3.3f** (30 % vs 27 %,[149] Scheme 3.5E), and **3.3g** (47 % vs 27 %,[145] Scheme 3.5F).



A Synthesis of **3.3a** under the new conditions:

3.4 (62%)+ **3.1a** —52%→ **3a** (32% overall yield)

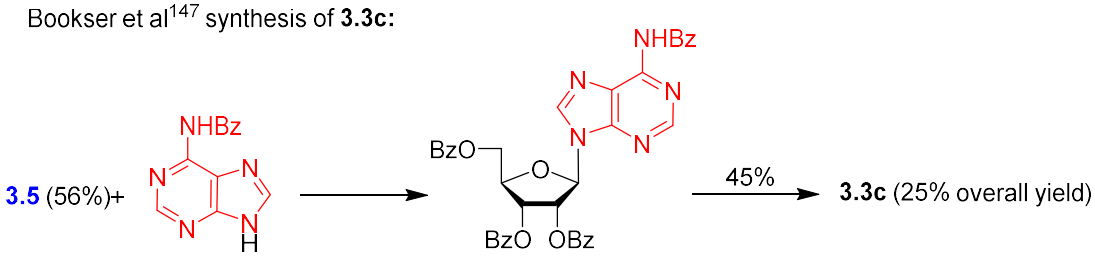
Yadav et al[146] synthesis of **3.3a:**



B Synthesis of **3.3c** under the new conditions:

3.4 (62%)+ **3.1c** —74%→ **3.3c** (46% overall yield)

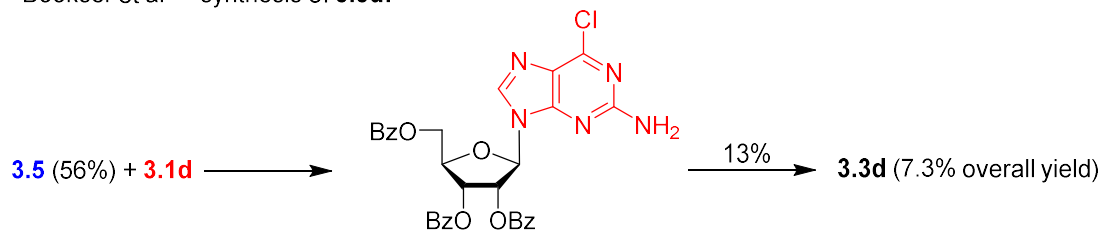
Bookser et al[147] synthesis of **3.3c:**

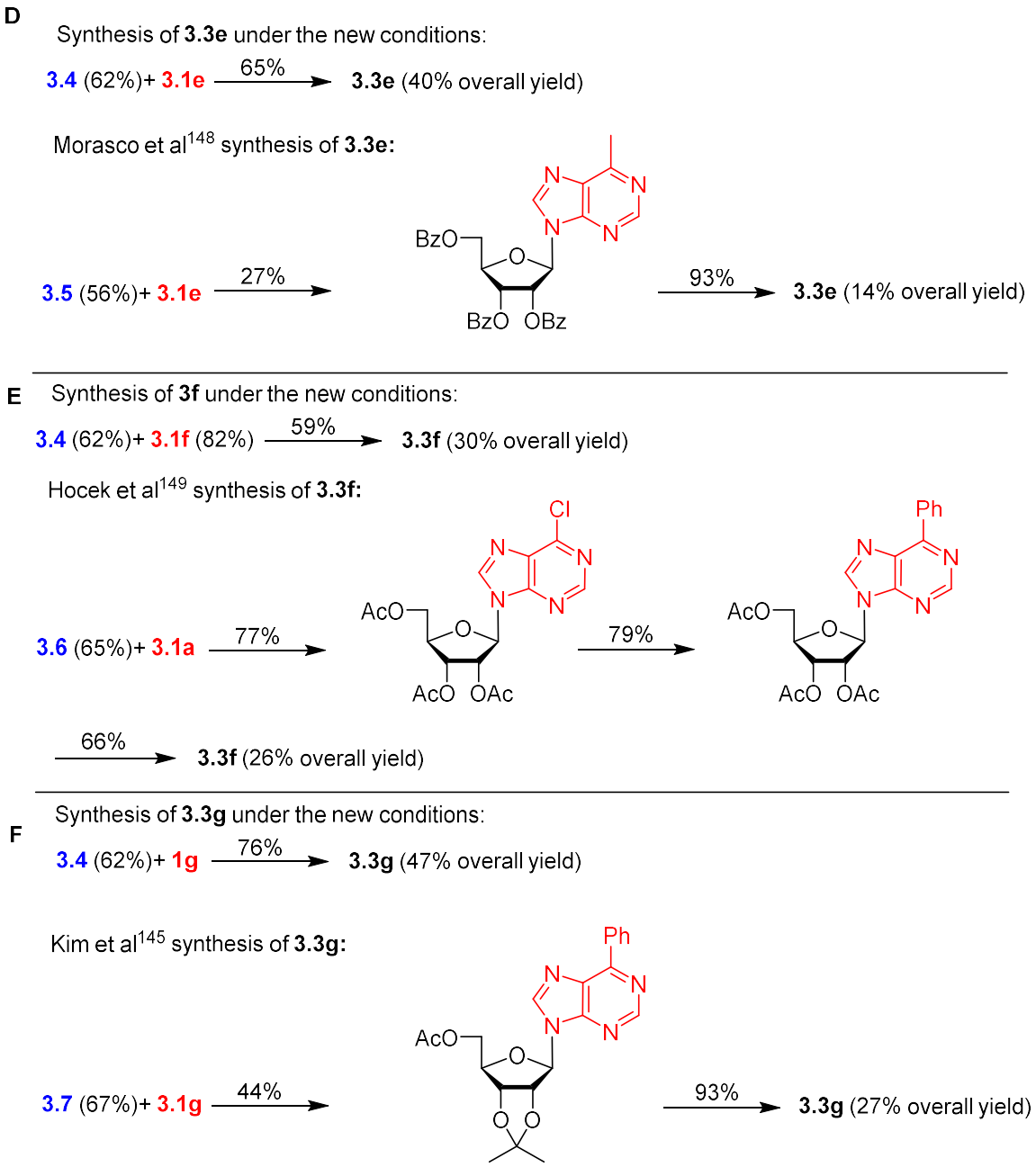


C Synthesis of **3.3d** under the new conditions:

3.4 (62%)+ **3.1d** —68%→ **3.3d** (42% overall yield)

Bookser et al[147] synthesis of **3.3d:**





Scheme 3.5: Comparison of yields to the new procedure versus recent published procedures.

3.1.4 Shortcomings

Despite the operationally simple and conceptually novel nature of this first study, some shortcomings and blockades were encountered. Firstly, cytosine and guanine were not compatible with the conditions. Guanine is known to have little to no solubility in most solvents and tends to be poorly regioselective due to competing glycosylation reactions at N^7.[150] These

observations were consistent with my study, and, thus, no product could ever be detected even if DMF was used as solvent. More surprisingly, was the incompatibility of cytosine in this study. I noted that no reaction took place at all suggesting a side reaction with the nucleobase itself took place before the ribosyl unit was even added although this could not be confirmed. 5-Fluorouracil, an anticancer drug so important it is on the World Health Organization (WHO)'s List of Essential Medicines, was also not compatible. I was also surprised to find out that 7-deazapurine moieties that are unsubstituted at position 7 (the two attempted substrates are shown in Figure 3.2) were also non-viable. In both instances, a reaction with DIAD took place at position 7 resulting in a ~3:2 mixture of the desired product and the undesired 7-subsituted nucleoside (~50 % overall yield). Moreover, I was unable to find other nucleophiles that were compatible with my conditions, such azido, cyano, thiolate, or fluoride. In all case no reaction took place. This perhaps indicated, once again, a side reaction with the Mitsunobu reagents, hence stopping the reaction before the sugar was even added.

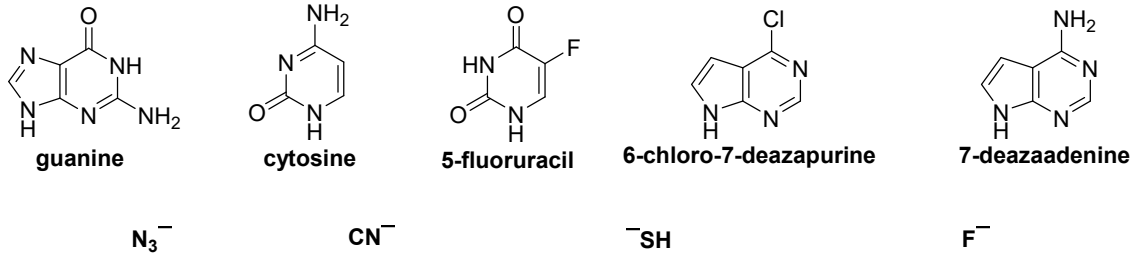


Figure 3.2: Incompatible substrates under the conditions in my initial study.

Due to these very clear shortcomings another investigation was necessitated to find conditions that were more general and resulted in fewer incompatibilities. I postulated that by first undertaking a mechanistic study to better understand what is occurring in the reaction, I would be aided in elucidating improved conditions for protecting group-free glycosylation.

3.2 Mechanistic investigation

3.2.1 Mitsunobu-like reaction

The obvious first step in the mechanistic study was to first confirm that all of the reagents were required for the reaction to take place, hence, indicating that the reaction proceeds through a Mitsunobu-like mechanism. Shown in Figure 3.3 is a TLC plate with four individual experiments run concurrently. It concluded that all reagents are required for a reaction to occur. Four reactions were run in parallel following the procedure for the synthesis of **3.2a**. The TLC

was developed in 9:1 CH_2Cl_2–MeOH; Ribose = D-ribose; 6-chloro = 6-chloropurine (**3.1a**); furanoside = previously isolated furanoside product **3.3a**; pyranoside = previously isolated pyranoside product **3.2a**. The first experiment (column 1 on the TLC plate) served as a negative control. Neither P(*n*Bu)$_3$ nor DIAD was added. No reaction took place. The second experiment (column 2 on the TLC plate) was run without P(*n*Bu)$_3$, but with DIAD, still no reaction took place. Only **3.1a** and ribose can be seen on the plate. The third experiment (column 3 on the TLC plate) was run with P(*n*Bu)$_3$ but in the absence of DIAD. Once again, no reaction took place. The final experiment (column 4 on the TLC plate) contained all of the optimized reaction components. Under UV light the pyranoside product **3.2a** is shown to form with nearly complete exhaustion of 6-chloropurine **3.1a**. Trace amounts of furanoside product **3.3a** can also be seen (Figure 3.3A). The TLC plate was then stained with the *p*-anisaldehyde stain (Figure 3.3B). As final evidence that no product formed when any component of the reaction was missing is seen by the deep black spots of unreacted ribose.

(A) TLC plate under UV light **B) TLC plate after *p*-anisaldehyde stain**

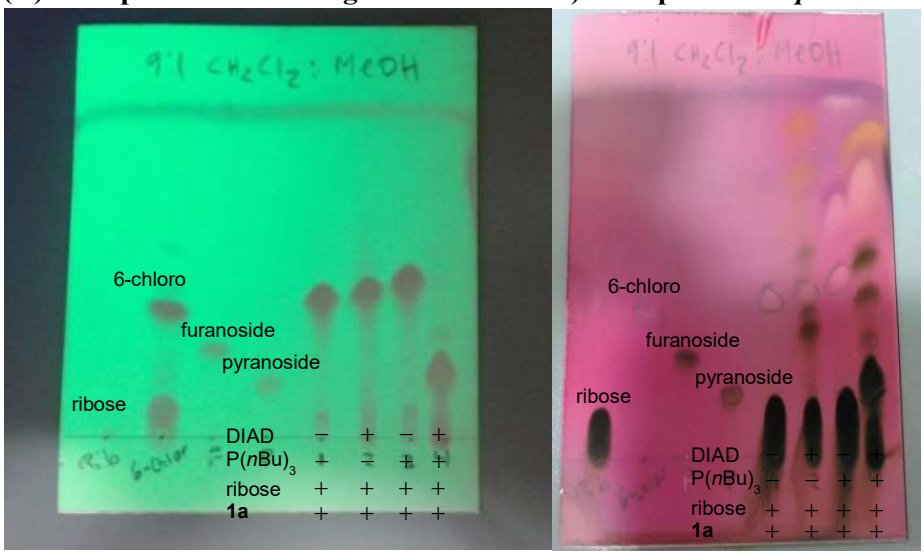
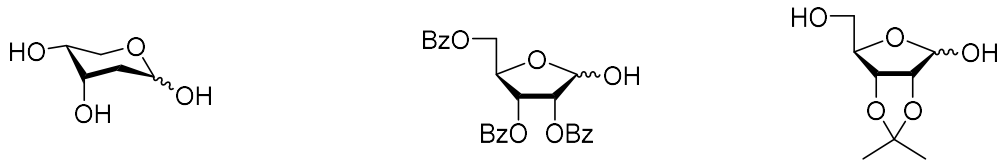


Figure 3.3: Evidence for a Mitsunobu-like reaction.

3.2.2 Elucidating the role of the *C*2-OH group

Once it was known that the reaction proceeds through a Mitsunobu-like mechanism, I sought to rationalize the perfect stereoselectivity of the reaction. The most obvious hypothesis was that the there was anchimeric assistance or a strong directing effect from the *C*2-OH group, to provide in all the instances the desired 1,2-*trans* diastereomer. To test this postulate, I carried out the optimized reaction with 6-chloropurine **3.1a** as the acceptor on three other donor saccharide substrates (Figure 3.4). In the case of 2-deoxyribose (**3.8**) the reaction yielded a

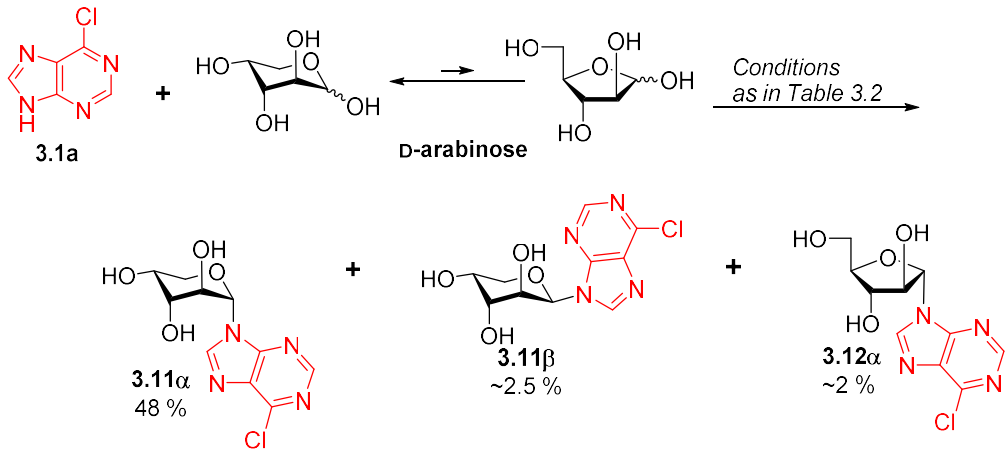
highly complex mixture of products that was impossible to purify. In the case of *C*2-OH-protected donors **3.9**[151] and **3.10**,[152] no traces of a reaction taking place or product forming could be seen by TLC and ^{1}H NMR analysis of the crude reaction mixture. This was highly indicative of a role of the *C*2-OH in the reaction.



3.8 *complex mixture* **3.9** *no reaction* **3.10** *no product*

Figure 3.4: 2-Deoxyribose and *C*2-OH protected donors are not compatible with my conditions.

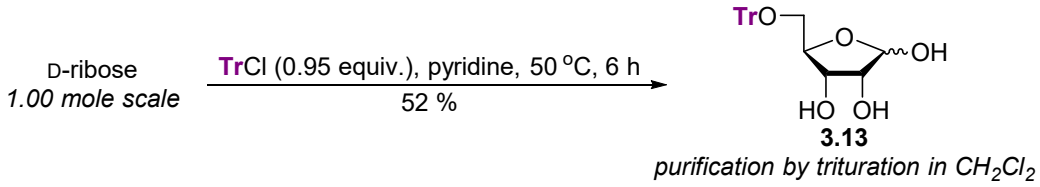
As a final experiment, I subjected D-arabinose to my conditions and **1a** as the donor expecting to obtain the α-anomeric product as the major one and confirm irrefutably that the *C2*-OH group provided neighboring group assistance or a strong directing effect (Scheme 3.6). Pleasingly, I did isolate in moderate yield (48 %) the α-arabinopyranosyl nucleoside (**3.11α**) accompanied by just a small amount of the α-furanoside (**3.12α**, ~2 %) and the β-pyranoside (**3.11β**, ~2.5 %) as determined using ROESY analysis. These data confirmed a role for the *C*2-OH group in the reaction. With these final experiments completed, this project was published in *Organic Letters* where I was the first author on the study.[153]



Scheme 3.6: Glycosylation of 6-chloropurine using D-arabinose as the donor.

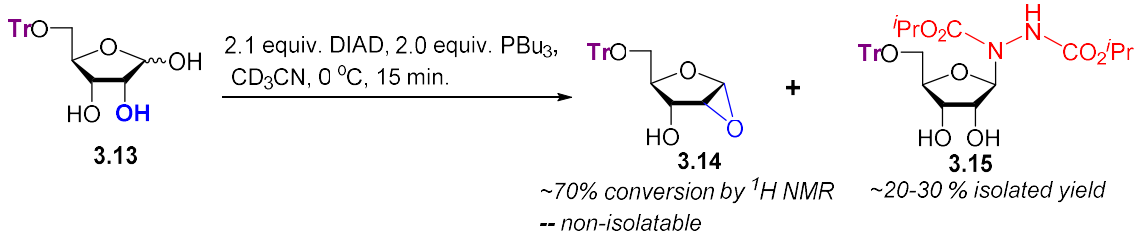
With this knowledge in hand, I endeavoured to elucidate a more thorough mechanism and to determine a method to enlarge the substrate scope on a preparative scale. In order to do this, I thought that I must first scale up the synthesis of the starting 5-*O*-monoprotected sugar. To

my initial dismay, I discovered that the MMTr group was not compatible with decagram scale preparation as it proved unstable and the oily nature of 5-*O*-MMTr ribose further complicated its use. I was pleased to discover, however, that the cheaper trityl group could be used instead and still offered similar acid lability. 5-*O*-Tritylribose (**3.13**) is also a solid, stable at room temperature indefinitely, and multimole syntheses have been described previously making it the obvious selection for seeking improved conditions.[154] I synthesized **3.13** on a one mole scale and purification did not require chromatography (Scheme 3.7).



Scheme 3.7: Mole-scale synthesis of 5-*O*-tritylribose **3.13**.

My initial hypothesis was that the *C*2-OH was offering anchimeric assistance and forming a 1,2-anhydrosugar *in situ* that was subsequently being opened by the nucleophilic nucleobase. In order to assay this, I removed the nucleobase from the reaction and subjected only the sugar to the Mitsunobu reagents (P(*n*Bu)$_3$ and DIAD) in CD_3CN. After 15 minutes, I analysed the reaction mixture by NMR. I was pleased to observe the formation of the 1,2-anhydrosugar (termed "anhydrose") in the ^{1}H NMR as characterized by the upfield shift of the *C*2-hydrogen atom. However, by TLC analysis there were two spots presents indicating an unwanted side-reaction had taken place. I first attempted to isolate the epoxide by column chromatography, but this failed – only starting material (~65 % yield) was found, indicating the anhydrose is unstable. I was able to isolate the unwanted side product (~20-30 % yield with contamination by phosphine oxide), on the other hand, and it was determined to be opening of the epoxide by the reduced hydrazine of DIAD to form dicarboxylate **3.15**, necessitating another electron acceptor be found (Scheme 3.8).



Scheme 3.8: Treatment of 5-*O*-tritylribose (**3.13**) using the first Mitsunobu conditions.

I was pleased to discover that this was not a difficult issue to circumvent: by simply lowering the molar equivalents of P(*n*Bu)$_3$ to 1.6 and substituting ADDP for DIAD (1.5 equivalents) no hydrazine by-product was observed (Figure 3.5A). I then re-ran the reaction in CD_3CN and analysed the reaction mixture by NMR. Gratifyingly, I noted nearly quantitative conversion of the saccharide **3.13** to the anhydrose **3.14** as can be seen in the ^{1}H NMR. I also note that in the absence of moisture, the epoxide was stable *in situ* for at least one month. With these new conditions in hand it was time to undertake an in-depth mechanistic study to confirm precisely the pathway for formation of anhydrose **3.14**.

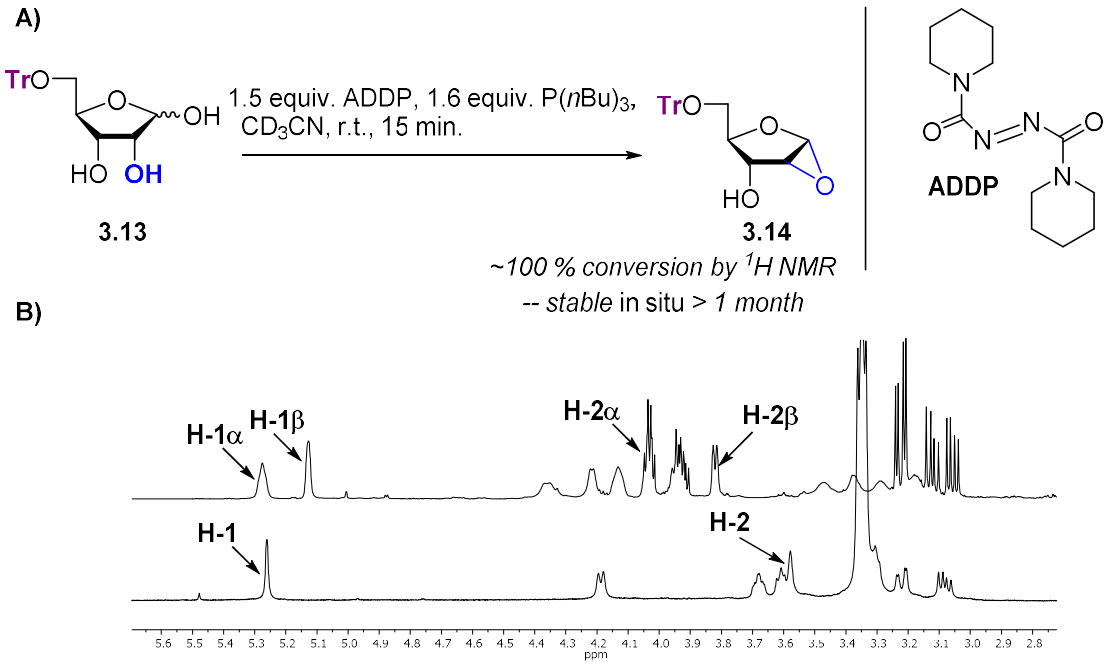
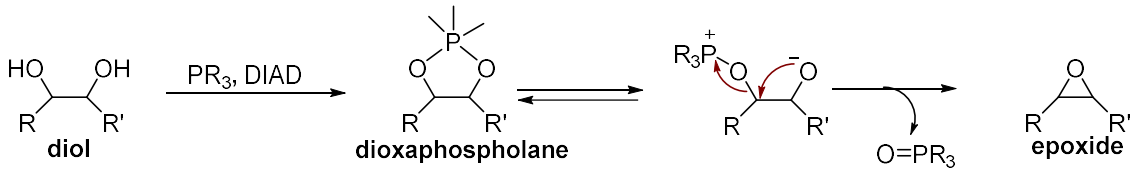


Figure 3.5: Optimizing the formation of anhydrose **3.14**. A) Changing the electron acceptor to ADDP removes any traces of a side reaction. B) The ^{1}H NMR spectrum before (top) and after (bottom) P(*n*Bu)$_3$ and ADDP were added showing nearly quantitative conversion to the anhydrose.

3.2.3 Elucidating the pathway for formation of the anhydrose

To the best of my knowledge no such method to access 1,2-anhydrosugars has been described or characterized even with the other hydroxyl groups protected, but I do note a 2016 study on the hydroxide-catalyzed hydrolysis of 4-nitrophenyl α-mannopyranoside where the intermediate structure was calculated (not experimentally observed) to be 1,2-β-anhydromannose.[155] What has been well accepted is the pathway with which Mitsunobu reaction with diols proceeds.[156-158] It is putative that the reaction progresses through a highly

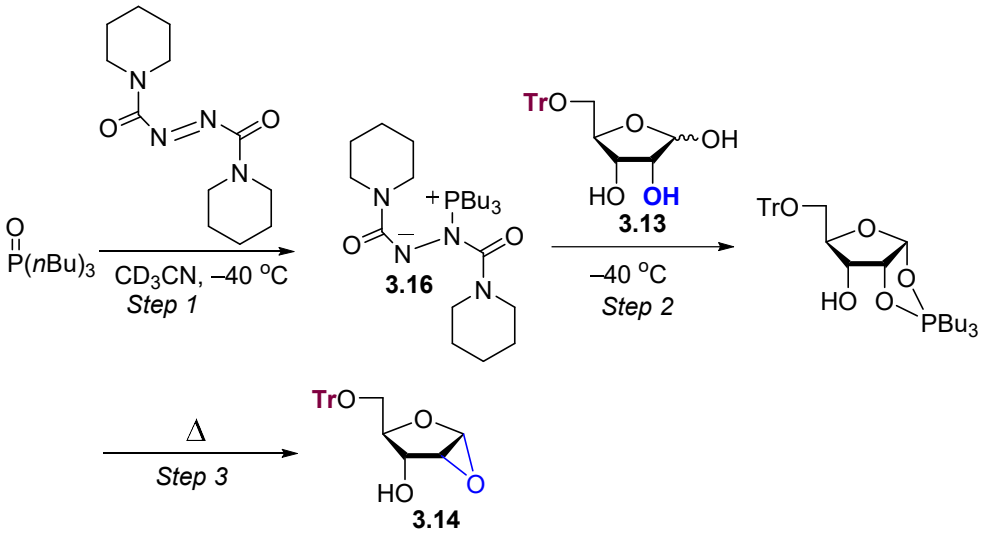
reactive dioxaphospholane intermediate that then cyclizes in an intramolecular fashion to provide the epoxide (Scheme 3.9).[158] Armed with this knowledge, I endeavoured to determine if the glycosylation reaction progresses through this dioxaphospholane intermediate using cryogenic NMR studies.



Scheme 3.9: Proposed mechanism for the conversion of a diol to an epoxide under Mitsunobu conditions.

P(*n*Bu)$_3$ is a highly reactive phosphine and observation of such a phosphorane, specifically a 1,3-dioxaphospholane, has been reported only twice previously when ethylene glycol was used as the diol.[159-160] I hoped that by using low temperature NMR experiments I would be able to increase the lifetime of the phosphorane long enough to confirm its existence and irrefutably determine the stereochemistry at the anomeric centre. Here I must acknowledge the assistance of Dr. R. Pohl (IOCB) in running these experiments with me. The experiments are shown in Scheme 3.10. We first observed that by sequential addition of the Mitsunobu reagents, first P(*n*Bu)$_3$ (δ = –33.37 ppm, Figure 3.6A, bottom) then ADDP, the intermediate phosphonium betaine species (**3.16**, Scheme 3.10) could be observed (δ = +60.66 ppm, Figure 3.6A, middle) by ^{31}P NMR. If the monoprotected ribose moiety **3.13** was then added carefully and quickly to the NMR tube, the key 1,3-dioxaphospholane **3.17** (δ = –14.94 ppm, Figure 3.6A, top) could be seen. These chemical shifts are consistent with previously reported chemical shifts of 1,2 diols reacting with PPh$_3$ and DIAD under Mitsunobu conditions allowing us to confirm the existence of both the betaine and the 1,3-dioxaphospholane.[161] We also observed a distinctive doublet (δ = 5.47 ppm, Figure 3.6B, bottom) in the ^{1}H NMR spectrum containing **3.17**, with a coupling constant of $J_{H,P}$ = 17.6 Hz. This was nearly identical to the H-1–P coupling constant ($J_{H,P}$ = 17.2 Hz) of known α-D-ribofuranosyl 1,2-cyclic monophosphate[162] indicating, but not confirming that the dioxaphospholane was in the *cis* conformation. Pleasingly, the phospholane appeared to be stable indefinitely at –40 °C, thus allowing us to carry out the necessary NOESY and ROESY experiments required to irrefutably establish the stereochemistry. We irradiated the H-1 proton and observed correlations between both the H-2 proton and the methylene protons of the butyl groups on the phosphorus atom, thus validating the formation of the

phospholane. Then, by employing ROESY we noted correlations between the H-1 and H-2 protons which confirmed the *cis* stereochemistry of the 5-membered dioxaphospholane. We then warmed the sample to room temperature and were very pleased to find out that the epoxide still formed in nearly 100 % yield as indicated by a sole anomeric proton and the disappearance of the starting material (Figure 3.6B, top).



Scheme 3.10: Representation of the NMR experiment used to confirm if the reaction goes through a *cis*-1,3-dioxaphospholane intermediate.

With these data in hand, a plausible reasonable reaction mechanism for the formation of anhydrose **3.14** could be posited. Importantly, the pKa of the anomeric hydroxyl proton has been determined by Raman spectroscopy to be only ~11.8,[163] which is much lower than OH-2 and OH-3. Hence, the phosphonium betaine formed in the first step of the Mitsunobu reaction would be able to deprotonate it as they are able to deprotonate nucleophiles up to pKa ~13–14 (Scheme 3.11). The alkoxide formed would then attack at the phosphorus resulting in an oxyphosphonium ion that could be then be attacked by *C*2-OH. Reduction to the hydrazine would occur resulting in dioxaphospholane **3.17**, which then converts to anhydrose **3.14** at higher temperatures extruding O=P(*n*Bu)$_3$ in a concerted process.

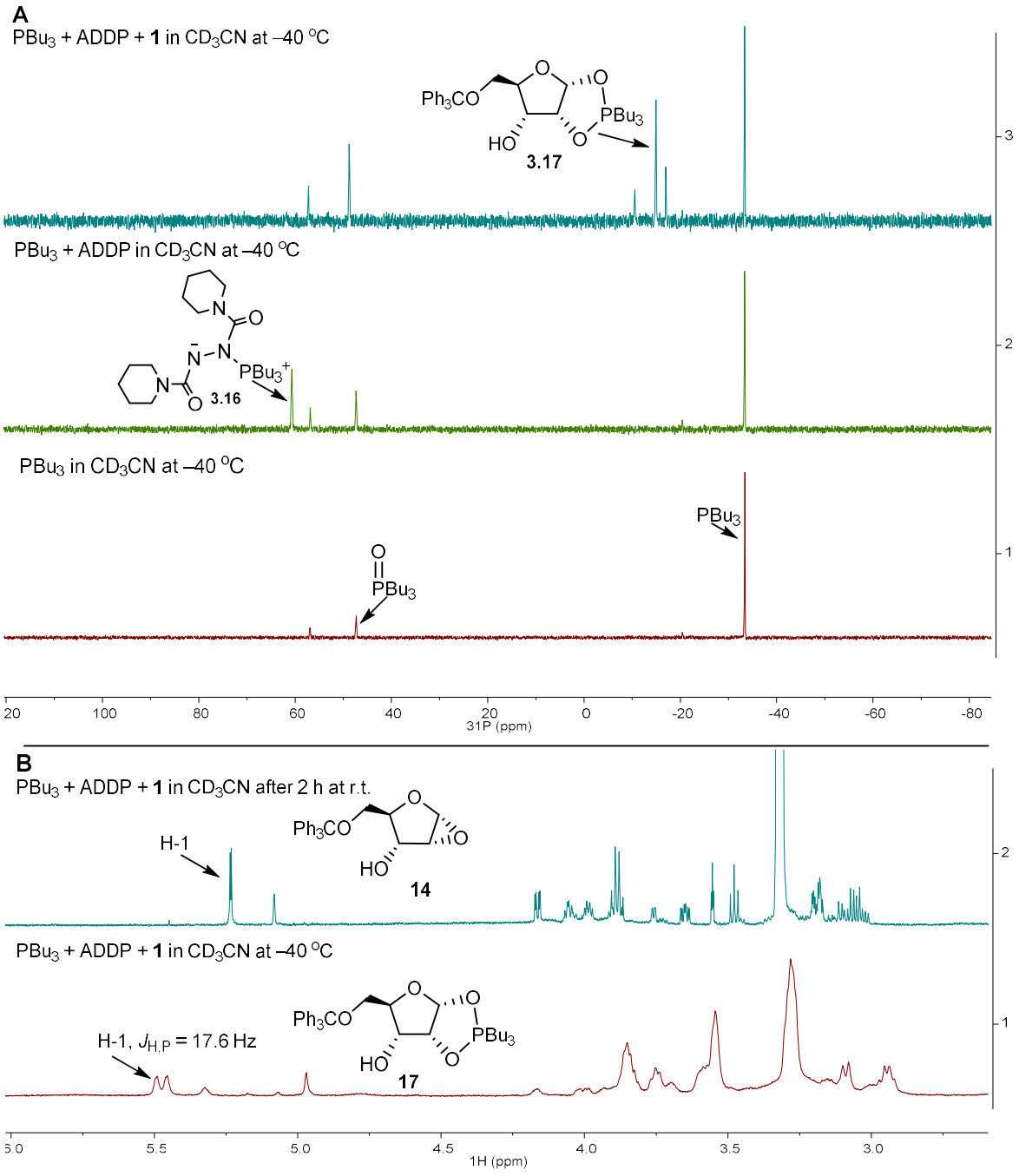
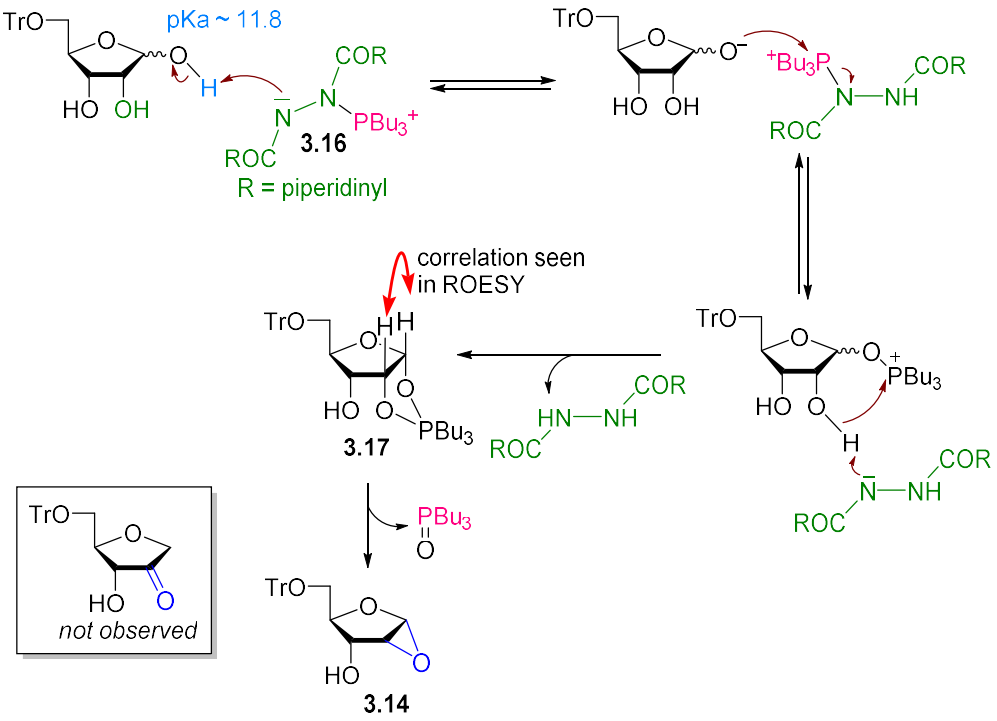


Figure 3.6: Cryogenic NMR experiments confirming the reaction proceeds through 1,3-dioxaphospholane intermediate. A) Overlaying ^{31}P NMR experiments. B) Overlaying ^{1}H NMR spectra.



Scheme 3.11: Plausible reaction mechanism.

Final mechanistic insight was gained by modelling the conversion of phospholane **3.17** to anhydrose **3.14** by density functional theory (DFT) calculations at the level of B3LYP-D3/6-311+G**. I am very grateful to Prof. J. Roithova (Charles University in Prague) for carrying out these calculations and designing Figure 3.7 on my behalf. We used more simplified substrates where a 5-*O*-methyl group was substituted for the 5-*O*-trityl group and methyl groups were modelled on the phosphorus atom instead of the butyl groups (Figure 3.7). The calculations determined that the extrusion of phosphine oxide and the closure of the epoxide ring occurs as a concerted process. The transition state structure is stabilized by participation of the non-bonding electrons of the ring-oxygen atom and hydrogen bonding between the *C*3-OH group and the oxygen atom at the *C*2 position. The energy barrier for the formation of anhydrose **3.14** is 16.1 kcal mol^{-1}. The alternative reaction pathway leads to the 3-tetrahydrofuranone derivative which is, in fact, thermodynamically favoured, however, it was never observed because it would proceed over an energy barrier of 18.4 kcal mol^{-1} which explains the complete preference for the formation of anhydrose **3.14**.

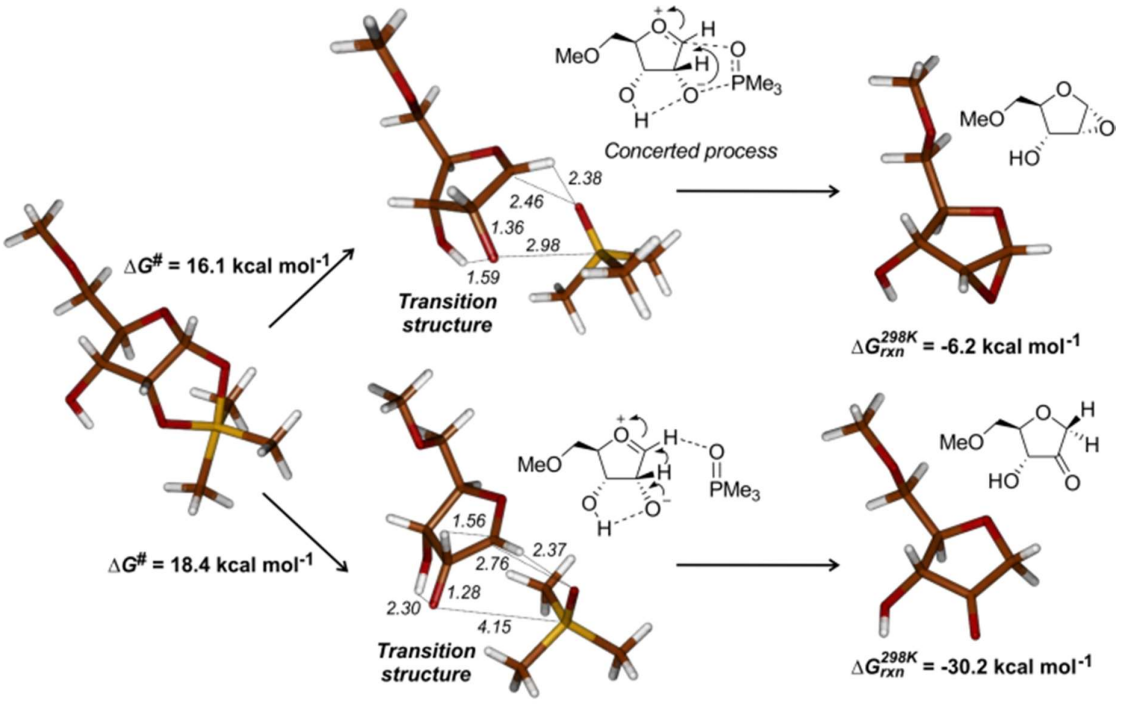


Figure 3.7: Mechanism of rearrangement of the phosphorane **3.17** to the anhydrose **3.14** or the tetrahydrofuranone (not observed) from B3LYP-D3/6-311+G** calculations with continuum model approximation of MeCN solvation. The selected interatomic distances at the transition structures are given in Å. Figure prepared by Prof. J. Roithová, taken from Downey, et al (2017).[164]

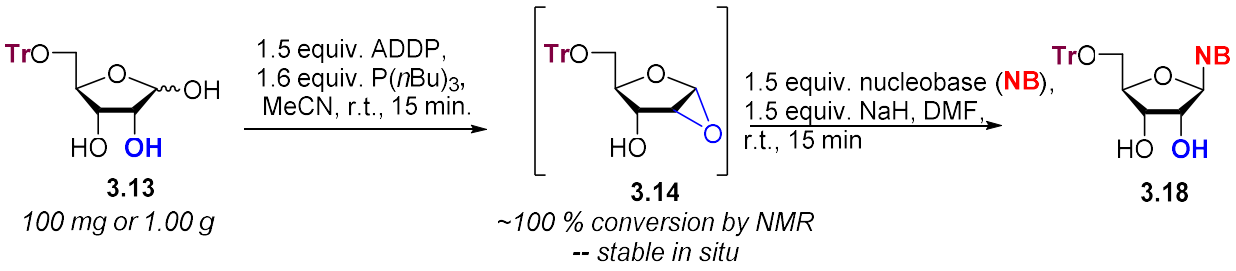
3.3 Development of an improved protocol

3.3.1 Synthesis and isolation of the 5-*O*-monoprotected nucleosides

Armed with a mechanism and the improved conditions, I sought to expand the substrate scope to include all of nucleosides that were not available in the first study on both a semi-preparative (0.25 mmol) and preparative scale (2.5 mmol). The new conditions dictated that the anhydrose be formed first *in situ* then the nucleophile (nucleobase) be added after the formation was complete. One very convenient aspect of using ADDP as the electron acceptor in the Mitsunobu reaction is that ADDP is an orange solid with high solubility in MeCN and the reduced hydrazine is a white solid with lower solubility so it can be easily determined when the Mitsunobu reaction-dictated conversion to the 1,2-anhydrosugar is complete (Figure 3.8).

Figure 3.8: Epoxide **3.14** formation on a 1.00 g scale: **(Left)** Flask prior to the addition of ADDP, **(Middle)** flask immediately after the addition of ADDP, **(Right)** flask 15 minutes after the addition of ADDP confirming **3.14** has formed.

With the epoxide forming in a separate flask, I would deprotonate the nucleobase and then add it to the epoxide to perform the glycosylation after the epoxide was formed. Despite the high boiling nature of DMF, I still determined that this solvent was best due to its increased solubilizing capacity of many of the nucleobases. I also discovered that NaH was the optimal base for deprotonation because in many cases the deprotected nucleoside was so polar, separation of the desired product from DBU proved to be extremely difficult or impossible using either reverse phase or normal phase chromatography. I also wanted to demonstrate the possibility of being able to isolate both the monoprotected and deprotected nucleoside on a preparative scale.



3.13
100 mg or 1.00 g

3.14
~100 % conversion by NMR
-- stable in situ

3.18

Scheme 3.12: Procedure for the glycosylation of nucleobases using 5-*O*-monoprotected ribose analog **3.13** as the donor.

With the improved conditions and order of addition of the reagents in hand, I synthesized a series of nucleosides to address the shortcomings of the previous study (Figure 3.13).

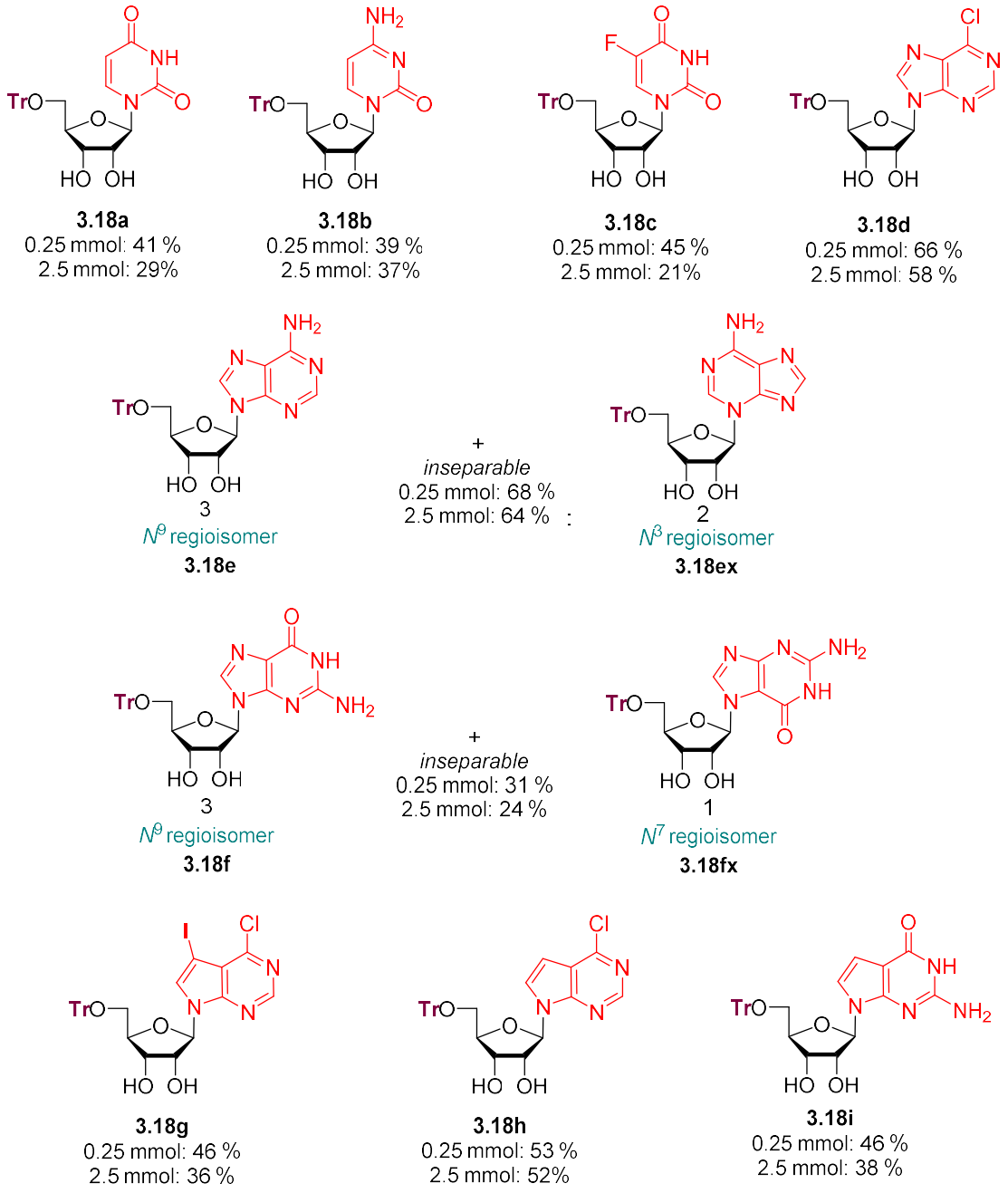
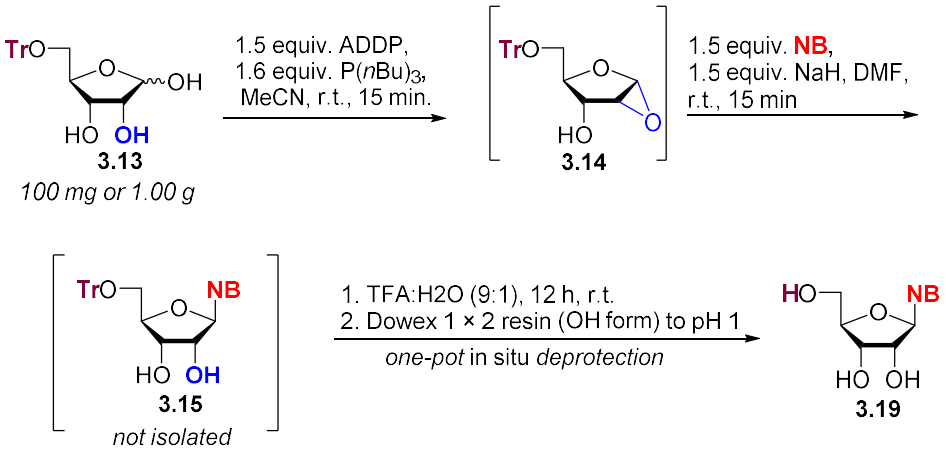


Figure 3.13: Expanded substrate scope under the improved conditions at both semi-preparative and preparative scale.

Once again, as in my first study, the reaction was perfectly stereoselective for the β-anomer as determined by ^{1}H NMR of the crude reaction mixture in each case. On the semi-preparative scale (100 mg or 0.25 mmol of **3.13**) yields of the pyrimides (41–45 %) were still lower than in the purines, however, pleasingly, their yield improved dramatically from the first study. Importantly, 5-fluorouridine, incompatible with the first conditions, was available as well in moderate yield. In the purine series, pleasingly, 6-chloropurinyl (**3.18d**), 6-chloro-7-deazapurinyl (**3.18h**), 6-chloro-7-iodo-7-deazapurinyl (**3.18g**) and 7-deazaguaninyl (**3.18i**) nucleosides were obtained cleanly as single regioisomers in moderate to good yields (46–66 %). On the other hand, in the case of adenine and guanine, inseparable mixtures of the desired N^9 nucleosides **3.18e** and **3.18f** with unwanted N^3 (in adenine, **3.18ex**) or N^7 (in guanine, **3.18fx**) regioisomers were obtained in 66 % and 31 % yield, respectively. I also note that in the case of guanine, DMSO had to be used as solvent instead of DMF due to its deplorable solubility in virtually all solvents. Although, this lack of regioselectivity was unfortunate, the desired N^9 isomer presented in excess and I believe this to be one of the only examples of guanine being shown to be chemically glycosylated at all. A more in-depth study on the regioselectivity is available in Section 3.3.3. When the reactions were scaled up one order of magnitude (1.00 gram or 2.5 mmol of **3.13**), encouragingly, with the exception of 5-fluorouridine a dramatic decrease in yield was not observed.

3.3.2 Synthesis of the free nucleoside in one-pot.

Analogous to our initial work, I wanted to prove that the deprotection step could be carried out in a two-step one-pot process. Initially, I tried to duplicate the conditions from the first investigation (1 M HCl(aq), pH ~1) at both room temperature or at 50 °C. Although these conditions proved to be compatible and no degradation of the anomeric centre was observed with many of the substrates, the 7-deaza-7-unsubstituted purines did show some destruction. As a result, I substituted the acid for 90 % trifluoroacetic acid (TFA) in water which solved this issue. I must note, however, that an increased length of time (12 h) at room temperature was required for detritylation. In order to avoid any problems of contamination of the products by trifluoroacetate salts and to avoid cleavage of the nucleobase during work-up, I increased the pH of the reaction mixture using Dowex 1 × 2 resin in the $^-$OH form to pH ~1. The reaction mixture could then be concentrated *in vacuo* under normal conditions (Scheme 3.13).



Scheme 3.13: The conditions for the two-step one-pot synthesis of fully deprotected nucleosides at a preparative scale using donor **3.13**.

In the instances of compounds **3.19a**–**3.19d**, **3.19g**–**3.19i** the isolated yields of the deprotected nucleosides were comparable to that of the yields of the monoprotected nucleosides, demonstrating that there was no degradation at the anomeric position (Figure 3.14). I was also pleased to observe that after this detritylation, adenosine **3.19e** could be separated from its N^3 regioisomer by crystallization after column chromatography. I mention lastly that guanosine **3.19f** was also provided in 14 % overall yield after iterative chromatography to separate it from its N^7 isomer.

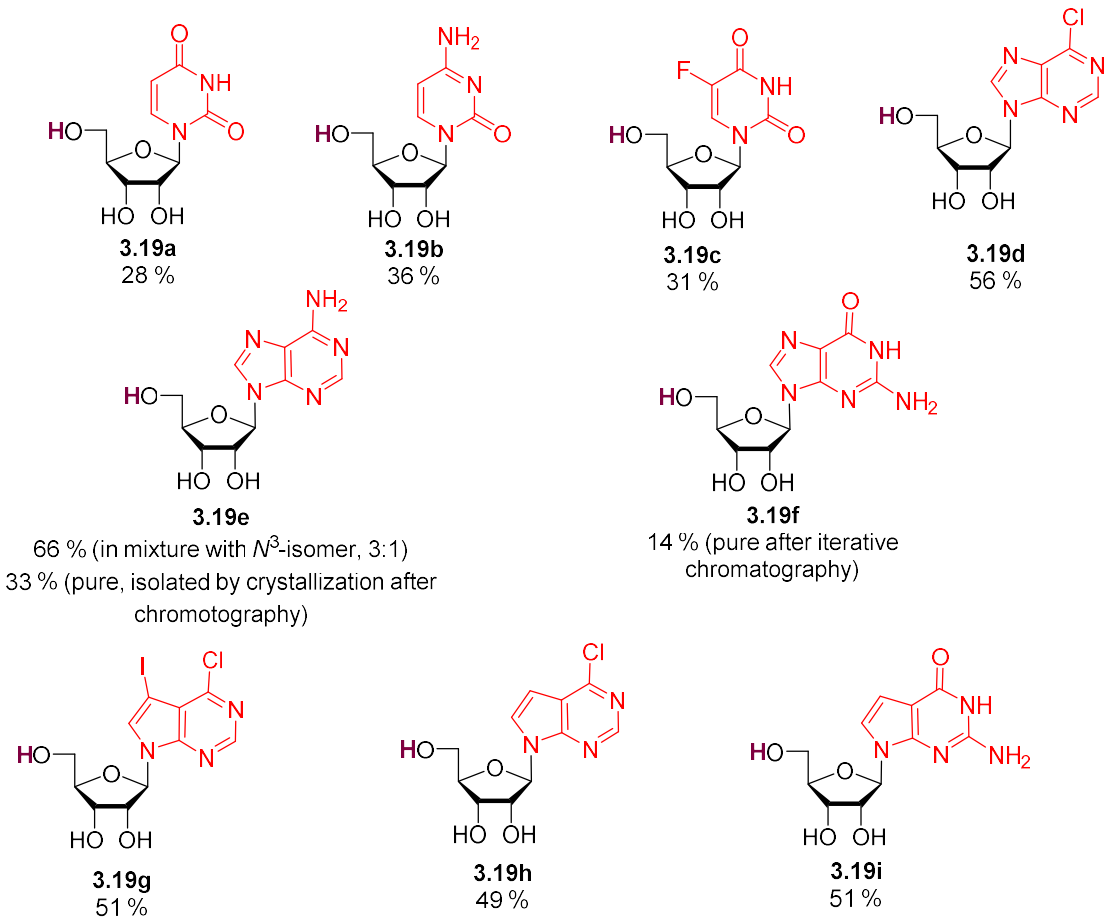


Figure 3.14: Substrate scope of the preparative scale one-pot glycosylation–deprotection strategy.

In our previous communication[153] I employed the slightly more acid-labile 4-methoxytrityl group for the 5-*O*-protecting group. I also employed a stronger acid (aq. HCl) to effect the deprotection. These two factors are the most probable reasons contributing to the more rapid deprotection rate (15 min to 1 h) than what was under these improved conditions (12 h). However, as discussed above, the substrate scope has been substantially broadened. I conclude, therefore, that this one minor setback is inconsequential as a result of the vastly improved substrates available.

3.3.3 Regioselectivity study using deazaadenines

As a result of this regiochemical drawback with adenine discussed in the previous two sections, I was compelled to carry out a systematic study of deazaadenines lacking the nitrogen atom at each of the 1, 3, or 7 positions to see what impact that would have on the products formed (Figure 3.15). For convenience, the purine nomenclature has been used, not the correct

IUPAC nomenclature which is used in the experimental section to follow. The reactions were only carried out at preparative scale using the improved conditions because I was anticipating multiple products and wanted to ensure enough material was generated to allow for isolation and characterization. Interestingly, in the case of 7-deazaadenine only the N^3 regioisomeric nucleoside (**3.18j**) was produced in very moderate yield (36 %). In the case of 1-deazaadenine the major product was still the N^3 regioisomer (**3.18kx**, 38 %), however, the desired product was also formed in very poor yield (**3.18k**, 17 %). Encouragingly, however, the isomers were separable using flash chromatography. Finally, in the case of 3-deazaadenine, I did observe the desire N^9 regioisomeric product (**3.18l**, 49 %) formed in large excess (7:1 ratio) over the N^7 isomer (**3.18l**, 8 %).

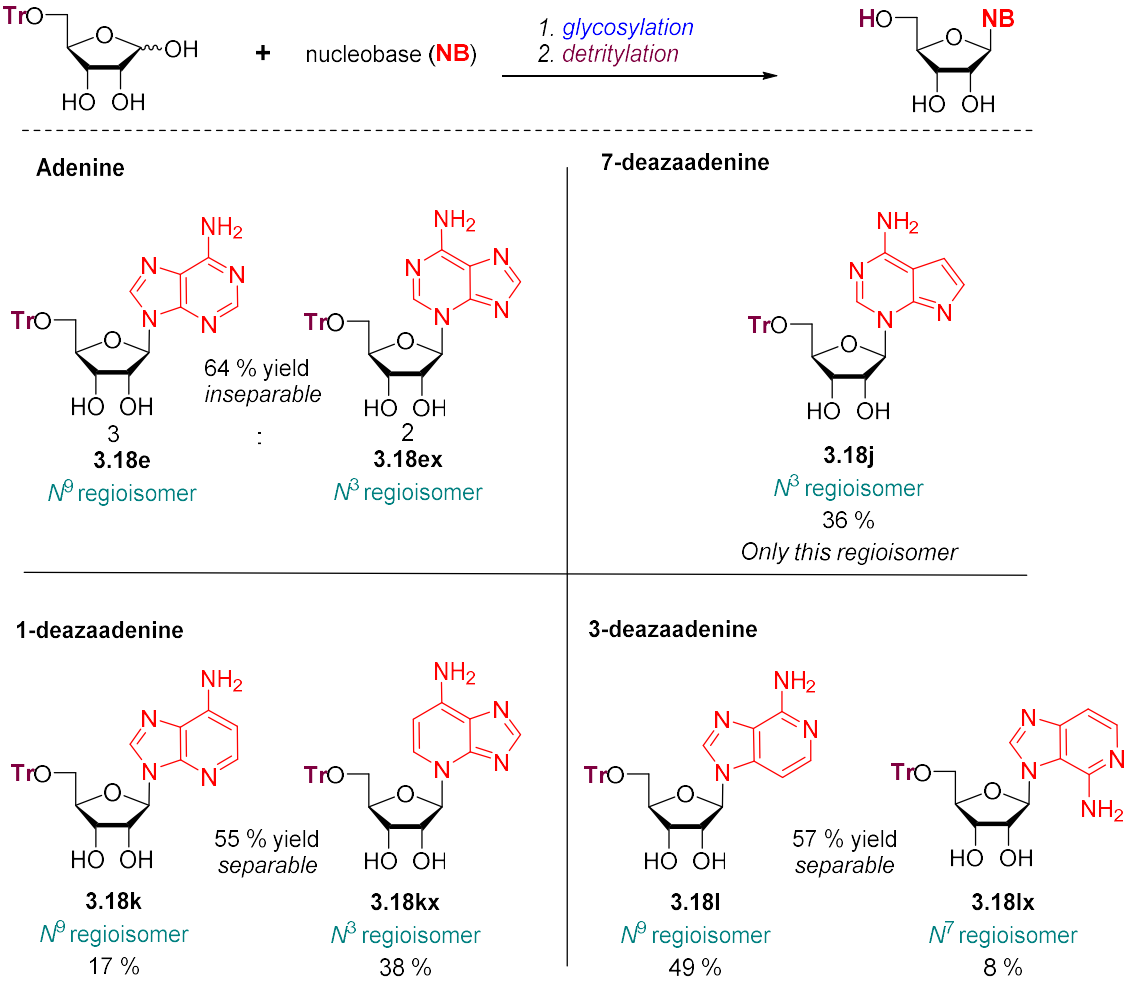


Figure 3.15. Systematic deazaadenine glycosylation study of the monoprotected nucleosides.

With the monoprotected study complete, I next sought to determine if these analogs could be separated and/or deprotected using the detritylation strategy from Section 3.3.2 of this thesis.

Surprisingly, once again in the systematic deazaadenine study (Figure 3.16), I observed that either partial or utter destruction of the undesired N^3 regioisomers was observed during the acid cleavage of the trityl group. In fact, regioisomer **3.19j** was not observed at all—only complete decomposition of the nucleoside. In the case of 1-deazaadenine, the N^3 regioisomer (**3.19kx**) was degraded to a loss of 26 % overall yield (only 12 % isolated yield). This implied that glycosylation at the N^3 position of the pyrimidine ring provides a much less stable product than at the desired position (N^9) on the pyrrole or imidazole ring. In the case of 3-deazaadenine, the desired N^9 analog suffered no loss of yield during deprotection, but once again the small amount of the isomeric N^7 nucleoside was decomposed to an extent that it could not be isolated in purely, in high enough yield for characterization. Only very small traces by NMR analysis could detect its presence. Due to the low yield of the deprotection of N^7 regioisomer, this datum is indicative of a similar phenomenon as the N^3 position of the pyrimidine ring, however, further research would be required to confirm this.

Another notable detail of this study is that it is not just academic, the fact that both deazaadenosine regioisomers **3.19k** and **3.19l** were not noticeably destroyed using the deprotection conditions could have implications in the study and development of ribozymes.[165-166]

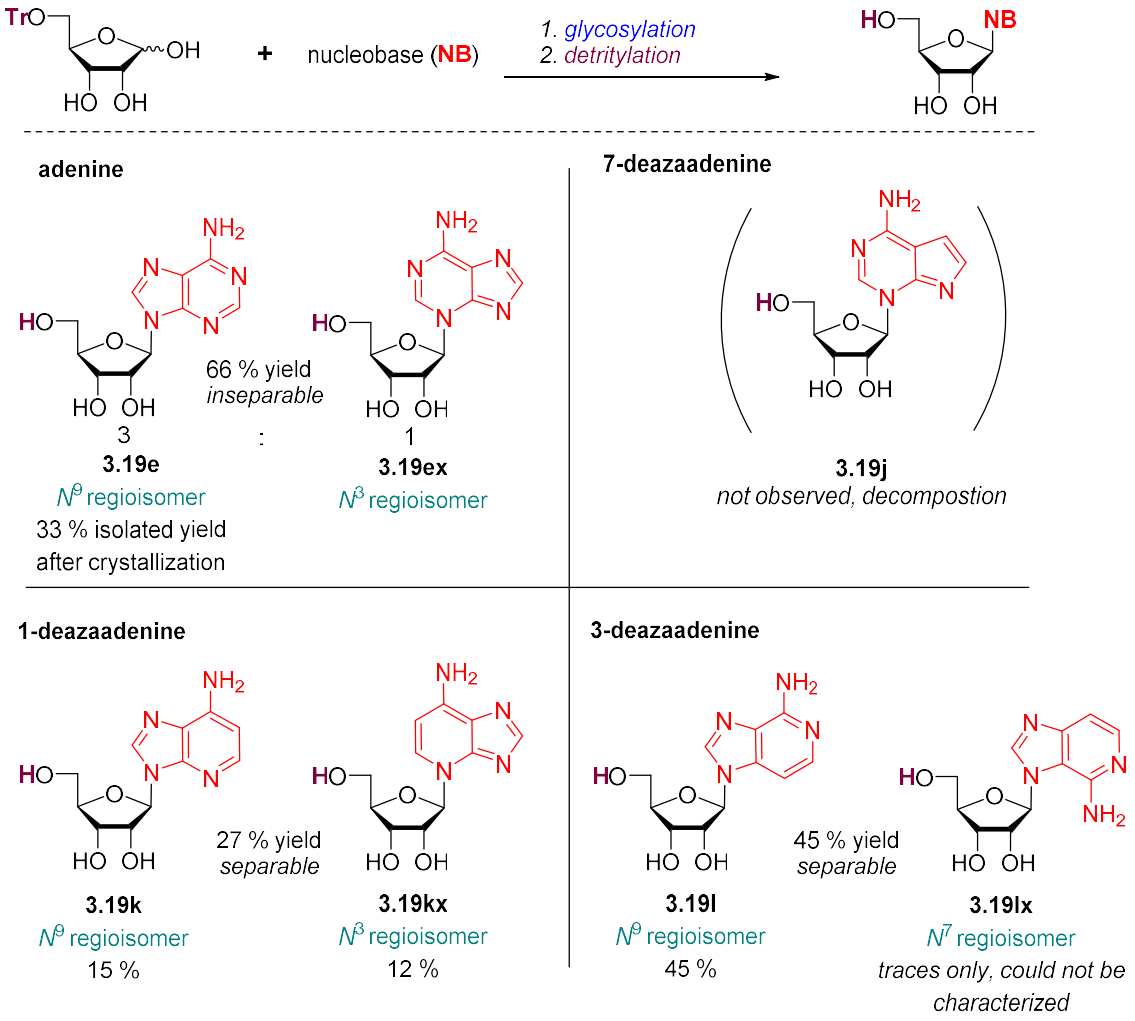
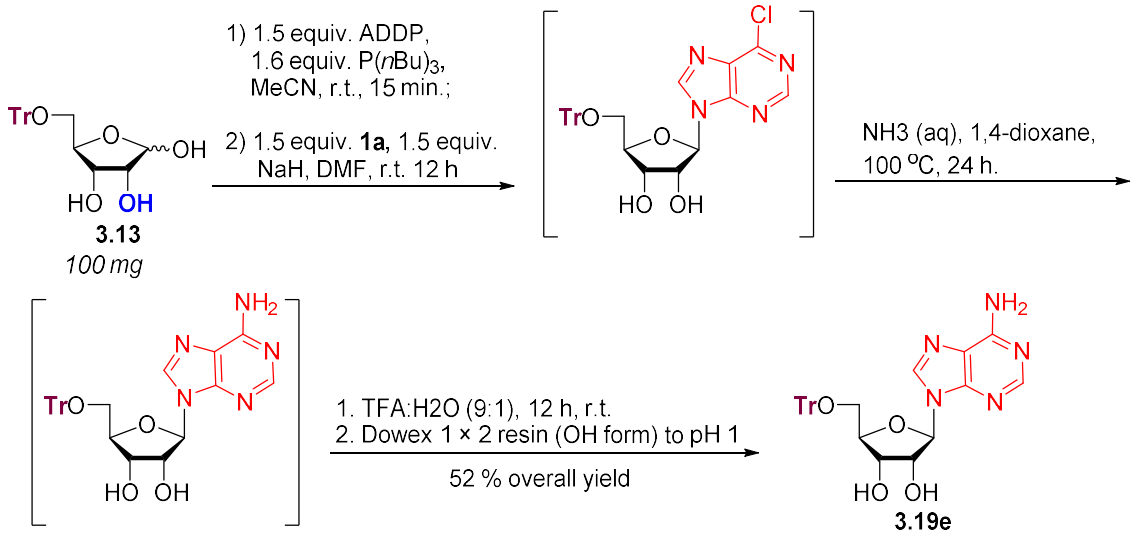


Figure 3.16: Systematic deazaadenine two-step one-pot glycosylation–deprotecton study to provide deprotected deazaadenosines.

3.3.4 Improved synthesis of adenosine

As a result of the diminished regiochemical control in the synthesis of adenosine, I endeavored to find alternate conditions to solve this issue. The obvious strategy was to try a three-step one-pot process where I would use 6-chloropurine as the donor, effect the glycosylation under my optimized conditions, perform standard amination of the heterocycle, and then cleave the trityl group using 90 % TFA in water. Knowing that the amination is best effected in NH_3(aq) and 1,4-dioxane, I hoped that the glycosylation could also take place in 1,4-dioxane as well. Unfortunately, the glycosylation does not take place to any extent in 1,4-dioxane so I quickly abandoned this strategy. What I was pleased to observe that if I simply switch back to the initial glycosylation solvent (4:1 MeCN/DMF), then evaporate the solvent after 12 h and take up the

crude reaction mixture in 1,4-dioxane/NH_3(aq), this problem was easily bypassed. After heating this mixture at 100 °C for 24 h, reevaporating the solvent and cleaving the trityl group as in Scheme 3.14, I could isolate adenosine in 52 % overall yield after purification by flash chromatography (Scheme 3.14).



Scheme 3.14: Three-step one-pot synthesis of adenosine.

3.3.5 Synthesis of medicinally- or biologically-relevant nucleosides.

To further demonstrate the utility of this methodology, I endeavored to expand the methodology to include medicinally-relevant and biologically relevant nucleosides. I also sought to demonstrate that this methodology could be amenable to the synthesis of building blocks for automated solid-phase phosphoramidite synthesis. Three obvious sugars to carry out this study were 5-deoxy-D-ribose, 5-deoxy-5-fluoro-D-ribose, 5-*O*-DMT-D-ribose; the reasons being because antitumor prodrug doxifluridine **13a**[167] and organic fluoride precursor 5′-deoxy-5′-fluoroadenosine[168] bear these sugar scaffolds (Figure 3.17, bottom). It is important to note that 5'-*O*-tritylated ribonucleosides in general are also useful intermediates in the synthesis of phosphoramidites for automated synthesis of oligonucleotides on solid support.[169] I briefly discuss the synthesis of the parent sugar before discussing the analogs available under the optimized conditions.

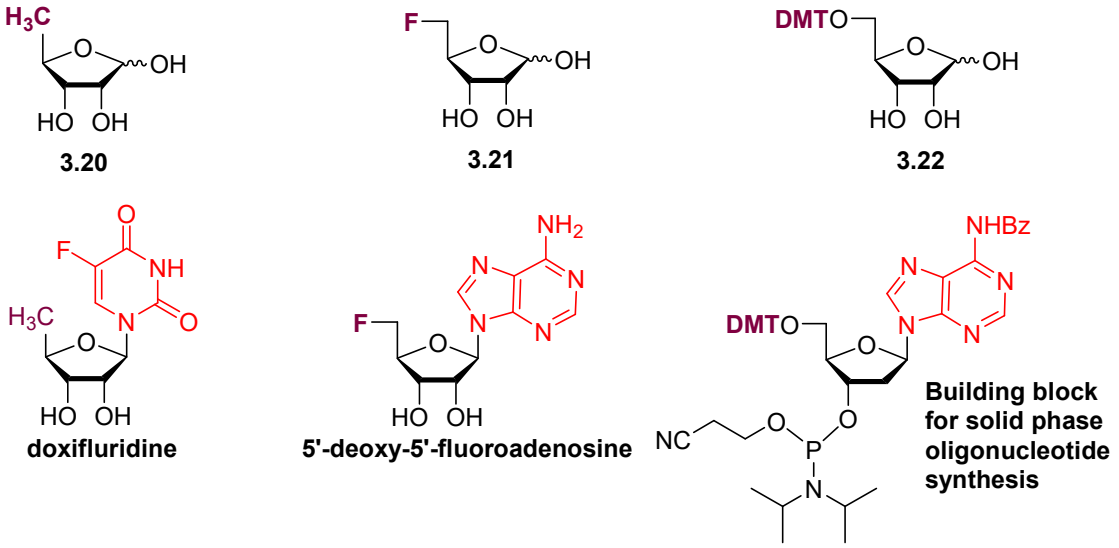
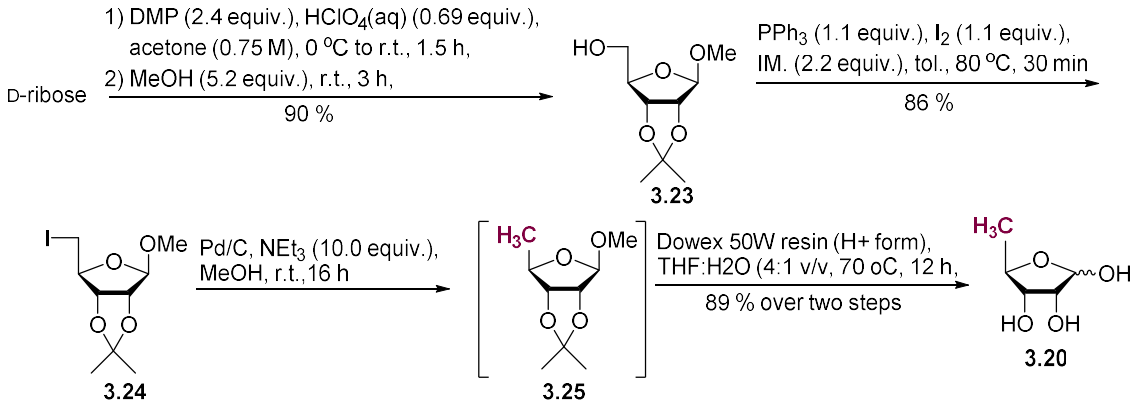


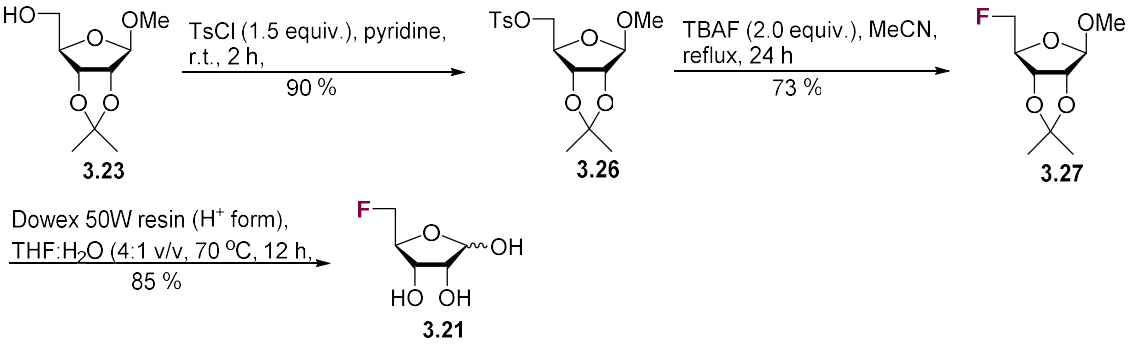
Figure 3.17: The structures of 5-deoxy-D-ribose, 5-deoxy-5-fluoro-D-ribose, 5-*O*-DMT-D-ribose and important nucleosides that contain their scaffolding.

5-deoxy-D-ribose is a known compound,[170] however, I devised a higher yielding synthetic pathway in fewer steps than reported previously to the best of my knowledge. Using a well described method from the Carrell group,[171] methyl 2,3-isopropylidine ribofuranoside **3.23 w**as synthesized in excellent yield. Subseqently, the free *C*5-OH group was converted *C*5-deoxy-5-iodo derivative **3.24**[172] in good yield using the Appel reaction. I then opted to utilize hydrogenolysis to cleave the iodo group followed by acidic removal of the 2,3-isopropylidene group (Dowex 50W resin, H^+ form) to provide 5-deoxy-D-ribose (**3.20**) in 89 % yield over the final two steps. The final two steps had not been described exactly as such previously and proved to be higher yielding (Scheme 3.15).



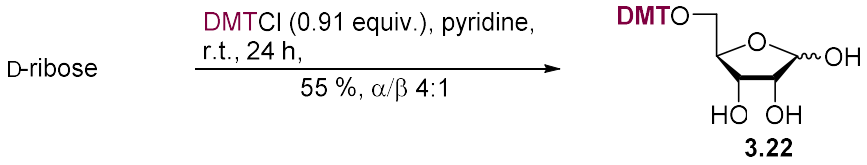
Scheme 3.15: Synthesis of 5-deoxy-D-ribose.

With 5-deoxyribose in hand, I synthesized 5-deoxy-5-fluoro-D-ribose as well (Scheme 3.16). Starting from partially protected methyl glycoside **3.23**, the free *C*5-OH was tosylated in excellent yield (**3.26**, 90 %) and then displaced nucleophilically by *tert*-butylammonium fluoride (TBAF) in a slightly modified procedure reported to what was reported by the O'Hagen group[173]. With protected intermediate **3.27** in hand, I effected the deprotection using the same conditions as in the synthesis of **3.20** to provide 5-deoxy-5-fluoro-D-ribose (**3.21**) in good yield and 4 steps from D-ribose.



Scheme 3.16: Synthesis of 5-deoxy-5-fluoro-D-ribose **3.21**.

I mention finally the synthesis of 5-*O*-DMT-D-ribose as this compound has been reported once previously by the Wengel group[174] in the literature, however, a complete experimental procedure was not provided, nor any characterization data for the molecule. I do remark that the yield reported by the Wengel group was reproducible in my hands, however. Simply, a slight excess of D-ribose was stirred in pyridine in the presence of dimethoxytrityl chloride (DMTCl) for 24 h at room temperature. I was pleased to observe that after work-up, the compound could be purified using normal phase (SiO_2) column chromatography and no cleavage of the DMT group was observed. Monoprotected compound **3.22** was isolated in a moderate 55 % yield (Scheme 3.17). I also provided a complete characterization of the molecule.



Scheme 3.17: Synthesis of 5-*O*-dimethoxytrityl-D-ribose **3.22**.

With these three monosaccharides in hand, I was ready to test the effectiveness of the glycosylation conditions on forming the corresponding anhydroses and then nucleosidation of

the activated acceptors. I subjected first 5-deoxyribose **3.20** to these conditions. I first carried out the NMR experiment to confirm that the epoxide formed quantitatively *in situ*. As in the case of 5-*O*-tritylribose **3.13** the reaction mixture turned from a homogenous bright orange color to a heterogenous white slurry, indicating that anhydrose **3.28** had formed (Figure 3.18, left). I was also pleased to find that it proved stable indefinitely like anhydrose **3.14**.

Figure 3.18: Pictures taken after formation of the anhydroses. **Left:** Completed conversion of **3.20** to anhydrose **3.28**. **Middle:** Completed conversion of **3.21** to anhydrose **3.30**. **Right:** Completed conversion of **3.22** to anhydrose **3.32**. All three pictures were taken after stirring at room temperature for 15 minutes.

On a semipreparative scale I then synthesized three nucleosides all in good yield and perfectly stereoselectively for the β-anomer. Most pleasingly, commercial anticancer drug, doxifluridine (**3.29a**), was available under these conditions in 54 % yield. This means that it can be made in 6 steps from 5-fluorouracil and ribose. The glycosylation also yielded a nucleoside in nearly twice the yield as in the 5-*O*-tritylribose series. Also, noteworthy is 7-deazapurine analog **3.29c** (63 % yield), which could be used as a synthetic precursor for the synthesis of nucleoside kinase inhibitors (Figure 3.19) was also isolatable.[175]

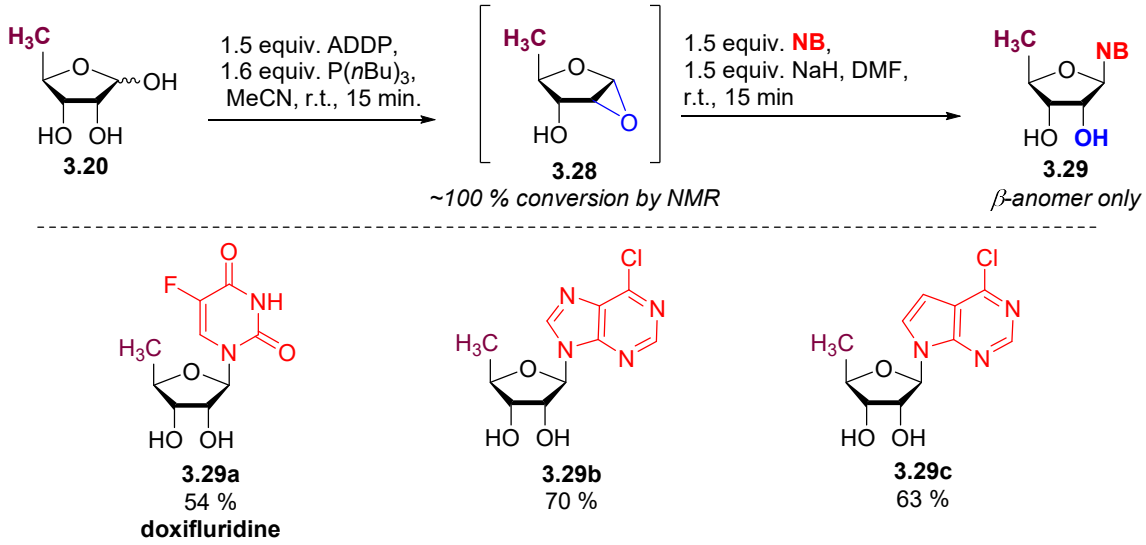


Figure 3.19: Synthesis of 5-deoxyribofuranosyl nucleosides.

I next tested 5-deoxy-5-fluoro-D-ribose **3.21** to analyze its ability to yield anhydrose **3.30** under the epoxide-forming conditions prior to glycosylation. As discussed above, we were interested in these nucleosides as 5-deoxy-5-fluoroadenosine has been shown to be an organic fluoride precursor so more analogs could be of interest in elucidating biosynthetic pathways (Figure 3.20).[168]

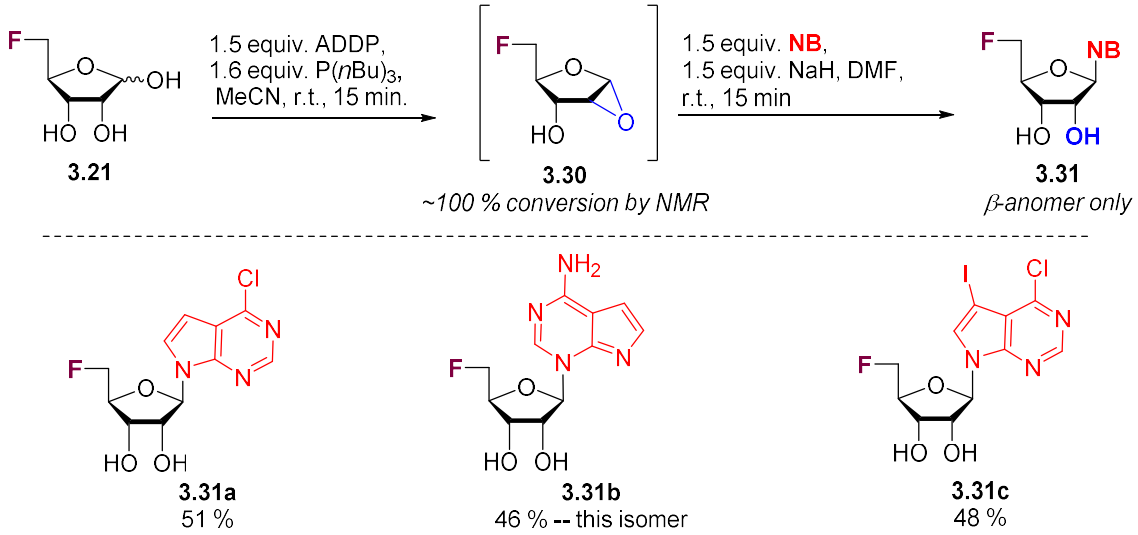


Figure 3.20: Synthesis of 5-deoxy-5-fluororibofuranosyl nucleosides.

I was once again delighted to observe that in CD_3CN the anhydrose **3.30** could still be observed to form quantitatively, however, its bench life was only hours instead of weeks. The formation of the anhydrose was deemed complete when after addition of the Mitsunobu reagents, the reaction turned from a homogenous orange solution to a heterogenous brown slurry containing a white solid (Figure 3.18, middle). The slightly lower stability of the anhydrose proved trivial as the conditions still allowed me to isolate 7-deazapurine analog **3.31a** in moderate to good yield (51 %) and likewise 7-deaza-7-iodopurine analog **3.31c** (48 %). I also sought to determine if the same phenomenon that was observed in the systematic deazaadenine series held true with other saccharide donors. Not surprisingly, when 7-deazaadenine was used as the acceptor the sole product observed was N^3-glycosylated regioisomer (**3.31b**, 46 %) suggesting the it is the electronic properties of the heterocycle that results in its regioisomeric preference not the sugar donor. In all three instances only the β-anomer formed.

My final study focused on the ability of the improved procedure to be valuable to automated solid-phase synthesis of oligonucleotides. As in the previous study, I first screened the efficiency of the epoxide formation on a small scale by NMR in CD_3CN. I was again pleased to observe quantitative conversion to anhydrose **3.32**. The completed formation of anhydrose **3.32** was monitored visually, as the reaction turned from orange to colorless over 15 minutes (Figure 3.18, right). I then screened two acceptors, uracil and N^6-benzoyladenine to assay the ability of the nucleosidation to proceed (Figure 3.21). Pleasingly, both nucleosides were formed in moderate yield: uridine analog **3.33a** in 54 % and the N^6 protected adenosine analog **3.33b** in 51 %. Most strikingly, the N^6-benzolyated analog was regioselective for the desired N^9-glycosylated product. I postulate that perhaps the electron withdrawing nature of the benzoyl group results in this perfect regioselectivity. There would be slightly less electron density available around the pyrimidine ring of the purine scaffold thus precluding nucleophilic attack from taking place at any other nitrogen other than the desired N^9 position.

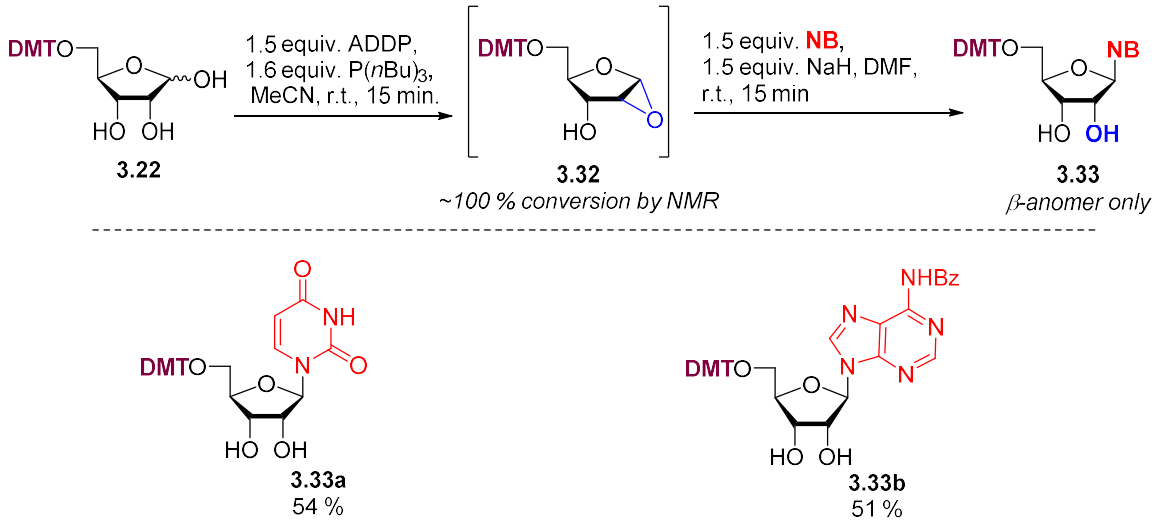


Figure 3.21: Synthesis of 5-*O*-DMTribofuranosyl nucleosides.

3.3.6 Synthesis of non-nucleoside ribofuranosyl glycosides.

3.3.6.1 Successful non-nucleoside nucleophiles

With a robust array of nucleosides, including analogs bearing synthetic bases and saccharide components, now available under my conditions, I endeavoured to effect the ring opening of the anhydrose with non-nucleobase nucleophiles that could act as precursors for other biologically relevant molecules. I envisioned, once again, forming the anhydrose *in situ*, and then adding the nucleophile, with some DMF to the epoxide (Figure 3.22). One very logical starting target was the N_3^- moiety as a substrate for click chemistry (Figure 3.22, **3.34a**). I first attempted simply to use NaN_3 as the nucleophile, however, no reaction took place even when DMF was used solely as the solvent. I postulated this was because of the poor solubility of NaN_3 in organic solvents at ambient temperature, however, any temperature increase beyond 45 °C resulted in total destruction of the anhydrose donor. Fortuitously, if I switched the nucleophile to 1,1,1′,1′-tetramethylguanidinium azide formed *in situ*, I could form the glycosyl azide in good yield when employing mild (45 °C) heating. Initially, I struggled to remove some of the guanidinium salt by-products from the reaction mixture even after purification by column chromatography. Luckily, however, if I treated the crude mixture with Dowex 50W resin in the Na^+ form I could remove enough of the salt that the product was not contaminated after chromatographic purification. Also, I note very encouragingly, that despite the small size of the nucleophile, the β-anomer was still formed perfectly stereoselectively as determined by NMR analysis of the crude reaction mixture.

I focused next on the possibility of installing a fluoride[176] or thiolate moiety at the anomeric position as they are known glycosyl donor moieties. Much to my delight, both were formed easily using the first nucleophile employed. I utilized TBAF as the nucleophile to provide smoothly in 70 % overall yield, the stereoselective β-glycosyl fluoride **3.35c**. Analogous to azide **3.35a**, I also needed to employ the resin to remove some of the tetrabutylammonium salts prior purification. To provide thiolate **3.35d**, I utilized sodium thiolphenolate as the nucleophile to smoothly form the product stereoselectively in moderate-to-good yield (52 %).

I next sought to examine the feasibility of forming the cyano glycoside as a precursor for *C*-nucleosides. As was the case for the azido glycoside, I tried first NaCN and KCN as the nucleophiles to open the epoxide, however, no product was formed. I again suppose this is due to low solubility of the nucleophile and the incompatibility of the method with high temperatures. I was very pleased, however, to observe that if I changed the nucleophile to freshly dried tetraethylammonium cyanide I could form the anomeric cyanide is good yield and perfect stereoselectivity for the β-anomer (**3.35b**, 75 % yield at 0.25 mmol scale, 66 % at 2.5 mmol scale).

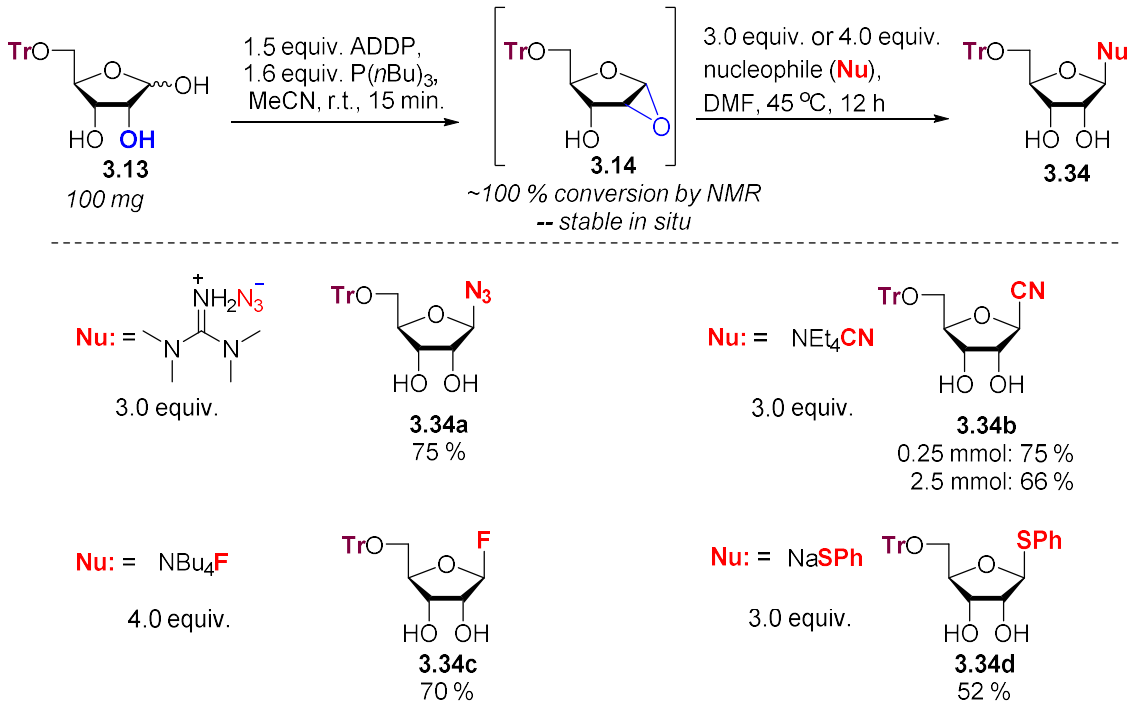
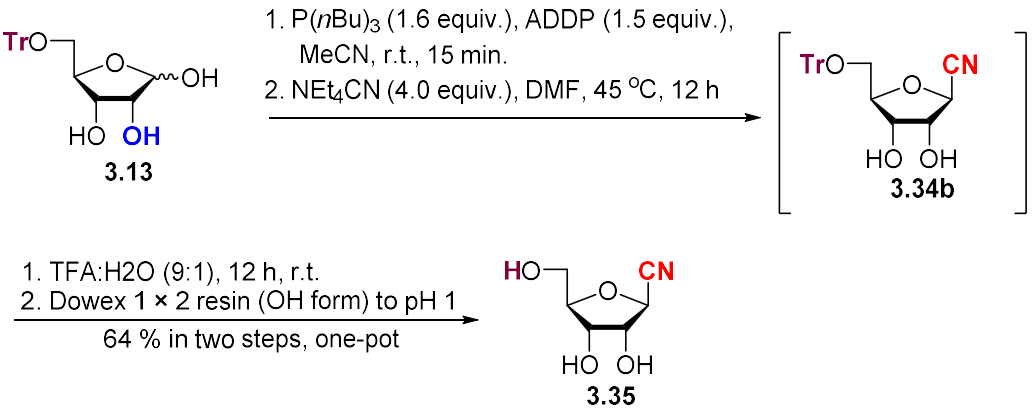


Figure 3.22: Formation of non-nucleoside glycosyl adducts via epoxide ring opening of anhydrose **3.14** at 0.25 mmol scale unless otherwise noted.

As final proof that my deprotection strategy extends beyond nucleosides, I deprotected cyanide **3.34b** using the same the conditions as described in section 3.6.2 to afford the deprotected cyano sugar **3.35** in 64 % overall yield. This, once again, showed that the deprotection step proceeds quantitively without compromising the anomeric position (Scheme 3.18). These experiments provided the final dataset before we published this work in *Chemistry—A European Journal* where I was again the first author of the study.[164]



Scheme 3.18: Synthesis of deprotected potential *C*-nucleoside precursor **3.35** in the two-step one-pot process.

3.3.6.2 Unsuccessful non-nucleoside nucleophiles

From outset of this investigation on non-nucleobase nucleophiles, I had an interest in using the methodology to create *C*-nucleosides, which could have tremendous implications in medicinal chemistry.[177-178] Unfortunately, all attempts to effect the anhydrose ring opening with an aryl nucleophile failed in my hands. Selected conditions included PhMgBr, PhLi, $LiCu(Ph)_2$,[179] and $PhBF_3K$ or $PhB(OH)_2$ under Lewis acid-catalyzed conditions.[180] Since all of these conditions failed to provide any products, I next endeavoured to try to install a boronic acid or boronic ester[181-182] under Cu(I)-catalyzed conditions at the anomeric position, as a precursor for Suzuki coupling to obtain the *C*-nucleoside. Once again, all attempts were unsuccessful.

With *C*-nucleosides not viable directly, I focused my attention on a few last efforts. I attempted epoxide ring opening of **3.14** using ethylacetomalonate as well as cyanoacetomalonate (both deprotonated with NaH), which could lead to *C*-nucleosides downstream. Regrettably, no product formation could be confirmed by ^1H NMR of the crude reaction mixture or by MS analysis. My last attempt, albeit an unsuccessful one, was to form a phosphonate at the anomeric position via anhydrose ring opening of silylated diethylphosphite,[183] however, once again, no reaction took place.

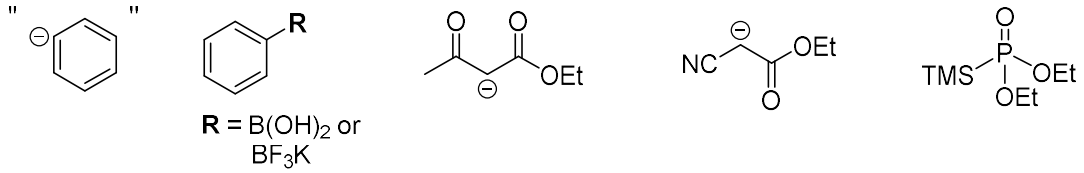


Figure 3.23: Unsuccessful nucleophiles for anhydrose ring opening to access other anomeric functionalizing groups.

4. Conclusions

In my thesis, I developed two novel methods for the stereoselective synthesis of nucleosides from either fully unprotected D-ribose or from 5-*O*-monoprotected ribose under modified Mitsunobu conditions. After extensive optimization, I determined that P(*n*Bu)$_3$ was the ideal phosphine source and DIAD the ideal electron acceptor. I optimized the order of addition of the reagents as well and I discovered that the nucleophilicity of the heterocycle could be improved by deprotonating the N^9 nitrogen with DBU. Using these conditions, in the first study, I determined that unprotected D-ribose can be used to glycosylate a series of purine and pyrimidine nucleobases to provide in moderate-to-good yield stereoselectively β-ribopyranosyl nucleosides as the major product.

This unprecedented reactivity and methodology served as a very convenient proof-of-principal, however, for biologically study, the ribofuranosyl nucleosides are more valuable. As a result, I chose to employ a bulky protecting group (MMTr) that reacts preferentially with primary alcohols that is known to be acid labile. Hence, when reacted with D-ribose in solution only the furanosyl conformation will react with this group, locking ribose in the furanose form. With the monoprotected ribosyl donor in hand, I demonstrated that the same conditions as in the pyranosyl series could still be used to glycosylate the same series of nucleobases. I then showed that the MMTr group could be cleaved with 1 M HCl(aq) *in situ* in a two-step one-pot glycosylation–deprotection strategy to provide β-ribofuranosyl nucleosides perfectly stereoselectively. I also demonstrated that the *C*2-OH group provides either a directing effect or anchimeric assistance in the reaction.

This study was the first of its kind and a very nice series of nucleosides could be obtained, however, there were still some shortcomings. Most notably cytosine, guanine, 5-fluorouracil, 7-deazapurines unsubstituted at the 7-position, as well as other non-heterocyclic nucleophiles were incompatible. A plausible reaction mechanism was also necessitated. By modifying the electron acceptor to ADDP and removing the nucleophile (nucleobase), I could observe the quantitative conversion of the starting monoprotected ribosyl moiety to a 1,2-anhydrosugar (termed “anhydrose”) via *cis*-dioxaphospholane intermediate using NMR spectroscopy, thus confirming the neighboring group participation of the *C*2-OH group.

Armed with this knowledge, I improved the reaction conditions to address the shortcomings of the previous study. I was successfully able to glycosylate cytosine, guanine, 5-fluorouracil, and 7-deazapurines unsubstituted at the 7-position using 5-*O*-tritylribose as the

donor. I also demonstrated that the 5′-*O*-monoprotected nucleoside could be isolated separately but also be cleaved in a two-step one-pot glycosylation–deprotection process using 90 % TFA in H_2O over 12 h without compromising the anomeric position at all. A further demonstration of the power of my methodology, was the three-step one-pot synthesis of adenosine starting from 6-chloropurine. Furthermore, I demonstrated that small nucleophiles such as N_3^-, CN^-, ^-SPh, and F^- could all nucleophilically open the epoxide of the anhydrose perfectly stereoselectively for the β-anomer. Still, some nucleophiles failed to open the epoxide and I hope this will be addressed in the future.

The final demonstration of the power of the methodology came by synthesizing medicinally active or potentially medicinally active *C*5-modified sugar nucleosides, including the valuable anticancer drug doxifluridine. I also proved that my procedure may be amenable to the synthesis of the building blocks for solid phase oligonucleotide synthesis as well by synthesizing β-5-*O*-DMTribosyl nucleosides that had surprising stability.

5. Experimental Section

5.1 General remarks

All reactions that required anhydrous conditions were carried out using flame-dried glassware and purged with argon several times prior to use. Anhydrous MeCN was purchased from Merck and anhydrous DMF from Acros Organics. All other reagents were purchased at the highest commercial quality and used without further purification. Yields refer to chromatographically and spectroscopically (^{1}H NMR) pure materials, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on aluminum-backed Merck silica gel plates (60F-254) using UV light as the visualizing agent and an acidic mixture of *p*-anisaldehyde or basic aqueous $KMnO_4$ and heat as the developing agent. NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer (^{1}H at 300 MHz, ^{13}C at 75 MHz), a Bruker Avance 400 MHz spectrometer (^{1}H at 400 MHz, ^{13}C at 100 MHz), or a Bruker Avance 500 MHz spectrometer (^{1}H at 500 MHz, ^{13}C at 125.7 MHz). Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. The following abbreviations (or a combination thereof) were used to explain signal multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured at 25 °C, and $[\alpha]_D^{20}$ values are given in 10^{-1} deg cm^2 g^{-1}. High resolution mass spectra were measured using electrospray ionization. FT IR spectra were measured as neat samples on a Bruker Alpha spectrometer using the ATR technique.

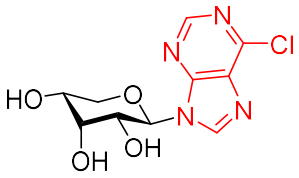
5.2 Procedures and the synthesis of nucleosides

5.2.1 General procedure for the synthesis of ribopyranosyl nucleosides

To a slurry of nucleobase (1.00 mmol, 1.0 equiv.) in MeCN (10 ml) in a dried 50 mL round bottom or pear-shaped flask was added DBU (150 μL, 1.00 mmol, 1.0 equiv.) at r.t. with stirring. The slurry quickly homogenized and stirring was continued for 15 min. After 15 min, the flask was cooled to 0 °C in an ice bath and to it DIAD (420 μL, 2.10 mmol, 2.1 equiv.) was added followed by P(*n*-Bu)$_3$ (93.5 %, 530 μL, 2.00 mmol, 2.0 equiv.) dropwise over 5 min. After 5 min, D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) was added all at once. The reaction was slowly allowed to come to r.t. overnight. After 12−16 h the reaction was neutralized with aqueous 1 M HCl (~10−20 drops) and concentrated *in vacuo*. The crude oil was subsequently dissolved in a minimum volume (~2 mL) of MeOH−CH_2Cl_2 (1:1) and triturated through

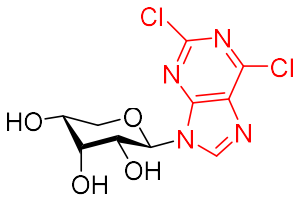
petroleum ether–diethyl ether (110–130 mL, 6:1). After stirring for 20 min, an oil formed and stuck on the inside wall of the flask that contained the product. The filtrate was filtered off and checked by TLC to ensure it contained no dissolved product. The crude oil was re-dissolved in MeOH and concentrated onto silica gel. Purification using flash column chromatography on silica gel (MeOH:CH_2Cl_2) provided the nucleoside pyranoside product.

6-Chloro-9-(β-D-ribopyranosyl)purine (3.2a)



Following the general procedure 6-chloropurine **3.1a** (155 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (10 mL). Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided two products: **3.3a** (10.1 mg, 3.4%) (first eluting, R_f = 0.36, 9:1 CH_2Cl_2–MeOH) and the major product **3.2a** (215 mg, 76%) (second eluting, R_f = 0.26, 9:1 CH_2Cl_2–MeOH) as a faint yellow foam that crystallized from water over 3 days at 4 °C. R_f = 0.26 (9:1 CH_2Cl_2–MeOH). m.p. 186–188 °C (decomp.). $[\alpha]_D$: –24.4 (*c* 0.242, CH_3OH). ^{1}H NMR (600.1 MHz, DMSO-d_6): δ 3.65 (ddd, 1H, J_{gem} = 10.0, $J_{5'b,4'}$ = 5.1, $J_{5'b,3'}$ = 1.2, H-5'b); 3.72 (t, 1H, J_{gem} = $J_{5'a,4'}$ = 10.0, H-5'a); 3.77 (dddd, 1H, $J_{4',5'}$ = 10.0, 5.1, $J_{4',OH}$ = 6.4, $J_{4',3'}$ = 2.4, H-4'); 4.06 (m, 1H, H-3'); 4.28 (ddd, 1H, $J_{2',1'}$ = 9.4, $J_{2',OH}$ = 6.8, $J_{2',3'}$ = 2.6, H-2'); 4.93 (d, 1H, $J_{OH,4'}$ = 6.4, OH-4'); 5.19 (d, 1H, $J_{OH,3'}$ = 3.7, OH-3'); 5.19 (d, 1H, $J_{OH,2'}$ = 6.8, OH-2'); 5.78 (d, 1H, $J_{1',2'}$ = 9.4, H-1'); 8.81 (s, 1H, H-2); 8.90 (s, 1H, H-8). ^{13}C NMR (150.9 MHz, DMSO-d_6): δ 65.49 (CH_2-5'); 66.71 (CH-4'); 68.44 (CH-2'); 71.21 (CH-3'); 80.91 (CH-1'); 131.22 (C-5); 146.64 (CH-8); 149.39 (C-6); 152.01 (CH-2); 152.38 (C-4). IR: (KBr) ν_{max} 3435, 3220, 3114, 3098, 3069, 1599, 1574, 1503, 1492, 1443, 1416, 1406, 1398, 1331, 1204, 1198, 1146, 1094, 1082, 1047, 1011, 791, 652, 637. HR ESIMS: *m/z* [M+Na$^+$] calcd for $C_{10}H_{11}ClN_4O_4Na$: 309.03610. Found: 309.03613.

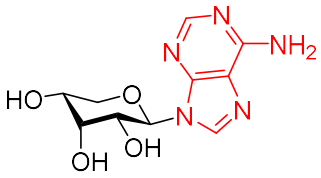
2,6-Dichloro-9-(β-D-ribopyranosyl)purine (3.2b)



Following the general procedure 2,6-dichloropurine **3.1b** (188 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (10 mL). Purification by flash column chromatography on silica

gel (0%–10% MeOH in CH_2Cl_2) provided the product **3.3b** (121 mg, 38%) as a colorless foam. R_f = 0.43 (9:1 CH_2Cl_2–MeOH). $[\alpha]_D$: –1.8 (*c* 0.447, CH_3OH).^{1}H NMR (500.0 MHz, DMSO-d_6) δ 3.66 (ddd, J_{gem} = 9.8, $J_{5'b,4'}$ = 5.0, $J_{5'b,3'}$ = 0.9 Hz, 1H, H-5'b), 3.71 (t, J_{gem} = $J_{5'a,4'}$ = 9.8 Hz, 1H, H-5'a); 3.76 (dddd, $J_{4',5'}$ = 9.8, 5.0, $J_{4',OH}$ = 6.2, $J_{4',3'}$ = 2.4 Hz, 1H, H-4'); 4.05 (m, 1H, H-3'), 4.19 (ddd, $J_{2',1'}$ = 9.4, $J_{2',OH}$ = 6.8, $J_{2',3'}$ = 2.6 Hz, 1H, H-2'), 4.96 (d, $J_{OH,4'}$ = 6.2 Hz, 1H, OH-4'), 5.24 (d, $J_{OH,3'}$ = 3.8 Hz, 1H, OH-3'), 5.24 (d, $J_{OH,2'}$ = 6.8 Hz, 1H, OH-2'), 5.70 (d, $J_{1',2'}$ = 9.4 Hz, 1H, H-1'), 8.95 (s, 1H, H-8). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 65.56 (CH_2-5'), 66.65 (CH-4'), 68.64 (CH-2'), 71.16 (CH-3'), 81.01 (CH-1'), 130.85 (C-5), 147.46 (CH-8), 150.12 (C-6), 151.49 (C-2), 153.83 (C-4). IR: (KBr) ν_{max} 3419, 3180, 3122, 2935, 1597, 1560, 1497, 1462, 1421, 1385, 1360, 1288, 1238, 1215, 1161, 1097, 1044, 1016, 960, 879, 847, 786, 771, 682, 627, 595. HR ESIMS: *m/z* [M+Na$^+$] calcd for $C_{10}H_{10}Cl_2N_4O_4Na$: 342.99713. Found: 342.99680.

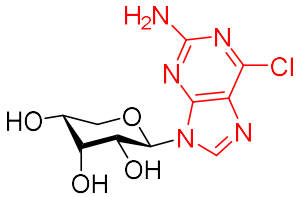
9-(β-D-Ribopyranosyl)adenine (3.2c)



This is a known compound.[137] Following the general procedure **EXCEPT** that DMF was used as solvent instead of MeCN: adenine **3.1c** (135 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in DMF (10 mL). Purification by flash column chromatography on silica gel (5%–20% MeOH in CH_2Cl_2) provided the product **3.2c** (187 mg, 70%) (first eluting, R_f = 0.19, 4:1 CH_2Cl_2–MeOH) as a colorless foam. The desired product could be crystallized from water over 3 days at 4 °C. R_f = 0.19 (4:1 CH_2Cl_2–MeOH). m.p. 238–241 °C (Lit. 239-241 °C).[137] $[\alpha]_D$: –27.7 (*c* 0.292, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.58 (ddd, 1H, J_{gem} = 10.0, $J_{5'b,4'}$ = 5.1, $J_{5'b,3'}$ = 1.1, H-5'b); 3.67 (t, 1H, J_{gem} = $J_{5'a,4'}$ = 10.0, H-5'a); 3.72 (dddd, 1H, $J_{4',5'}$ = 10.0, 5.1, $J_{4',OH}$ = 6.4, $J_{4',3'}$ = 2.4, H-4'); 4.03 (m, 1H, H-3'); 4.23 (ddd, 1H, $J_{2',1'}$ = 9.6, $J_{2',OH}$ = 7.3, $J_{2',3'}$ = 2.6, H-2'); 4.89 (d, 1H, $J_{OH,4'}$ = 6.4, OH-4'); 5.06 (d, 1H, $J_{OH,2'}$ = 7.3, OH-2'); 5.12 (d, 1H, $J_{OH,3'}$ = 3.6, OH-3'); 5.62 (d, 1H, $J_{1',2'}$ = 9.6, H-1'); 7.22 (bs, 2H, NH_2); 8.13 (s, 1H, H-2); 8.29 (s, 1H, H-8). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 65.31 (CH_2-5'); 66.87 (CH-4'); 68.35 (CH-2'); 71.42 (CH-3'); 79.80 (CH-1'); 118.92 (C-5); 139.89 (CH-8); 150.19 (C-4); 152.75 (CH-2); 156.14 (C-6). IR: (KBr) ν_{max} 3470, 3404, 3339, 3265, 3220, 3118, 2937, 1643, 1599, 1584, 1507, 1478, 1424, 1338, 1372, 1091, 1045, 1011, 979, 905, 797, 729. HR ESIMS:

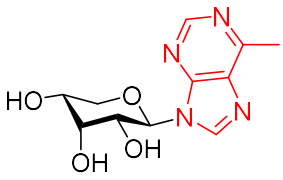
m/z [M+H$^+$] calcd for $C_{10}H_{14}N_5O_4$ 268.10403. Found: 268.10408. [M+Na$^+$] calcd for $C_{10}H_{13}N_5O_4Na$: 290.08598. Found: 290.08603.

2-Amino-6-chloro-9-(β-D-ribopyranosyl)purine (3.2d)



Following the general procedure 2-amino-6-chloropurine **3.1d** (155 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (10 mL). Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided two products: **3.3d** (15.0 mg, 5.0%) (first eluting, R_f = 0.36, 9:1 CH_2Cl_2–MeOH) and the major product **3.2d** (255 mg, 85%) (second eluting, R_f = 0.21, 9:1 CH_2Cl_2–MeOH) as a colorless foam that crystallized from water over 3 days at 4 °C. R_f = 0.21 (9:1 CH_2Cl_2–MeOH). m.p. 188–191 °C (decomp. 194–195 °C). [α]$_D$: +6.3 (*c* 0.271, DMSO). ^{1}H NMR (500.0 MHz, DMSO-*d*$_6$) δ 3.59 (ddd, J_{gem} = 10.3, $J_{5'b,4'}$ = 5.6, $J_{5'b,3'}$ = 0.8 Hz, 1H, H-5'b), 3.64 (t, J_{gem} = $J_{5'a,4'}$ =10.3 Hz, 1H, H-5'a), 3.71 (dddd, $J_{4',5'}$ = 10.3, 5.6, $J_{4',OH}$ = 6.4, $J_{4',3'}$ = 2.4 Hz, 1H, H-4'), 4.02 (m, 1H, H-3'), 4.14 (ddd, $J_{2',1'}$ = 9.5, $J_{2',OH}$ = 6.9, $J_{2',3'}$ = 2.6 Hz, 1H, H-2'), 4.91 (d, $J_{OH,4'}$ = 6.4 Hz, 1H, OH-4'), 5.11 (d, $J_{OH,3'}$ = 3.5 Hz, 1H, OH-3'), 5.15 (d, $J_{OH,2'}$ = 6.9 Hz, 1H, OH-2'), 5.56 (d, $J_{1',2'}$ = 9.5 Hz, 1H, H-1'), 6.99 (bs, 2H, NH_2), 8.30 (s, 1H, H-8). ^{13}C NMR (125.7 MHz, DMSO-*d*$_6$) δ 65.33 (CH_2-5'), 66.76 (CH-4'), 68.32 (CH-2'), 71.33 (CH-3'), 79.58 (CH-1'), 123.45 (C-5), 141.84 (CH-8), 149.55 (C-6), 154.77 (C-4), 160.06 (C-2). IR: (KBr) ν_{max} 3409, 3334, 3216, 2925, 1655, 1617, 1568, 1517, 1410, 1293, 1222, 1152, 1093, 1045, 921, 785, 733, 645, 604. HR ESIMS: *m/z* [M+Na$^+$] calcd for $C_{10}H_{12}N_5O_4ClNa$: 324.04700. Found: 324.04710.

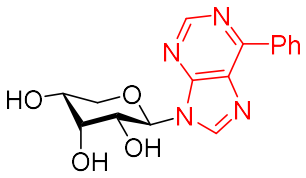
6-Methyl-9-(β-D-ribopyranosyl)purine (3.2e)



Following the general procedure 6-methylpurine **3.1e** (219 mg, 1.63 mmol, 1.0 equiv.), DBU (243 µL, 1.63 mmol, 1.0 equiv.), DIAD (670 µL, 3.42 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 860 µL, 3.26 mmol, 2.0 equiv.), and D-ribose (490 mg, 3.26 mmol, 2.0 equiv.) were reacted in MeCN (16 mL). Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided two products: **3.3e** (21 mg, 5.0%) (first eluting, R_f = 0.37, 9:1 CH_2Cl_2–MeOH) and the major product **3.2e** (358 mg, 82%) (second eluting, R_f = 0.14, 9:1 CH_2Cl_2–MeOH) as a colorless foam that was contaminated with DBU. Removal of

DBU by semi-preparative RP HPLC (0% MeOH in H_2O to 100% MeOH, linear gradient, 10 mL/min) furnished the product (235 mg, 54%) as a colorless foam. R_f = 0.14 (9:1 CH_2Cl_2–MeOH). $[\alpha]_D$: −13.3 (*c* 0.323, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 2.72 (s, 3H, CH_3); 3.62 (ddd, 1H, J_{gem} = 9.9, $J_{5'b,4'}$ = 5.0, $J_{5'b,3'}$ = 1.0, H-5'b); 3.70 (t, 1H, J_{gem} = $J_{5'a,4'}$ = 9.9, H-5'a); 3.75 (dddd, 1H, $J_{4',5'}$ = 9.9, 5.0, $J_{4',OH}$ = 6.3, $J_{4',3'}$ = 2.7, H-4'); 4.06 (m, 1H, H-3′); 4.29 (ddd, 1H, $J_{2',1'}$ = 9.6, $J_{2',OH}$ = 7.2, $J_{2',3'}$ = 2.6, H-2′); 4.93 (d, 1H, $J_{OH,4'}$ = 6.3, OH-4′); 5.14 (d, 1H, $J_{OH,2'}$ = 7.2, OH-2′); 5.18 (d, 1H, $J_{OH,3'}$ = 3.7, OH-3′); 5.75 (d, 1H, $J_{1',2'}$ = 9.6, H-1′); 8.69 (s, 1H, H-8); 8.77 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 19.25 (CH_3); 65.42 (CH_2-5′); 66.81 (CH-4′); 68.34 (CH-2′); 71.33 (CH-3′); 80.19 (CH-1′); 132.69 (C-5); 144.55 (CH-8); 150.86 (C-4); 151.95 (CH-2); 158.19 (C-6). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{11}H_{15}N_4O_4$: 267.10878. Found: 267.10883. [M+Na$^+$] calcd for $C_{11}H_{14}N_4O_4Na$: 289.09073. Found: 289.09078.

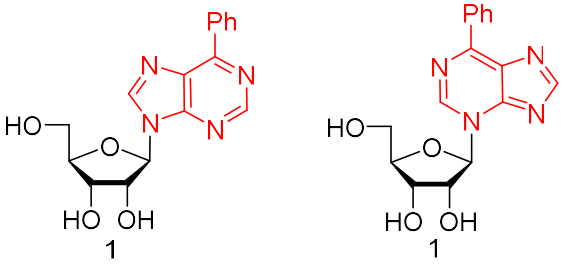
6-Phenyl-9-(β-D-ribopyranosyl)purine (3.2f)



Following the general procedure 6-phenylpurine **3.1f** (196 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (10 mL). Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided three products: **3.3f** (51 mg, 15%) and its N-3 glycosylated isomer (determined by ^{1}H–^{13}C HMBC correlation, see below) that co-eluted in ~1:1 ratio (first eluting, R_f = 0.46, 9:1 CH_2Cl_2–MeOH) and the major product **3.2f** (218 mg, 66%) (second eluting, R_f = 0.33, 9:1 CH_2Cl_2–MeOH) as a colorless foam that crystallized from water over 3 days at 4 °C. R_f = 0.33 (9:1 CH_2Cl_2–MeOH). m.p. 150–152 °C. $[\alpha]_D$: −29.5 (*c* 0.234, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.66 (ddd, 1H, J_{gem} = 9.9, $J_{5'b,4'}$ = 4.9, $J_{5'b,3'}$ = 1.3, H-5'b); 3.74 (t, 1H, J_{gem} = $J_{5'a,4'}$ = 9.7, H-5'a); 3.79 (dddd, 1H, $J_{4',5'}$ = 9.7, 4.9, $J_{4',OH}$ = 6.3, $J_{4',3'}$ = 2.3, H-4'); 4.09 (m, 1H, H-3′); 4.33 (ddd, 1H, $J_{2',1'}$ = 9.5, $J_{2',OH}$ = 7.1, $J_{2',3'}$ = 2.6, H-2′); 4.95 (d, 1H, $J_{OH,4'}$ = 6.3, OH-4′); 5.21 (d, 1H, $J_{OH,2'}$ = 7.1, OH-2′); 5.22 (d, 1H, $J_{OH,3'}$ = 4.2, OH-3′); 5.85 (d, 1H, $J_{1',2'}$ = 9.5, H-1′); 7.55 – 7.65 (m, 3H, H-*m,p*-Ph); 8.83 (m, 2H, H-*o*-Ph); 8.88 (s, 1H, H-8); 9.01 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 65.50 (CH_2-5′); 66.82 (CH-4′); 68.45 (CH-2′); 71.35 (CH-3′); 80.21 (CH-1′); 128.91 (CH-*m*-Ph); 129.55 (CH-*o*-Ph); 130.68 (C-5); 131.32 (CH-*p*-Ph); 135.47 (C-*i*-Ph); 145.57 (CH-8); 152.23 (CH-2); 152.93 (C-6); 153.02 (C-4). IR: (KBr) ν_{max} 3425, 1603, 1588, 1588, 1568, 1503, 1488, 1458, 1331, 1331,

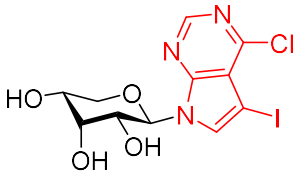
1219, 1183, 1139, 1096, 1084, 1045, 839, 650, 579. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{16}H_{17}N_4O_4$: 329.12443. Found: 329.12447. [M+Na$^+$] calcd for $C_{16}H_{16}N_4O_4Na$: 351.10638. Found: 351.10642.

Charaterization data for 3f + N^3 glycosylated furanosyl isomer (1:1)



Complete characterization of **3.3f** is found in the furanoside section 5.2.2. Data for the N^3 isomer: R_f = 0.46 (9:1 CH_2Cl_2–MeOH). Data for the N^3 isomer: ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.70 (ddd, 1H, J_{gem} = 12.4, $J_{5'b,OH}$ = 7.1, $J_{5'b,4'}$ = 2.6, H-5′b); 3.86 (ddd, 1H, J_{gem} = 12.4, $J_{5'a,OH}$ = 4.0, $J_{5'a,4'}$ = 2.6, H-5′a); 4.17 (dt, 1H, $J_{4',3'}$ = 4.6, $J_{4',5'}$ = 2.6, H-4′); 4.25 (dt, 1H, $J_{3',OH}$ = 5.1, $J_{3',2'}$ = $J_{3',4'}$ = 4.6, H-3′); 4.67 (dt, 1H, $J_{2',OH}$ = 5.5, $J_{2',1'}$ = $J_{2',3'}$ = 4.6, H-2′); 5.30 (d, 1H, $J_{OH,3'}$ = 5.1, OH-3′); 5.76 (d, 1H, $J_{OH,2'}$ = 5.5, OH-2′); 5.96 (dd, 1H, $J_{OH,5'}$ = 7.1, 4.0, OH-5′); 6.35 (d, 1H, $J_{1',2'}$ = 4.6, H-1′); 7.57-7.66 (m, 3H, H-*m,p*-Ph); 8.46 (s, 1H, H-8); 8.99 (m, 2H, H-*o*-Ph); 9.41 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 60.74 (CH_2-5′); 69.90 (CH-3′); 74.38 (CH-2′); 86.90 (CH-4′); 94.77 (CH-1′); 129.02 (CH-*m*-Ph); 130.55 (CH-*o*-Ph); 132.08 (CH-*p*-Ph); 134.91 (C-*i*-Ph); 136.48 (C-5); 139.79 (CH-2); 150.68 (C-6); 153.86 (C-4); 160.83 (CH-8).

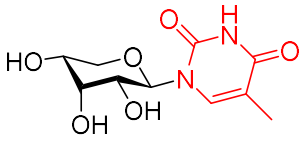
4-Chloro-5-iodo-7-(β-D-ribopyranosyl)-7*H*-pyrrolo[2,3-d]pyrimidine (3.2g)



Following the general procedure: **3.1g** (279 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5 %, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (10 mL). Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided two products: **3.3g** (15 mg, 4%) (first eluting, R_f = 0.60, 9:1 CH_2Cl_2–MeOH) and the major product **3.2g** (269 mg, 65%) (second eluting, R_f = 0.46, 9:1 CH_2Cl_2–MeOH) as a white semi-solid that crystallized from water over 3 days at 4 °C. R_f = 0.46 (9:1 CH_2Cl_2–MeOH). m.p. 196–199 °C (decomp.). [α]$_D$: –19.1 (*c* 0.388, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.58 (ddd, 1H, J_{gem} = 9.7, $J_{5'b,4'}$ = 4.7, $J_{5'b,3'}$ = 1.3, H-5'b); 3.68 (t, 1H, J_{gem} = $J_{5'a,4'}$ = 9.7, H-5'a); 3.73 (dddd, 1H, $J_{4',5'}$ = 9.7, 4.7, $J_{4',OH}$ = 6.0, $J_{4',3'}$ = 2.3, H-4'); 4.02 (m, 1H, H-3'); 4.10 (ddd, 1H, $J_{2',1'}$ = 9.4, $J_{2',OH}$ = 7.1, $J_{2',3'}$ = 2.7, H-2'); 4.91 (d, 1H, $J_{OH,4'}$ = 6.0, OH-4'); 5.07 (d, 1H, $J_{OH,2'}$ = 7.1, OH-2'); 5.15 (d, 1H, $J_{OH,3'}$ = 3.7, OH-3'); 5.92 (d, 1H,

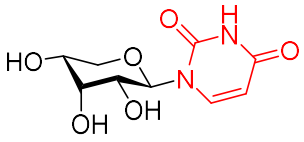
$J_{1',2'}$ = 9.4, H-1'); 8.18 (s, 1H, H-6); 8.67 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 53.46 (C-5); 65.34 (CH_2-5'); 66.74 (CH-4'); 68.65 (CH-2'); 71.44 (CH-3'); 80.00 (CH-1'); 116.60 (C-4a); 134.36 (CH-6); 150.92 (CH-2); 151.17 (C-4); 151.67 (C-7a). IR: (KBr) ν_{max} 3405, 3123, 2933, 1578, 1539, 1505, 1448, 1428, 1340, 1093, 1077, 1043, 958, 953, 873, 781, 600, 587. HR ESIMS: *m/z* [M+Na^+] calcd for $C_{11}H_{11}ClN_3O_4INa$: 433.93751. Found: 433.93721.

1-(β-D-Ribopyranosyl)thymine (3.2h)



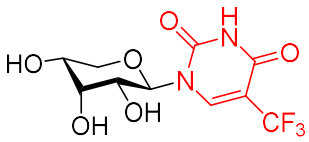
This is a known compound.[137-138] Following the general procedure thymine **3.1h** (126 mg, 1.00 mmol, 1.0 equiv.), DBU (150 μL, 1.00 mmol, 1.0 equiv.), DIAD (420 μL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 μL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (5 mL). Purification by flash column chromatography on silica gel (0%–20% MeOH in CH_2Cl_2) provided the major product **3.2h** and the co-eluting furanoside **3.3h** (**3.2h/3.3h**: 10/1.5, 99 mg, 41%) as a colorless foam. R_f = 0.40 (4:1 CH_2Cl_2–MeOH). $[\alpha]_D$: –6.1 (*c* 1.00, CH_3OH). ^{1}H NMR (300.0 MHz, CD_3OD): δ 7.50 (d, *J* = 1.2 Hz, 1H), 5.76 (d, *J* = 9.5 Hz, 1H), 4.20 – 4.16 (m, 1H), 3.85 – 3.71 (m, 4H), 1.89 (d, *J* = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 166.2, 153.1, 138.3, 111.8, 81.5, 72.8, 69.7, 68.1, 66.6, 12.3. HR ESIMS: *m/z* [M+H^+] calcd for $C_{10}H_{15}N_2O_6$: 257.0779. Found: 257.0779.

1-(β-D-Ribopyranosyl)uracil (3.2i)



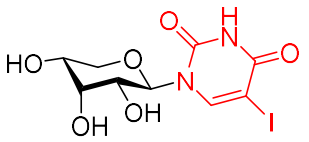
This is a known compound.[137-138] Following the general procedure uracil **3.1i** (112 mg, 1.00 mmol, 1.0 equiv.), DBU (150 μL, 1.00 mmol, 1.0 equiv.), DIAD (420 μL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 μL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (5 mL). Purification by flash column chromatography on silica gel (0%–20% MeOH in CH_2Cl_2) provided the major product **3.2i** and the co-eluting furanoside **3.3i** (**3.2i/3.3i**: 10/0.8, 88 mg, 36%) as a colorless foam. R_f = 0.33 (4:1 CH_2Cl_2–MeOH). $[\alpha]_D$: –5.8 (*c* 1.00, CH_3OH). ^{1}H NMR (500.0 MHz, CD_3OD): δ 7.67 (d, *J* = 8.1 Hz, 1H), 5.77 (d, *J* = 9.5 Hz, 1H), 5.73 (d, *J* = 8.1 Hz, 1H), 4.20 – 4.17 (m, 1H), 3.83 – 3.70 (m, 4H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.0, 152.9, 142.7, 103.1, 81.6, 72.8, 69.9, 68.0, 66.7. HR ESIMS: *m/z* [M+H^+] calcd for $C_9H_{13}N_2O_6$: 243.0623. Found: 243.0623.

1-(β-D-Ribopyranosyl)trifluorothymine (3.2j)



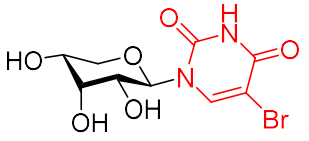
Following the general procedure trifluorothymine **3.1j** (181 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (5 mL). Purification by flash column chromatography on silica gel (0%–20% MeOH in CH_2Cl_2) provided the major product **3.2j** and the co-eluting furanoside **3.3j** (**3.2j/3.3j**: 10/1.1, 106 mg, 34%) as a colorless foam. R_f = 0.30 (4:1 CH_2Cl_2–MeOH). $[\alpha]_D$: –12.4 (*c* 1.00, CH_3OH). ^{1}H NMR (300.0 MHz, CD_3OD): δ 8.09 (m, 1H), 5.81 (d, *J* = 9.4 Hz, 1H), 4.18 (t, *J* = 2.6 Hz, 1H), 3.96 – 3.71 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD): δ 159.4, 150.4, 142.3, 122.3 (d, *J* = 269.5 Hz), 104.8 (d, *J* = 34.2 Hz) 80.9, 71.4, 68.7, 66.5, 65.5. ^{19}F NMR (471 MHz, CD_3OD): δ –64.27. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{10}H_{12}F_3N_2O_6$: 311.0496. Found: 311.0496.

5-Iodo-1-(β-D-ribopyranosyl)uracil (3.2k)



Following the general procedure 5-iodouracil **3.1k** (238 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (5 mL). Purification by flash column chromatography on silica gel (0%–20% MeOH in CH_2Cl_2) provided the major product **3.2k** and the co-eluting furanoside **3.3k** (**3.2k/3.3k**: 10/0.8, 85 mg, 23%) as a colorless foam. R_f = 0.38 (4:1 CH_2Cl_2–MeOH). $[\alpha]_D$: –26.0 (*c* 1.00, CH_3OH). ^{1}H NMR (500.0 MHz, CD_3OD): δ 8.05 (s, 1H), 5.74 (d, *J* = 9.4 Hz, 1H), 4.18 (d, *J* = 2.6 Hz, 1H), 3.83 (dd, *J* = 8.3, 2.6 Hz, 1H), 3.79 (dd, *J* = 9.4, 2.8 Hz, 1H), 3.77 – 3.74 (m, 2H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 162.6, 152.6, 147.2, 81.9, 72.8, 69.9, 69.2, 67.9, 66.7. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_9H_{12}N_2O_6I$: 368.9589. Found: 368.9588.

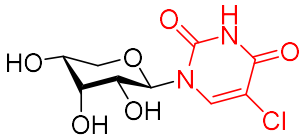
5-Bromo-1-(β-D-ribopyranosyl)uracil (2l)



Following the general procedure, 5-bromouracil **3.1l** (191 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00

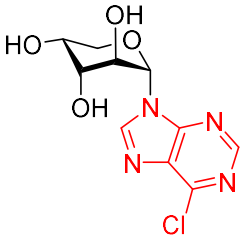
mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (5 mL). Purification by flash column chromatography on silica gel (0%–20% MeOH in CH_2Cl_2) provided the major product **3.2l** and the co-eluting furanoside **3.3l** (**3.2l/3.3l**: 10/1, 103 mg, 32%) as a colorless foam. R_f = 0.36 (4:1 CH_2Cl_2–MeOH). $[\alpha]_D$: –23.1 (*c* 1.00, CH_3OH). ^1H NMR (300.0 MHz, CD_3OD): δ 8.04 (s, 1H), 5.76 (d, *J* = 9.4 Hz, 1H), 4.18 (s, 1H), 3.90 – 3.69 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD): δ 160.0, 150.9, 140.9, 96.3, 80.6, 71.4, 68.6, 66.6, 65.4. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_9H_{12}N_2O_6Br$: 320.9728. Found: 320.9728.

5-Chloro-1-(β-D-ribopyranosyl)uracil (3.2m):



Following the general procedure 5-chlorouracil, **3.1m** (147 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 530 µL, 2.00 mmol, 2.0 equiv.), and D-ribose (300 mg, 2.00 mmol, 2.0 equiv.) were reacted in MeCN (5 mL). Purification by flash column chromatography on silica gel (0%–20% MeOH in CH_2Cl_2) provided the major product **3.2m** and the co-eluting furanoside **3.3m** (**3.2m/3.3m**: 10/2, 86 mg, 31%) as a colorless foam. R_f = 0.36 (4:1 CH_2Cl_2–MeOH). $[\alpha]_D$: –17.2 (*c* 1.00, CH_3OH). ^1H NMR (300.0 MHz, CD_3OD): δ 7.96 (s, 1H), 5.76 (d, *J* = 9.4 Hz, 1H), 4.20 – 4.17 (m, 1H), 3.87 – 3.72 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD): δ 161.3, 152.0, 139.6, 109.9, 82.0, 72.7, 69.9, 67.9, 66.7. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_9H_{12}N_2O_6Cl$: 277.0233. Found: 277.0234.

6-Chloro-9-(α-D-arabinopyranosyl)purine (9)

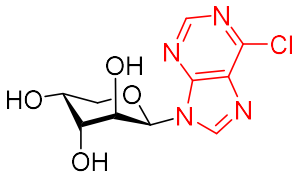


Following the general procedure, 6-chloropurine **1a** (460 mg, 3.00 mmol, 1.0 equiv.), DBU (450 µL, 3.00 mmol, 1.0 equiv.), DIAD (1.3 mL, 6.30 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (93.5%, 1.6 mL, 6.00 mmol, 2.0 equiv.), and D-arabinose (900 mg, 6.00 mmol, 2.0 equiv.) were reacted in MeCN (25 mL). Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) enabled the isolation of two TLC spots: The first eluting*** (57 mg, 6.6%) (first eluting, R_f = 0.35, 9:1 CH_2Cl_2–MeOH) and the major, desired product **9** (602 mg, 70%) (second eluting, R_f = 0.20, 9:1 CH_2Cl_2–MeOH) as a faint yellow foam contaminated with DBU. The yellow foam was further purified to remove the DBU impurities by reverse phase high performance flash chromatography on a Biotage SP1 apparatus (KP-C18-HS column, 0–100% MeOH in water) to provide pure **9** (408 mg, 48%) as

a white foam. R_f = 0.20 (9:1 CH_2Cl_2–MeOH). $[\alpha]_D$: –40.8 (*c* 0.321, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.61 (app. dt, 1H, *J* = 8.7, 4.0, H-3'); 3.79–3.88 (m, 3H, H-4', H-5'a, H-5'b); 4.27 (app. td, 1H, $J_{2',1'}$ = 9.1, *J* = 4.9, H-2'); 4.82 (d, 1H, $J_{OH,4'}$ = 4.8, OH-4'); 5.07 (d, 1H, $J_{OH,3'}$ = 5.4, OH-3'); 5.31 (d, 1H, $J_{OH,5'}$ = 5.4, OH-5'); 5.48 (d, 1H, $J_{1',2'}$ = 9.1, H-1'); 8.81 (s, 1H, H-2); 8.86 (s, 1H, H-8). ^{13}C NMR (100 MHz, DMSO-d_6): δ 68.92 (CH-4'); 69.36 (CH-2'); 70.02 (CH_2-5'); 73.66 (CH-3'); 84.64 (CH-1'); 131.31 (C-5); 146.46 (CH-8); 149.67 (C-6); 152.35 (CH-2); 152.54 (C-4).

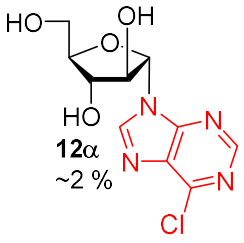
HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{10}H_{12}N_4O_4Cl$: 287.05416. Found: 287.05423. [M+Na$^+$] calcd for $C_{10}H_{11}ClN_4O_4Na$: 309.03619. Found: 309.03610.

***As discussed in Section 3.2.2, the first eluting spot was an inseparable mixture in roughly ~4:3 ratio of two products: the β-pyranoside (major) and α-furanoside (minor) as confirmed by ROESY-NMR analysis contaminated with a small amount of the by-product hydrazine from the Mitsunobu reaction. The spot was isolated as a faint yellow oil. R_f = 0.35, 9:1 CH_2Cl_2–MeOH). NMR for the slightly **major β-pyranoside product**:



^{1}H NMR (400 MHz, DMSO-d_6): δ 3.71–3.83 (m, 3H, H-2', H-5'a, H-5'b); 3.92 (app. q, *J* = 3.6 Hz, H-4'), 3.98–4.07 (m, 1H, H-3'), 4.85 (d, 1H, *J* = 6.6 Hz, OH-3'), 5.37 (d, 1H, *J* = 5.6 Hz, OH-4'), 5.69 (d, 1H, *J* = 5.6 Hz, OH-2'), 6.13 (d, 1H, *J* = 1.5 Hz, H-1'); 8.67 (s, 1H, H-8); 8.80 (s, 1H, H-2).

^{13}C NMR (100 MHz, DMSO-d_6): δ 63.25 (CH-3'); 66.38 (CH_2-5'); 70.67 (CH-2' + CH-4'); 80.12 (CH-1'); 130.91 (C-5); 146.19 (CH-8, absent in APT, assigned by HMBC); 149.48 (C-6); 151.44 (C-4); 152.2 (CH-2). NMR for the slightly minor α-furanoside product:

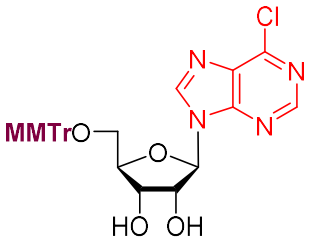


^{1}H NMR (400 MHz, DMSO-d_6): δ 3.50–3.56 (m, 1H, H-5'b); 3.60–3.65 (m, 1H, H-5'a); 3.98–4.07 (m, 1H, H-3'), 4.27 (ddd, 1H, *J* = 11.9, 5.3, 3.8 Hz, H-4'), 4.67 (app. q, 1H, *J* = 4.9 Hz, H-2'); 4.96 (m, 1H, OH-4'); 5.59 (d, 1H, *J* = 4.6 Hz, OH-3'), 5.85 (d, *J* = 5.3 Hz, OH-2'), 6.02 (d, 1H, *J* = 4.8 Hz, H-1'); 8.83 (s, 1H, H-2); 8.88 (s, 1H, H-8).

^{13}C NMR (100 MHz, DMSO-d_6): δ 61.50 (CH_2-5'); 75.32 (CH-3'); 79.76 (CH-2'); 86.59 (CH-4'); 89.78 (CH-1'); 131.86 (C-5); 146.47 (CH-8, absent in APT, assigned by HMBC); 149.70 (C-6); 151.92 (C-4); 152.2 (CH-2).

5.2.2 Procedure for the two-step synthesis of 6-Chloro-1-(β-D-ribofuranosyl)purine (3a)

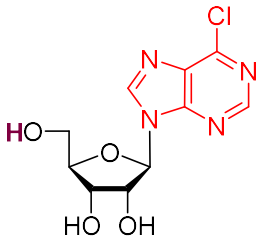
6-Chloro-1-(5'-*O*-monomethoxytrityl)-β-D-ribofuranosyl)purine (5a)



To a slurry of 6-chloropurine **1a** (128 mg, 0.828 mmol, 1.0 equiv.) in MeCN (6 mL) under argon with stirring was added DBU (125 µL, 0.828, 1.0 equiv.) at r.t. The slurry quickly homogenized and stirring was continued for 15 min. After 15 min, the reaction vessel was cooled to 0 °C in an ice bath and to it DIAD (340 µL, 1.743, 2.1 equiv.) was added, followed by P(*n*-Bu)$_3$ (93.5%, 440 µL, 1.66 mmol, 2.0 equiv.) dropwise over 5 min. After 5 min, a solution of 5-*O*-(4-methoxytrityl)-D-ribofuranose **4** (351 mg, 0.828 mmol, 1.0 equiv.) in MeCN (3 mL) was added all at once *via cannula* and the flask rinsed with a small volume of MeCN (~1 mL). The reaction was slowly allowed to come to room temperature overnight. After 12 h, the reaction was neutralized (pH ~7) with 1 M HCl(aq) and concentrated *in vacuo*. The crude red oil was dissolved in a minimal volume of CH_2Cl_2 (~1.5 mL) and triturated through petroleum ether (100 mL). After stirring for 20 min an oil formed on the outside of the flask that contained the product. The filtrate was filtered off and checked by TLC to ensure it contained no dissolved product. The crude oil was re-dissolved in CH_2Cl_2 and concentrated onto silica gel. Purification using flash column chromatography on silica gel (3:2 EtOAc–Pet. ether) provided the product **5a** (246 mg, 53%) as a faint yellow foam. R_f = 0.22 (3:2 EtOAc–Pet. ether). $[\alpha]_D$: +6.2 (*c* 0.451, CH_2Cl_2). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.24 (dd, 1H, J_{gem} = 10.4, $J_{5'b,4'}$ = 3.6, H-5′b); 3.27 (dd, 1H, J_{gem} = 10.4, $J_{5'a,4'}$ = 5.4, H-5′a); 3.73 (s, 3H, CH_3O-MTr); 4.14 (td, 1H, $J_{4',3'}$ = 5.4, $J_{4',5'}$ = 5.4, 3.6, H-4′); 4.35 (dt, 1H, $J_{3',OH}$ = 5.9, $J_{3',2'}$ = $J_{3',4'}$ = 5.4, H-3′); 4.75 (ddd, 1H, $J_{2',3'}$ = 5.4, $J_{2',OH}$ = 5.2, $J_{2',1'}$ = 4.2, H-2′); 5.31 (d, 1H, $J_{OH,3'}$ = 5.9, OH-3′); 5.67 (d, 1H, $J_{OH,2'}$ = 5.2, OH-2′); 6.08 (d, 1H, $J_{1',2'}$ = 4.2, H-1′); 6.82 (m, 2H, H-*m*-C_6H_4OMe); 7.17 – 7.28 (m, 8H, H-*m,p*-Ph, H-*o*-C_6H_4OMe); 7.34 (s, 2H, H-*o*-Ph); 8.73 (s, 1H, H-2); 8.83 (s, 1H, H-8). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 55.20 (CH_3O-MTr); 63.87 (CH_2-5′); 70.38 (CH-3′); 73.14 (CH-2′); 83.51 (CH-4′); 85.94 (C-MTr); 89.11 (CH-1′); 113.32 (CH-*m*-C_6H_4OMe); 127.02, 127.06 (CH-*p*-Ph); 127.98, 128.01 (CH-*m*-Ph); 128.09, 128.14 (CH-*o*-Ph); 130.20 (CH-*o*-C_6H_4OMe); 131.74 (C-5); 135.04 (C-*i*-C_6H_4OMe); 144.39 144.40 (C-*i*-Ph); 146.43 (CH-8); 149.58 (C-4); 151.60 (C-6); 151.85 (CH-2); 158.33 (C-*p*-C_6H_4OMe). IR: (KBr) ν_{max} 3434, 3062, 3034, 3002, 2926, 2838, 1609, 1594, 1563, 1510, 1491, 1463, 1447, 1423, 1402, 1340, 1301, 1252, 1216, 1203, 1203, 1181, 1148, 1118, 1082,

1035. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{30}H_{28}N_4O_5Cl$: 559.17427. Found: 559.17448. *m/z* [M+Na$^+$] calcd for $C_{30}H_{28}N_4O_5ClNa$: 581.15622. Found: 581.15638.

6-Chloro-1-(β-D-ribofuranosyl)purine (3a)



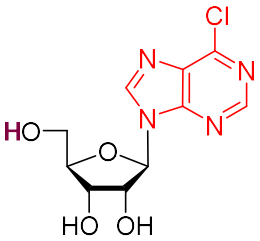
This is a commercially available compound (Sigma-Aldrich). To a stirring solution of **5a** (170 mg, 0.303 mmol, 1.0 equiv.) in MeCN (3 mL) at r.t. was added 1 M HCl(aq) until pH ~1. Stirring was continued for 15 min at which time TLC analysis indicated complete consumption of starting material. The reaction was neutralized (pH ~7) with 1M NaOH(aq) and concentrated directly onto silica gel. Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided the product **3a** (83 mg, 93%) as a pale yellow foam that crystallized from water after 3 days at 4 °C to afford a pale yellow crystalline solid. The data were in accordance with a commercially available sample. R_f = 0.36 (9:1 CH_2Cl_2–MeOH). Melting point: 178–180 °C (decomp.) (lit. 161-163 °C).[146] HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{10}H_{12}N_4O_4Cl$: 287.05416. Found: 287.05420. [M+Na$^+$] calcd for $C_{10}H_{11}N_4O_4ClNa$: 309.03610. Found: 309.03619.

5.2.3 General procedure for the synthesis of ribofuranosyl nucleosides

To a slurry of nucleobase **1a–1n** (1.0 equiv.) in MeCN under argon with stirring was added DBU (1.0 equiv.) at room temperature. The slurry quickly homogenized and stirring was continued for 15 min. After 15 min, the reaction vessel was cooled to 0 °C in an ice bath and to it DIAD (2.1 equiv.) was added, followed by P(*n*-Bu)$_3$ (93.5%, 2.0 equiv.) dropwise over 5 min. After 5 min, a solution of 5-*O*-(4-methoxytrityl)-D-ribofuranose **4** (1.0 or 1.5 equiv.) in MeCN was added all at once *via cannula* and the flask rinsed with a small volume of MeCN. The reaction was slowly allowed to come to r.t. overnight. After 12 h, one drop of reaction mixture was removed and diluted in a vial and set aside. The pH of the reaction was then lowered to ~1 with aqueous 1 M HCl and either heated at 60 °C for exactly 15 min. or stirred at room temperature for 1 h. After this time a TLC plate spotted with the reaction mixture was developed (9:1 CH_2Cl_2–MeOH) and compared to the diluted, unacidified reaction mixture in the vial to ensure complete cleavage of the MMTr group. The reaction was then neutralized with aqueous 1 M NaOH and concentrated *in vacuo*. The crude oil was subsequently dissolved in a minimum volume (~2 mL) of MeOH–CH_2Cl_2 (1:1) and triturated through petroleum ether–diethyl ether (110–130 mL, 6:1). After stirring for 20 min an oil formed and stuck on the

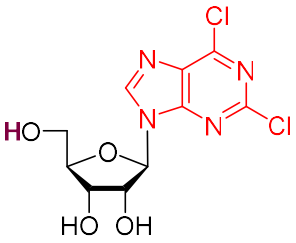
inside wall of the flask that contained the product. The filtrate was filtered off and checked by TLC to ensure it contained no dissolved product. The crude oil was re-dissolved in MeOH and concentrated onto silica gel. Purification using flash column chromatography on silica gel (MeOH:CH_2Cl_2) provided the nucleoside furanoside product.

6-Chloro-1-(β-D-ribopyranosyl)purine (3a)



Following the general procedure 6-chloropurine **1a** (123 mg, 0.800 mmol, 1.0 equiv.), DBU (120 µL, 0.800 mmol, 1.0 equiv.), DIAD (330 µL, 1.68 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (430 µL, 1.60 mmol, 2.0 equiv.), and **4** (336 mg, 0.800 mmol, 1.0 equiv.) dissolved in MeCN (2.5 mL) in MeCN (5.5 mL) were reacted. Cleavage of the MMTr group was effected by heating the acidified reaction mixture at 60 °C for exactly 15 min. Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided the product **3a** (120 mg, 52 %) as a pale yellow foam that crystallized from water after 3 days at 4 °C. Once again, the analytical data were in accordance with a commercially available sample (Sigma-Aldrich).

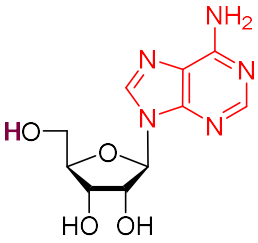
2,6-Dichloro-1-(β-D-ribofuranosyl)purine (3b)



Following the general procedure 2,6-dichloropurine **1b** (197 mg, 1.05 mmol, 1.0 equiv.), DBU (157 µL, 1.05 mmol, 1.0 equiv.), DIAD (434 µL, 2.20 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (560 µL, 2.10 mmol, 2.0 equiv.), and **4** (444 mg, 1.05 mmol, 1.0 equiv.) dissolved in MeCN (4 mL) in MeCN (7 mL) were reacted. Cleavage of the MMTr group was effected by heating the acidified reaction mixture at 60 °C for exactly 15 min. Purification by flash column chromatography on silica gel (0%–5% MeOH in CH_2Cl_2) provided the product **3b** contaminated with an unidentifiable side product (112 mg, 35%). Purification using semi-preparative RP HPLC (0% MeOH in H_2O to 100% MeOH, linear gradient, 10 mL/min) provided **3b** (94 mg, 28 %) as a colorless oil. 0.43 (9:1 CH_2Cl_2–MeOH). $[\alpha]_D$: −13.3 (*c* 0.323, MeOH). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.59 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 4.0, H-5′b); 3.71 (dd, 1H, J_{gem} = 11.9, $J_{5'a,4'}$ = 4.0, H-5′a); 3.99 (q, 1H, $J_{4',3'} = J_{4',5'}$ = 4.0, H-4′); 4.18 (dd, 1H, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 4.0, H-3′); 4.51 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 4.9, H-2′); 5.09 (bs, 1H, OH-5′); 5.28 (bs, 1H, OH-3′); 5.61 (bs, 1H, OH-2′); 5.97 (d, 1H, $J_{1',2'}$ = 4.9, H-1′); 8.98 (s,

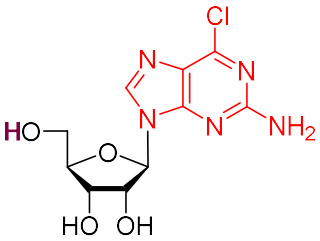
1H, H-8). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 60.92 (CH_2-5′); 70.01 (CH-3′); 74.23 (CH-2′); 85.86 (CH-4′); 88.44 (CH-1′); 131.18 (C-5); 146.57 (CH-8); 150.03 (C-2); 151.30 (C-6); 153.24 (C-4). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{10}H_{10}Cl_2N_4O_4Na$: 342.99713. Found: 342.99692.

Adenosine (3c)



Following the general procedure adenine **1c** (80 mg, 0.595 mmol, 1.0 equiv.), DBU (90 µL, 0.595 mmol, 1.0 equiv.), DIAD (250 µL, 1.25 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (320 µL, 1.19 mmol, 2.0 equiv.), and **4** (251 mg, 0.680 mmol, 1.0 equiv.) dissolved in MeCN (2 mL) in MeCN (5 mL) were reacted. Cleavage of the MMTr group was effected by heating the acidified reaction mixture at 60 °C for exactly 15 min. Purification by flash column chromatography on silica gel (0%–20% MeOH in CH_2Cl_2) provided adenosine (108 mg, 68 %) as a white foam that was slightly contaminated with an unidentifiable side product and DBU. Crystallization from MeOH provided pure adenosine (**1c**) as a white solid (63 mg, 40 %). Spectral data were in accordance with a commercially available sample (Sigma-Aldrich). R_f = 0.54 (4:1 CH_2Cl_2–MeOH). HR ESIMS: *m/z* [M+H^+] calcd for $C_{10}H_{14}N_5O_4$: 268.10403. Found: 268.10411. [M+Na^+] calcd for $C_{10}H_{13}N_5O_4Na$: 290.08598. Found: 290.08604.

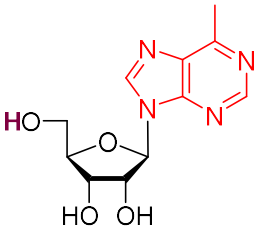
2-Amino-6-chloro-1-(β-D-ribofuranosyl)purine (3d)



This is a known compound.[147] Following the general procedure 2-amino-6-chloropurine **1c** (115 mg, 0.680 mmol, 1.0 equiv.), DBU (101 µL, 0.680 mmol, 1.0 equiv.), DIAD (280 µL, 1.43 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (360 µL, 1.36 mmol, 2.0 equiv.), and **4** (287 mg, 0.680 mmol, 1.0 equiv.) dissolved in MeCN (2 mL) in MeCN (5 mL) were reacted. Cleavage of the MMTr group was effected by heating the acidified reaction mixture at 60 °C for exactly 15 min. Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided the product **3c** (152 mg, 74 %) as a white solid that recrystallized from water after 3 days at 4 °C to afford a white powder. R_f = 0.25 (9:1 CH_2Cl_2–MeOH). m.p. 163–166 °C. **[α]D:** −11.0 (*c* 0.282, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.54 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 5.4, $J_{5'b,4'}$ = 4.1, H-5′b); 3.64 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 5.4, $J_{5'a,4'}$ = 4.3, H-5′a); 3.91 (ddd, 1H, $J_{4',5'}$ = 4.3, 4.1, $J_{4',3'}$ = 3.6, H-4′); 4.12

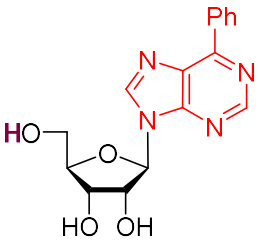
(td, 1H, $J_{3',2'} = J_{3',OH} = 4.8$, $J_{3',4'} = 3.6$, H-3′); 4.48 (td, 1H, $J_{2',1'} = J_{2',OH} = 5.8$, $J_{2',3'} = 4.8$, H-2′); 5.04 (t, 1H, $J_{OH,5'} = 5.5$, OH-5′); 5.18 (d, 1H, $J_{OH,3'} = 4.8$, OH-3′); 5.47 (d, 1H, $J_{OH,2'} = 5.8$, OH-2′); 5.81 (d, 1H, $J_{1',2'} = 5.8$, H-1′); 6.98 (s, 2H, NH_2); 8.38 (s, 1H, H-8). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 61.39 (CH_2-5′); 70.44 (CH-3′); 73.76 (CH-2′); 85.56 (CH-4′); 86.90 (CH-1′); 123.68 (C-5); 141.37 (CH-8); 149.69 (C-6); 154.26 (C-4); 160.00 (C-2). IR: (KBr) ν_{max} 3408, 3335, 3219, 2928, 1620, 1567, 1514, 1470, 1409, 1285, 1220, 1150, 1121, 1085, 1053, 920, 784, 635. HR ESIMS:. *m/z* [M+Na$^+$] calcd for $C_{10}H_{12}N_5O_4ClNa$: 324.04700. Found: 324.04704.

6-Methyl-1-(β-D-ribofuranosyl)purine (3e)



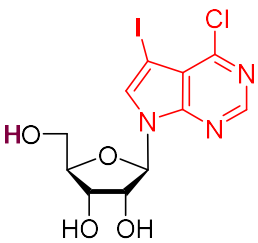
This is a known compound.[148] Following the general procedure 6-methylpurine **3e** (101 mg, 0.753 mmol, 1.0 equiv.), DBU (112 µL, 0.753 mmol, 1.0 equiv.), DIAD (310 µL, 1.58 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (400 µL, 1.51 mmol, 2.0 equiv.), and **4** (318 mg, 0.777 mmol, 1.0 equiv.) dissolved in MeCN 2.5 mL) in MeCN (5 mL) were reacted. Cleavage of the MMTr group was effected by heating the acidified reaction mixture at 60 °C for exactly 15 min. Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided the product **3e** (131 mg, 65 %) as a colorless foam that was slightly contaminated with DBU. Pure nucleoside (93 mg, 47 %) was furnished by washing the foam with CH_2Cl_2 to yield a white solid. R_f = 0.37 (9:1 CH_2Cl_2–MeOH). 92–194 °C. [α]$_D$: –37.0 (*c* 0.327, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 2.73 (s, 3H, CH_3); 3.57 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH} = 6.1$, $J_{5'b,4'} = 4.0$, H-5′b); 3.69 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH} = 5.2$, $J_{5'a,4'} = 4.0$, H-5′a); 3.97 (td, 1H, $J_{4',5'} = 4.0$, $J_{4',3'} = 3.5$, H-4′); 4.18 (td, 1H, $J_{3',2'} = J_{3',OH} = 5.0$, $J_{3',4'} = 3.5$, H-3′); 4.62 (td, 1H, $J_{2',1'} = J_{2',OH} = 5.9$, $J_{2',3'} = 5.0$, H-2′); 5.11 (dd, 1H, $J_{OH,5'} = 6.1, 5.2$, OH-5′); 5.22 (d, 1H, $J_{OH,3'} = 5.0$, OH-3′); 5.50 (d, 1H, $J_{OH,2'} = 5.9$, OH-2′); 6.01 (d, 1H, $J_{1',2'} = 5.9$, H-1′); 8.75 (s, 1H, H-8); 8.79 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 19.28 (CH_3); 61.46 (CH_2-5′); 70.49 (CH-3′); 73.80 (CH-2′); 85.85 (CH-4′); 87.78 (CH-1′); 133.07 (C-5); 144.17 (CH-8); 150.24 (C-4); 151.82 (CH-2); 158.51 (C-6). IR: (KBr) ν_{max} 3361, 3241, 2977, 2897, 1626, 1608, 1584, 1501, 1461, 1375, 1235, 1156, 1095, 1095, 1049, 867, 717, 528. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{11}H_{15}N_4O_4$: 267.10878. Found: 267.10881. [M+Na$^+$] calcd for $C_{11}H_{14}N_4O_4Na$: 289.09073. Found: 289.09074.

6-Phenyl-1-(β-D-ribofuranosyl)purine (3f)



This is a known compound.[149] Following the general procedure 6-phenylpurine **1f** (164 mg, 0.840 mmol, 1.0 equiv.), DBU (126 µL, 0.840 mmol, 1.0 equiv.), DIAD (350 µL, 1.76 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (450 µL, 1.55 mmol, 2.0 equiv.), and **4** (353 mg, 0.777 mmol, 1.0 equiv.) dissolved in MeCN (3 mL) in MeCN (6 mL) were reacted. Cleavage of the MMTr group was effected by heating the acidified reaction mixture at 60 °C for exactly 15 min. Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided the product 3f (162 mg, 59 %) as a colorless foam that recrystallized from MeOH after 3 days at –20 °C. R_f = 0.46 (9:1 CH_2Cl_2–MeOH). m.p. 220–221 °C. $[\alpha]_D$: –51.1 (c 0.487 DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.60 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.0, $J_{5'b,4'}$ = 3.9, H-5′b); 3.72 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 5.2, $J_{5'a,4'}$ = 3.9, H-5′a); 4.01 (q, 1H, $J_{4',3'} = J_{4',5'}$ = 3.9, H-4′); 4.22 (td, 1H, $J_{3',2'} = J_{3',OH}$ = 5.0, $J_{3',4'}$ = 3.9, H-3′); 4.66 (ddd, 1H, $J_{2',OH}$ = 5.9, $J_{2',1'}$ = 5.6, $J_{2',3'}$ = 5.0, H-2′); 5.15 (dd, 1H, $J_{OH,5'}$ = 6.0, 5.2, OH-5′); 5.27 (d, 1H, $J_{OH,3'}$ = 5.0, OH-3′); 5.58 (d, 1H, $J_{OH,2'}$ = 5.9, OH-2′); 6.10 (d, 1H, $J_{1',2'}$ = 5.6, H-1′); 7.56-7.64 (m, 3H, H-*m,p*-Ph); 8.83 (m, 2H, H-*o*-Ph); 8.94 (s, 1H, H-8); 9.02 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 61.40 (CH_2-5′); 70.44 (CH-3′); 73.94 (CH-2′); 85.86 (CH-4′); 87.85 (CH-1′); 128.92 (CH-*m*-Ph); 129.59 (CH-*o*-Ph); 131.05 (C-5); 131.38 (CH-*p*-Ph); 135.43 (C-*i*-Ph); 145.15 (CH-8); 152.12 (CH-2); 152.42 (C-4); 153.16 (C-6). IR: (KBr) ν_{max} 3397, 1588, 1566, 1501, 1483, 1460, 1331, 1305, 1215, 1171, 1104, 1057, 1043, 695, 642, 579. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{16}H_{17}N_4O_4$: 329.12443. Found: 329.12447. [M+Na$^+$] calcd for $C_{16}H_{16}N_4O_4Na$: 351.10638. Found: 351.10638.

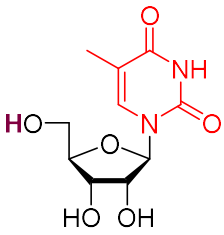
4-Chloro-5-iodo-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-d]pyrimidine (3g)



This is a known compound.[145] Following the general procedure **1g** (217 mg, 0.777 mmol, 1.0 equiv.), DBU (117 µL, 0.777 mmol, 1.0 equiv.), DIAD (320 µL, 1.63 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (410 µL, 1.55 mmol, 2.0 equiv.), and **4** (328 mg, 0.777 mmol, 1.0 equiv.) dissolved in MeCN (2 mL) in MeCN (5 mL) were reacted. Cleavage of the MMTr group was effected by heating the acidified reaction mixture at 60 °C for exactly 15 min. Purification by flash column chromatography on silica gel (0%–10% MeOH in CH_2Cl_2) provided the product 3g (242 mg, 76 %) as a white solid that recrystallized from water after 3 days at 4 °C

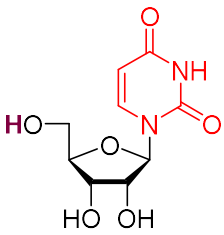
to afford a white solid. R_f = 0.60 (9:1 CH_2Cl_2–MeOH). m.p. 187–189 °C. $[\alpha]_D$: –50.9 (*c* 0.287, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 3.57 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 5.4, $J_{5'b,4'}$ = 3.8, H-5′b); 3.65 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 5.4, $J_{5'a,4'}$ = 4.0, H-5′a); 3.93 (ddd, 1H, $J_{4',5'}$ = 4.0, 3.8, $J_{4',3'}$ = 3.3, H-4′); 4.10 (td, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 4.9, $J_{3',4'}$ = 3.3, H-3′); 4.38 (td, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 6.1, $J_{2',3'}$ = 4.9, H-2′); 5.11 (t, 1H, $J_{OH,5'}$ = 5.4, OH-5′); 5.21 (d, 1H, $J_{OH,3'}$ = 4.9, OH-3′); 5.44 (d, 1H, $J_{OH,2'}$ = 6.1, OH-2′); 6.19 (d, 1H, $J_{1',2'}$ = 6.1, H-1′); 8.25 (s, 1H, H-6); 8.68 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 53.75 (C-5); 61.41 (CH_2-5′); 70.54 (CH-3′); 74.52 (CH-2′); 85.73 (CH-4′); 87.22 (CH-1′); 116.77 (C-4a); 133.71 (CH-6); 150.94 (CH-2); 151.20, 151.28 (C-4,7a). IR: (KBr) ν_{max} 3426, 2930, 1582, 1541, 1505, 1445, 1445, 1428, 1343, 1109, 1074, 1029, 958, 781, 599, 590. HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{11}H_{12}ClN_3O_4I$: 411.9555. Found: $C_{11}H_{12}ClN_3O_4I$: 411.9561. *m/z* [M+Na$^+$] calcd for $C_{11}H_{11}ClN_3O_4INa$: 433.93750. Found: 433.93756.

1-(β-D-Ribofuranosyl)thymine (3h)



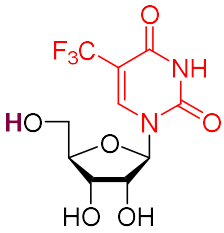
This compound is commercially available (Sigma-Aldrich). Following the general procedure thymine **1h** (126 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (530 µL, 2.00 mmol, 2.0 equiv.), and **4** (845 mg, 1.50 mmol, 1.5 equiv.) dissolved in MeCN (3 mL) in MeCN (7 mL) were reacted. Cleavage of the MMTr group was effected by stirring the acidified reaction mixture at room temperature for 1 h. Purification by flash column chromatography on silica gel (10%–15% MeOH in CH_2Cl_2) provided the desired product **3h** (101 mg, 39%) as a colorless foam. Spectral and HR ESIMS data were in accordance with the literature.[184] R_f = 0.40 (4:1 CH_2Cl_2–MeOH). ^{1}H NMR (500.0 MHz, CD_3OD): δ 7.85 (d, *J* = 1.2 Hz, 1H), 5.90 (d, *J* = 4.6 Hz, 1H), 4.18 (td, *J* = 9.8, 5.3 Hz, 2H), 4.00 (dt, *J* = 4.0, 2.9 Hz, 1H), 3.85 (dd, *J* = 12.2, 2.7 Hz, 1H), 3.74 (dd, *J* = 12.2, 3.1 Hz, 1H), 1.88 (d, *J* = 1.2 Hz, 3H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.4, 152.7, 138.4, 111.5, 90.4, 86.3, 75.5, 71.3, 62.3, 12.4.

Uridine (3i)



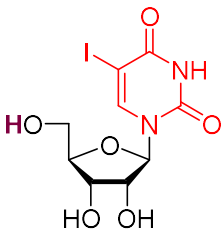
This compound is commercially available. Following the general procedure uracil **1i** (110 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (530 µL, 2.00 mmol, 2.0 equiv.), and **4** (845 mg, 1.50 mmol, 1.5 equiv.) dissolved in MeCN (3 mL) in MeCN (7 mL) were reacted. Cleavage of the MMTr group was effected by stirring the acidified reaction mixture at room temperature for 1 h. Purification by flash column chromatography on silica gel (10%–15% MeOH in CH_2Cl_2) provided the desired product **3i** (76 mg, 31%) as a colorless foam. Spectral and HR ESIMS data were in accordance with the literature.[185] R_f = 0.33 (4:1 CH_2Cl_2–MeOH). ^{1}H NMR (500.0 MHz, CD_3OD): δ 8.01 (d, *J* = 8.1 Hz, 1H), 5.90 (d, *J* = 4.7 Hz, 1H), 5.70 (d, *J* = 8.1 Hz, 1H), 4.20 – 4.16 (m, 1H), 4.16 – 4.13 (m, 1H), 4.01 (dt, *J* = 4.6, 2.9 Hz, 1H), 3.84 (dd, *J* = 12.2, 2.7 Hz, 1H), 3.73 (dd, *J* = 12.2, 3.1 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.2, 152.5, 142.7, 102.7, 90.7, 86.4, 75.7, 71.3, 62.3.

5-Trifluoromethyluridine (3j)



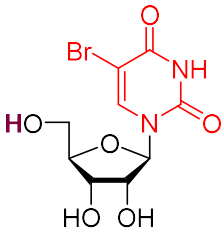
This is a known compound.[186] Following the general procedure trifluorothymine **1j** (181 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (530 µL, 2.00 mmol, 2.0 equiv.), and **4** (845 mg, 1.50 mmol, 1.5 equiv.) dissolved in MeCN (3 mL) in MeCN (7 mL) were reacted. Cleavage of the MMTr group was effected by stirring the acidified reaction mixture at room temperature for 1 h. Purification by flash column chromatography on silica gel (10%–15% MeOH in CH_2Cl_2) provided the product **3j** (97 mg, 31%) as a colorless foam. Spectral and HR ESIMS data were in accordance with the literature. R_f = 0.30 (4:1 CH_2Cl_2–MeOH). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 11.85 (s, 1H), 8.87 (s, 1H), 5.71 (d, *J* = 3.1 Hz, 1H), 5.51 (d, *J* = 4.6 Hz, 1H), 5.35 (t, *J* = 4.2 Hz, 1H), 5.08 (d, *J* = 4.6 Hz, 1H), 4.06 (dd, *J* = 7.3, 4.1 Hz, 1H), 4.00 (dd, *J* = 9.3, 5.3 Hz, 1H), 3.90 (dt, *J* = 6.1, 2.0 Hz, 1H), 3.77 – 3.72 (m, 1H), 3.62 – 3.57 (m, 1H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 159.1, 149.7, 142.4 (q, *J* = 5.5 Hz), 122.8 (q, *J* = 269.3 Hz), 102.8 (q, *J* = 31.9 Hz), 89.4, 84.3, 74.4, 68.5, 59.3. ^{19}F NMR (471 MHz, DMSO-d_6) δ = –61.19.

5-Iodouridine (3k)



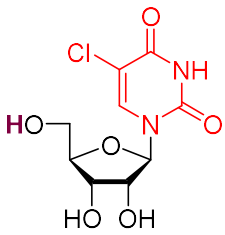
This compound is commercially available (Sigma-Aldrich). Following the general procedure 5-iodouracil **1k** (238 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (530 µL, 2.00 mmol, 2.0 equiv.), and **4** (845 mg, 1.50 mmol, 1.5 equiv.) dissolved in MeCN (3 mL) in MeCN (7 mL) were reacted. Cleavage of the MMTr group was effected by stirring the acidified reaction mixture at room temperature for 1 h. Purification by flash column chromatography on silica gel (10%–15% MeOH in CH_2Cl_2) provided the desired product **3k** (74 mg, 20%). Spectral and HR ESIMS data were in accordance with the literature.[186] R_f = 0.33 (4:1 CH_2Cl_2–MeOH). ^{1}H NMR (500.0 MHz, CD_3OD): δ 8.60 (s, 1H), 5.91 – 5.79 (m, 1H), 4.23 – 4.12 (m, 2H), 4.03 (dd, *J* = 4.7, 2.4 Hz, 1H), 3.88 (dd, *J* = 12.2, 2.6 Hz, 1H), 3.74 (dd, *J* = 12.2, 2.5 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 162.8, 152.2, 147.3, 91.0, 86.3, 76.1, 70.9, 68.3, 61.7.

5-Bromouridine (3l)



This compound is commercially available (Sigma-Aldrich). Following the general procedure 5-bromouracil **1l** (191 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (530 µL, 2.00 mmol, 2.0 equiv.), and **4** (845 mg, 1.50 mmol, 1.5 equiv.) dissolved in MeCN (3 mL) in MeCN (7 mL) were reacted. Cleavage of the MMTr group was effected by stirring the acidified reaction mixture at room temperature for 1 h. Purification by flash column chromatography on silica gel (10%–15% MeOH in CH_2Cl_2) provided the desired product **3l** (90 mg, 28%). Spectral and HR ESIMS data were in accordance with the literature.[185] R_f = 0.36 (4:1 CH_2Cl_2–MeOH). ^{1}H NMR (500.0 MHz, CD_3OD): δ 8.55 (s, 1H), 5.88 (d, *J* = 3.5 Hz, 1H), 4.23 – 4.12 (m, 2H), 4.08 – 3.98 (m, 1H), 3.89 (dd, *J* = 12.2, 2.6 Hz, 1H), 3.75 (dd, *J* = 12.2, 2.5 Hz, 1H) (DMSO = 2.66 ppm). ^{13}C NMR (125.7 MHz, CD_3OD): δ 161.6, 151.8, 142.2, 97.1, 91.0, 86.3, 76.1, 70.9, 61.7, (DMSO = 40.4 ppm).

5-Chlorouridine (3m)



This compound is commercially available (Carbosynth). Following the general procedure 5-chlorouracil **1m** (147 mg, 1.00 mmol, 1.0 equiv.), DBU (150 µL, 1.00 mmol, 1.0 equiv.), DIAD (420 µL, 2.10 mmol, 2.1 equiv.), P(*n*-Bu)$_3$ (530 µL, 2.00 mmol, 2.0 equiv.), and **4** (845 mg, 1.50 mmol, 1.5 equiv.) dissolved in MeCN (3 mL) in MeCN (7 mL) were reacted. Cleavage of the MMTr group was effected by stirring the acidified reaction mixture at room temperature for 1 h. Purification by flash column chromatography on silica gel (10 %–15 % MeOH in CH_2Cl_2) provided the desired product **3m** (70 mg, 28%). Spectral and HR ESIMS data were in accordance with the literature.[185] R_f = 0.36 (4:1 CH_2Cl_2–MeOH). ^{1}H NMR (500.0 MHz, CD_3OD): δ 8.46 (s, 1H), 5.88 (d, *J* = 3.7 Hz, 1H), 4.21 – 4.12 (m, 2H), 4.02 (dd, *J* = 2.8, 1.7 Hz, 1H), 3.88 (dd, *J* = 12.2, 2.6 Hz, 1H), 3.75 (dd, *J* = 12.2, 2.5 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 161.6, 151.6, 139.6, 109.4, 91.0, 86.4, 76.1, 71.0, 61.8.

5.2.4 Improved protocol for the synthesis of 5′*O*-monoprotected furanosyl nucleosides via anhydrose ring opening.

General procedure for the *in situ* formation of 1,2-anhydro-5-*O*-trityl-α-D-ribofuranose followed by nucleophilic ring opening (100 mg, 0.25 mmol scale).

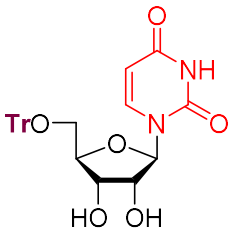
An argon-purged, dried 25 mL round bottom or pear-shaped flask containing a stir bar was charged with 5-*O*-trityl-D-ribose (**1**) (100 mg, 0.254 mmol, 1.0 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added MeCN (4 mL, 0.06 M with respect to the sugar) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 110 µl, 0.406 mmol, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (96 mg, 0.381 mmol, 1.5 equiv.) at r.t. Over the course of ~15 minutes the reaction turned from a homogenous orange color to a heterogeneous white color. The precipitate formed was the reduced ADDP hydrazine product which indicated conversion to the 1,2-anhydrosugar. In parallel, an argon-purged, dried 25 mL round bottom or pear-shaped flask containing a stir bar was charged with the nucleobase (2.0 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added DMF (0.33 M with respect to the nucleobase) via syringe. To this stirring slurry or solution was added NaH (60 % in mineral oil, 2.0 or 4.0 equiv. as indicated) at r.t. Stirring was continued for 15 min before the contents of the flask were transferred via syringe or cannula to the epoxide. After stirring at r.t. for 12 h, the reaction was quenched by decreasing the pH to ~7 using 1 M HCl(aq). The solvent was removed *in vacuo* coevaporating with toluene

several times. The crude oil was dissolved in a minimal volume MeOH (~3 mL) and concentrated directly onto silica gel. Purification of the product using either normal phase (silica gel) or reverse phase (C-18) column chromatography was carried out as indicated.

General procedure for the in situ formation of 1,2-anhydro-5-*O*-trityl-α-D-ribofuranose followed by nucleophilic ring opening (1.00 g, 2.5 mmol scale).

An argon-purged, dried 250 mL round bottom flask containing a stir bar was charged with 5-*O*-trityl-D-ribose (1.00 g, 2.54 mmol, 1.0 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added MeCN (40 mL, 0.06 M with respect to the sugar) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 1.1 ml, 4.06 mmol, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (965 mg, 3.81 mmol, 1.5 equiv.) at room temperature. Over the course of ~15 minutes the reaction turned from a homogenous orange color to a heterogeneous white color. The precipitate formed was the reduced ADDP hydrazine product which indicated conversion to the 1,2-anhydrosugar. In parallel, an argon-purged, dried 50 mL round bottom or pear-shaped flask containing a stir bar was charged with the nucleobase (1.5 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added DMF (0.33 M with respect to the nucleobase) via syringe. To this stirring slurry or solution was added NaH (60 % in mineral oil, 1.5 or 3.0 equiv. as indicated) at r.t. Stirring was continued for 15 min before the contents of the flask were transferred via syringe or cannula to the epoxide. After stirring at r.t. for 12 h, the reaction was quenched by decreasing the pH to ~7 using 1 M HCl(aq). The solvent was removed *in vacuo* coevaporating with toluene several times. The crude oil was dissolved in a minimal volume MeOH and concentrated directly onto silica gel. Purification of the product using either normal phase (silica gel) or reverse phase (C-18) column chromatography was carried out as indicated.

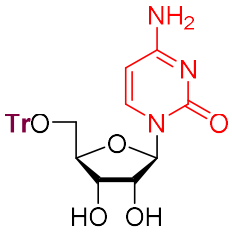
5′-*O*-Trityluridine (3.18a)



0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure uracil (57 mg, 0.508 mmol, 2.0 equiv.) and NaH (60% in mineral oil, 42 mg, 1.02 mmol, 4.0 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 19:1 CH_2Cl_2:MeOH) furnished the product **3.18a** (51 mg, 41 %) as a colorless foam.

2.5 mmol scale: According to the general nucleobase epoxide ring opening procedure uracil (426 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 320 mg, 7.62 mmol, 3.0 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 19:1 CH_2Cl_2:MeOH) furnished the product **3.18a** (353 mg, 29 %) as a colorless foam. R_f = 0.33 (CH_2Cl_2: MeOH = 19:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: +16.1 (*c* 0.402, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.29 (s, 1H, NH); 8.03 (d, 1H, $J_{6,5}$ = 8.1 Hz, H-6); 7.45–7.25 (m, 15H, H-Ar); 5.92 (d, $J_{1',2'}$ = 2.4 Hz, H-1′); 5.48 (bs, OH-2′); 5.36 (dd, $J_{5,6}$ = 8.1, $J_{5,HN}$ = 1.9, H-5); 4.47 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 6.0 Hz, H-3′); 4.37 (dd, 1H, $J_{2',3'}$ = 5.4, $J_{2',1'}$ = 2.5 Hz, H-2′); 4.20 (dt, 1H, $J_{4',3'}$ = 6.8, $J_{4',5'}$ = 2.6 Hz, H-4′); (m, 1H, H-2′); 3.55 (d, 2H, $J_{5',4'}$ = 2.6 Hz, H-5a′, H-5b′); 3.41 (bs, 1H, OH-3′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.9 (C-4); 151.1 (C-2); 146.8 (C-Ar); 143.2 (C-Ar) 140.4 (CH-6); 128.7 (C-Ar); 128.0 (C-Ar); 127.9 (C-Ar); 127.4 (C-Ar); 127.2 (C-Ar); 102.2 (CH-5); 90.6 (CH-1′); 87.6 (CPh_3); 83.6 (CH-4′); 75.4 (CH-2′); 69.5 (CH-3′); 61.9 (CH_2-5′). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{28}H_{26}N_2O_6$: 485.1718. Found: 485.1720.

5′-*O*-Tritylcytidine (3.18b)

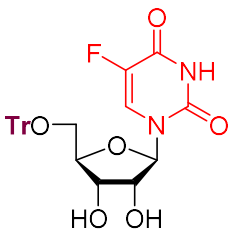


0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure cytosine (56 mg, 0.508 mmol, 2.0 equiv.) and NaH (60% in mineral oil, 21 mg, 0.508 mmol, 2.0 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the product **3.18b** (48 mg, 39 %) as a colorless foam.

2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure cytosine (422 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the product **3.18b** (453 mg, 37 %) as a white powder. R_f = 0.33 (CH_2Cl_2: MeOH = 9:1, v/v). $[\alpha]_D$: +6.1 (*c* 0.307, DMSO). ^{1}H NMR (400 MHz, DMSO–d_6): δ 7.73 (d, 1H, $J_{6,5}$ = 7.4 Hz, H-6); 7.42–7.25 (m, 15H, H-Ar); 7.17, 7.11 (s, 2 x 1H, NH_2); 5.77 (d, $J_{1',2'}$ = 2.9 Hz, H-1′); 5.51 (d, $J_{5,6}$ = 7.4, H-5); 5.45 (bs, OH-2′); 5.08 (bs, 1H, OH-3′); 4.11–4.05 (m, 1H, H-3′); 3.97–3.92 (m, 2H, H-2′, H-4′); 3.29–3.22 (m, 2H, H-5a′, H-5b′). ^{13}C NMR (100 MHz, DMSO–d_6): 165.5 (C-4); 155.1 (C-2); 143.5 (C-Ar); 140.9 (CH-6); 128.3 (C-

Ar); 128.0 (C-Ar); 127.2 (C-Ar); 93.7 (CH-5); 89.9 (CH-1′); 86.3 (CPh_3); 81.5 (CH-4′); 74.1 (CH-2′); 69.2 (CH-3′); 63.0 (CH_2-5′). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{28}H_{27}N_5O_5Na$: 508.18429. Found: 508.18432.

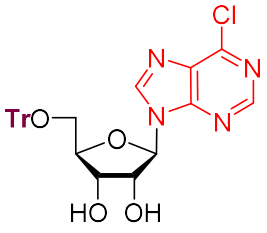
5-Fluoro-1-(5′-*O*-trityl-β-D-ribofuranosyl)uracil (4c)



0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure 5-fluorouracil (66 mg, 0.508 mmol, 2.0 equiv.) and NaH (60% in mineral oil, 42 mg, 1.016 mmol, 4.0 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the product **3.18c** (58 mg, 45 %) as a colorless foam.

2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure 5-fluorouracil (500 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 320 mg, 7.62 mmol, 3.0 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the product **3.18c** (269 mg, 21 %) as a white powder. R_f = 0.33 (CH_2Cl_2: MeOH = 9:1, v/v). $[\alpha]_D$: −7.5 (*c* 0.200, DMSO). ^{1}H NMR (400.0 MHz, DMSO-d_6): δ 12.10–11.70 (br. s, 1H, HN); 7.91 (d, 1H, $J_{6,F}$ = 6.8 Hz, H-6); 7.45–7.25 (m, 15H, H-Ar); 5.68 (dd, 1H, $J_{1',2'}$ = 3.9, $J_{1',F}$ = 1.7, H-1′); 5.53 (br. s, 1H, OH); 5.19 (br. s, 1H, OH); 4.15–4.07 (m, 2H, H-2′, H-3′); 3.97 (td, $J_{4',3'} = J_{4',5a'}$ = 5.0, $J_{4',5b'}$ = 2.5 Hz, H-4′); 3.33–3.28 (m, 1H, H-5a′); 3.20–3.15 (m, 1H, H-5b′). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.1 (d, $J_{C4,F}$ = 26.0 Hz, C-4); 149.3 (C-2); 143.4 (C-Ar) 140.2 (d, $J_{C5,F}$ = 231.7 Hz, C-5); 128.4 (C-Ar); 128.2 (C-Ar); 127.3 (C-Ar); 124.8 ($J_{C6,F}$ = 34.1 Hz, C-6); 89.3 (CH-1′); 86.6 (CPh_3); 82.7 (CH-4′); 73.4 (CH-2′); 69.7 (CH-3′); 63.5 (CH_2-5′). ^{19}F NMR (376 Hz, CD_3OD): δ -167.3 (d, $J_{F,H6}$ = 6.8 Hz). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{28}H_{25}O_6N_2FNa$: 527.15889. Found: 527.15900.

6-Chloro-9-(5′-*O*-trityl-β-D-ribofuranosyl)purine (3.18d)

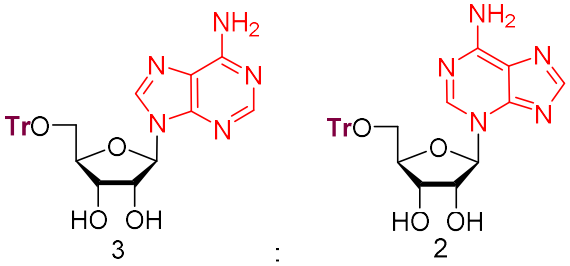


0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure 6-chloropurine (76 mg, 0.508 mmol, 2.0 equiv.) and NaH (60% in mineral oil, 21 mg, 3.81 mmol, 2.0 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column

chromatography (silica gel, 1:1 EtOAc:petroleum ether to 100 % EtOAc) furnished the product **3.18d** (88 mg, 66 %) as a waxy, white solid.

2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure 6-chloropurine (587 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 1:1 EtOAc:petroleum ether to 100 % EtOAc) furnished the product **3.18d** (829 mg, 62 %) as a waxy, white solid. R_f = 0.64 (EtOAc) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: –4.8 (*c* 0.229, MeOH). ^{1}H NMR (400.0 MHz,CD_3OD): δ 8.65 (s, 1H, H-8); 8.62 (s, 1H, H-2); 7.43–7.18 (m, 15H, H-Ar); 6.14 (d, 1H, $J_{1',2'}$ = 4.9, H-1′); 4.96 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 4.9 Hz, H-2′); 4.53 (t, 1H $J_{3',2'} = J_{3',4'}$ = 5.0 Hz, H-3′); 4.26 (q, $J_{4',5a'} = J_{4',5b'} = J_{4',3'}$ = 4.3 Hz, H-4′); 3.44–3.42 (m, 2H, H-5a′, H-5b′). ^{13}C NMR (100 MHz, CD_3OD): δ 151.6 (CH-2); 151.4 (C-4); 150.1 (C-6); 145.8 (CH-8); 143.7 (C-Ar); 131.7 (C-5); 128.4 (C-Ar); 127.4 (C-Ar); 126.8 (C-Ar) 89.7 (CH-1′); 86.8 (CPh_3); 84.2 (CH-4′); 73.6 (CH-2′); 70.7 (CH-3′); 63.4 (CH_2-5′). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{29}H_{26}N_4O_4Cl$: 529.16371. Found: 529.16394.

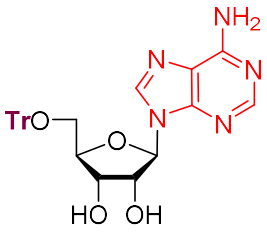
5′-*O*-Trityladenosine (3.18e) and 3-(5′-*O*-trityl-β-D-ribofuranosyl)adenine (3.18ex)



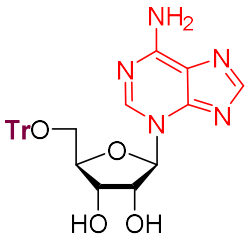
0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure adenine (68 mg, 0.508 mmol, 2.0 equiv.) and NaH (60% in mineral oil, 20 mg, 0.508 mmol, 2.0 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 50:1 to 19:1 CH_2Cl_2:MeOH) furnished the desired product **3.18e** and the N^3-glycosylated regioisomer **3.18ex** (ratio ~3:2) (75 mg, 58 %) that was an inseparable mixture as a colorless foam.

2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure adenine (513 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 50:1 to 19:1 CH_2Cl_2:MeOH) product **3.18e** and the N3-glycosylated regioisomer **3.18ex** (ratio ~3:2) (831

mg, 64 %) that was an inseparable mixture as a colorless foam. R_f = 0.35 (19:1 CH_2Cl_2:MeOH v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: −16.2 (*c* 0.419, DMSO).



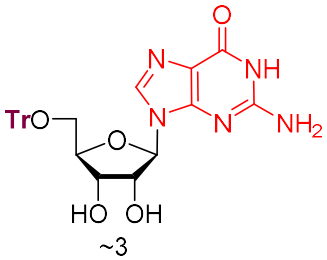
^{1}H NMR for the N^9 isomer **3.18e** (500.0 MHz, DMSO-d_6): δ 8.25 (s, 1H, H-2); 8.09 (s, 1H, H-8); 7.42–7.20 (m, 17H, H-Tr, H_2N); 5.93 (d, 1H, $J_{1',2'}$ = 4.6 Hz, H-1′); 5.55 (d, 1H, $J_{OH,2'}$ = 5.6 Hz, OH-2′); 5.22 (d, 1H, $J_{OH,3'}$ = 5.3 Hz, OH-3′); 4.70 (ddd, 1H, $J_{2',OH}$ = 5.6, $J_{2',3'}$ = 5.3, $J_{2',1'}$ = 4.6 Hz, H-2′); 4.32 (q, 1H, $J_{3',2'} = J_{3',4'} = J_{3',OH}$ = 5.3 Hz, H-3′); 4.07 (td, 1H, $J_{4',5'}$ = 5.3, 3.9, $J_{4',3'}$ = 5.3 Hz, H-4′); 3.24 (dd, 1H, J_{gem} = 10.3, $J_{5'a,4'}$ = 3.9, H-5′a); 3.21 (dd, 1H, J_{gem} = 10.3, $J_{5'b,4'}$ = 5.3, H-5′b). ^{13}C NMR for the N^9 isomer **3.18e** (125.7 MHz, DMSO-d_6): δ 156.3 (C-6); 152.8 (CH-2); 149.5 (C-4); 143.8 (C-*i*-Tr); 139.8 (CH-8); 128.4 (CH-*o*-Tr); 128.1 (CH-*m*-Tr); 127.2 (CH-*p*-Tr); 119.4 (C-5); 88.2 (CH-1′); 86.2 (C-Tr); 83.0 (CH-4′); 73.2 (CH-2′); 70.5 (CH-3′); 64.1 (CH_2-5′).

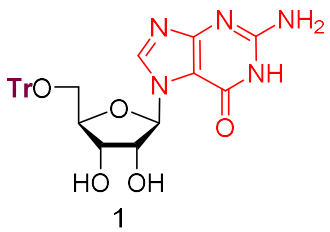


^{1}H NMR for the N^3 isomer **3.18ex** (125.7 MHz, DMSO-d_6): δ 8.50 (s, 1H, H-2); 8.13 (s, 1H, N**H$_a$**H$_b$); 8.04 (s, 1H, NH$_a$**H$_b$**); 7.74 (s, 1H, H-8); 7.42–7.20 (m, 15H, H-Ar); 6.06 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′); 5.78 (d, 1H, $J_{OH,2'}$ = 5.1 Hz, OH-2′); 5.21 (d, 1H, $J_{OH,3'}$ = 5.3 Hz, OH-3′); 4.68 (ddd,1H, $J_{2',3'}$ = 5.3, $J_{2',OH}$ = 5.1, $J_{2',1'}$ = 3.7 Hz, H-2′); 4.34 (q, 1H, $J_{3',2'} = J_{3',4'} = J_{3',OH}$ = 5.3 Hz, H-3′); 4.15 (td, 1H, $J_{4',5'}$ = 5.3, 3.3, $J_{4',3'}$ = 5.3 Hz, H-4′); 3.34 (dd, 1H, J_{gem} = 10.3, $J_{5'a,4'}$ = 3.3 Hz, H-5′a); 3.29 (dd, 1H, J_{gem} = 10.6, $J_{5'b,4'}$ = 5.3 Hz, H-5′b). ^{13}C NMR for the N^3 isomer **3.18ex** (125.7 MHz, DMSO-d_6): δ 155.3 (C-6); 152.6 (CH-8); 148.8 (C-4); 143.8 (C-*i*-Tr); 141.7 (CH-2); 128.4 (CH-*o*-Tr); 128.1 (CH-*m*-Tr); 127.2 (CH-*p*-Tr); 120.5 (C-5); 93.88 (CH-1′); 86.4 (C-Tr); 83.4 (CH-4′); 73.4 (CH-2′); 70.1 (CH-3′); 63.7 (CH_2-5′).

HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{29}H_{28}O_4N_5$: 510.21358. Found: 510.21362. *m/z* [M+Na$^+$] calcd for $C_{29}H_{27}O_4N_5Na$: 532.19553. Found: 532.19548.

5′-*O*-Tritylguanosine (3.18f) and 7-(5′-*O*-trityl-β-D-ribofuranosyl)guanine (3.18fx)

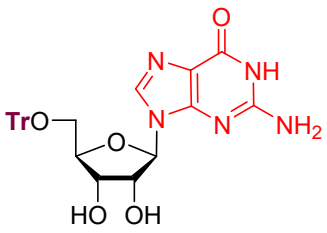




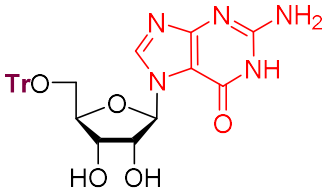
0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure **except that DMSO was used as solvent,** guanine (76 mg, 0.508 mmol, 2.0

equiv.) and NaH (60% in mineral oil, 21 mg, 0.508 mmol, 2.0 equiv.) were reacted in DMSO (1.5 mL) and subsequently added to the pre-formed epoxide. After removal of the MeCN *in vacuo* on a rotary evaporator, the crude reaction mixture was frozen and the DMSO removed on a lyophilizer. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the desired product **3.18f** and the N^7-glycosylated regioisomer **3.18fx** (ratio ~3:1) (34 mg, 26 %) that was an inseparable mixture as a colorless foam.

2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure **except that DMSO was used as solvent,** guanine (574 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMSO (10 mL) and subsequently added to the pre-formed epoxide. After removal of the MeCN *in vacuo* on a rotary evaporator, the crude reaction mixture was frozen and the DMSO removed on a lyophilizer. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the desired product **3.18f** and the N^7-glycosylated regioisomer **3.18fx** (ratio ~3:1) (316 mg, 24 %) that was an inseparable mixture as a colorless foam. R_f = 0.19 (9:1 CH_2Cl_2:MeOH v/v) UV active, one faint black spot with *p*-anisaldehyde stain.



^{1}H NMR for the N^9 isomer **3.18f** (500.0 MHz, DMSO-d_6): δ 10.92 (bs, 1H, HN); 7.79 (s, 1H, H-8); 7.38–7.23 (m, 15H, H-Ar); 6.57 (bs, 2H, H_2N); 5.74 (d, 1H, $J_{1',2'}$ = 4.9 Hz, H-1′); 5.56 (bs, 1H, OH-2′); 5.18 (bs, 1H, OH-3′); 4.43 (dd,1H, $J_{2',3'}$ = 5.1, $J_{2',1'}$ = 4.9 Hz, H-2′); 4.16 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 5.1 Hz, H-3′); 3.99 (td, 1H, $J_{4',5'}$ = 5.1, 3.7, $J_{4',3'}$ = 5.1 Hz, H-4′); 3.21–3.16 (m, 2H, H-5a′, H-5b′). ^{13}C NMR for the N^9 isomer **3.18f** (125.7 MHz, DMSO-d_6): δ 157.18 (C-6); 154.1 (C-2); 151.6 (C-4); 143.8 (C-*i*-Tr); 135.2 (CH-8); 128.4 (CH-*o*-Tr); 128.1 (CH-*m*-Tr); 127.2 (CH-*p*-Tr); 116.8 (C-5); 86.8 (CH-1′); 86.3 (C-Tr); 82.93 (CH-4′); 73.6 (CH-2′); 70.5 (CH-3′); 64.33 (CH_2-5′).

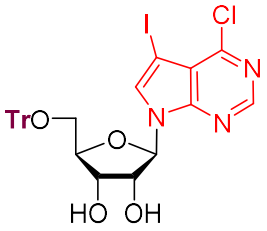


^{1}H NMR for the N^7 isomer **3.18fx** (500.0 MHz, DMSO-d_6): δ 10.92 (bs, 1H, HN); 8.10 (s, 1H, H-8); 7.38–7.23 (m, 15H, H-Ar); 6.23 (bs, 2H, H_2N); 6.06 (d, 1H, $J_{1',2'}$ = 4.7 Hz, H-1′); 5.56 (bs, 1H, OH-2′); 5.18 (bs, 1H, OH-3′); 4.42 (dd, 1H, $J_{2',3'}$ = 6.0, $J_{2',1'}$ = 4.7 Hz, H-2′); 4.09 (dd, 1H, $J_{3',2'}$ = 6.0, $J_{3',4'}$ = 5.5 Hz, H-3′); 4.01 (td, 1H, $J_{4',5'}$ = 5.5, 3.4, $J_{4',3'}$ = 5.5 Hz, H-4′); 3.21–3.16 (m, 2H, H-5a′, H-5b′). ^{13}C NMR for the N^7 isomer **3.18fx** (125.7

MHz, DMSO-d_6): δ 160.7 (C-4); 154.9 (C-6); 153.5 (C-2); 143.8 (C-*i*-Tr); 141.7 (CH-8); 128.4 (CH-*o*-Tr); 128.1 (CH-*m*-Tr); 127.2 (CH-*p*-Tr); 108.0 (C-5); 89.8 (CH-1′); 86.2 (C-Tr); 83.04 (CH-4′); 74.2 (CH-2′); 70.3 (CH-3′); 64.3 (CH_2-5′).

HR ESIMS: *m/z* [M+H^+] calcd for $C_{29}H_{28}O_5N_5$: 526.20850. Found: 526.20840. *m/z* [M+Na^+] calcd for $C_{29}H_{27}O_5N_5Na$: 548.19044. Found: 548.19040.

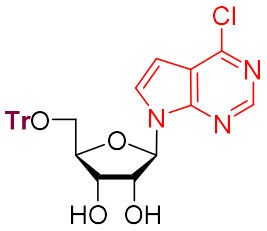
4-Chloro-7-(5′-*O*-trityl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (3.18g)



0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (78 mg, 0.508 mmol, 2.0 equiv.) and NaH (60% in mineral oil, 21 mg, 0.508 mmol, 2.0 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.18g** (71 mg, 53 %) as a waxy, white solid.

2.50 mmol scale: According to the general procedure 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (580 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.18g** (686 mg, 51 %) as a waxy, white solid. R_f = 0.49 (CH_2Cl_2: MeOH = 19:1, v/v) UV active. $[\alpha]_D$: –4.0 (*c* 0.175, MeOH). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ 8.65 (s, 1H, H-2); 7.84 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 7.34–7.40 (m, 6H, H-*o*-Tr); 7.22–7.33 (m, 9H, H-*m,p*-Tr); 6.71 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 6.22 (d, 1H, $J_{1',2'}$ = 5.0 Hz, H-1′); 5.62, 5.32 (2 × bs, 2 × 1H, OH-2′,3′); 4.53 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.0 Hz, H-2′); 4.24 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 5.0 Hz, H-3′); 4.09 (dt, 1H, $J_{4',3'}$ = 5.0, $J_{4',5'}$ = 4.1 Hz, H-4′); 3.21–3.28 (m, 2H, H-5′). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 151.2 (C-7a); 151.0 (C-4); 150.8 (CH-2); 143.8 (C-*i*-Tr); 128.9 (CH-6); 128.5 (CH-*o*-Tr); 128.1 (CH-*m*-Tr); 127.3 (CH-*p*-Tr); 117.6 (C-4a); 99.9 (CH-5); 88.2 (CH-1′); 86.3 (C-Ph_3); 83.1 (CH-4′); 73.8 (CH-2′); 70.5 (CH-3′); 64.2 (CH_2-5′). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{30}H_{26}N_3O_4ClNa$: 550.15041. Found: 550.15060.

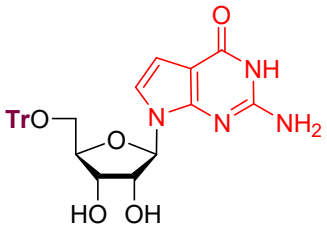
4-Chloro-5-iodo-7-(5′-*O*-trityl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (3.18h)



0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure 4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine (142 mg, 0.508 mmol, 2.0 equiv.) and NaH (60% in mineral oil, 21 mg, 0.508 mmol, 2.0 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.18h** (61 mg, 46 %) as a colourless foam.

2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure 4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine (1.06 g, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.18h** (802 mg, 48 %) as a colourless foam. R_f = 0.55 (CH_2Cl_2: MeOH = 19:1, v/v) UV active. $[\alpha]_D$: –4.7 (*c* 0.234, MeOH). ^{1}H NMR (400.0 MHz, DMSO-d_6): δ 8.66 (s, 1H, H-2); 8.05 (s, 1H, H-6); 7.41–7.24 (m, 15H, H--Tr); 6.19 (d, 1H, $J_{1',2'}$ = 5.2 Hz, H-1′); 5.59 (d, 1H, J = 5.6 Hz, OH-2′), 5.29 (d, 1H, J = 5.4 Hz, OH-3′); 4.53 (d, 1H, $J_{2',1'} = J_{2',3'}$ = 5.0 Hz, H-2′); 4.22 (d, 1H, $J_{3',2'} = J_{3',4'}$ = 5.0 Hz, H-3′); 4.07 (td, 1H, $J_{4',3'} = J_{4',5a'}$ = 5.0, $J_{4',5b'}$ = 3.2 Hz, H-4′); 3.28 (dd, 1H, J_{gem} = 10.6, $J_{5a',4'}$ = 5.0 Hz, H-5a′); 3.18 (dd, 1H, J_{gem} = 10.6, $J_{5b',4'}$ = 3.2 Hz, H-5b′). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 151.2 (C-4); 150.9 (C-7a); 150.8 (CH-2); 143.5 (C-Ar); 133.4 (CH-6); 128.2 (C-Ar); 128. (C-Ar); 127.1 (C-Ar); 116.7 (C-4a); 87.8 (CH-1′); 86.2 (C-Ph_3); 83.2 (CH-4′); 73.8 (CH-2′); 70.3 (CH-3′); 63.9 (CH_2-5′); 53.7 (C-5). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{30}H_{25}O_4N_3ClINa$ = 676.04705. Found: 676.04721.

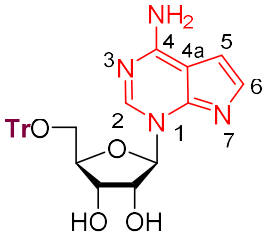
2-Amino-7-(5′-*O*-trityl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4(3H)-one (3.18i)



0.25 mmol scale: According to the general nucleobase epoxide ring opening procedure 2-amino-7H-pyrrolo[2,3-d]pyrimidine-4(3H)-one (75 mg, 0.508 mmol, 2.0 equiv.) and NaH (60% in mineral oil, 21 mg, 0.508 mmol, 2.0 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.18i** (61 mg, 46 %) as a pale brown foam.

2.50 mmol scale: According to the general procedure 2-amino-7H-pyrrolo[2,3-d]pyrimidine-4(3H)-one (570 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.18i** (506 mg, 38 %) as a pale brown foam. R_f = 0.45 (CH_2Cl_2: MeOH = 19:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: –40.3 (*c* 0.221, MeOH). ^{1}H NMR (400.0 MHz, CD_3OD): δ 6.77 (d, 1H, $J_{6,5}$ = 3.6 Hz, H-6); 7.55–7.07 (m, 15H, H-Tr); 6.52 (s, 1H, H-1′); 6.22 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5); 4.55 (dd, 1H, $J_{3',4'}$ = 6.0, $J_{3',4'}$ = 4.6 Hz, H-3′); 4.35 (d, 1H, $J_{2',3'}$ = 4.6 Hz, H-2′); 4.25 (td, 1H, $J_{4',3'} = J_{4',5b'}$ = 6.0, $J_{4',5a'}$ = 2.5 Hz, H-4′); 3.38–3.36 (m, 1H, H-5a′); 3.12 (dd, 1H, J_{gem} = 10.4, $J_{5b',4'}$ = 6.0 Hz, H-5b′). ^{13}C NMR (100 MHz, CD_3OD): δ 163.3 (C-7a); 160.6 (CH-2); 150.8 (CH-4); 145.2 (C-Tr); 129.8 (CH-Tr); 128.6 (CH-Tr); 127.8 (CH-Tr); 120.9 (CH-6); 103.6 (CH-1′); 100.1 (CH-5); 99.5 (C-4a); 87.6 (C-Tr); 84.2 (CH-4′); 76.2 (CH-2′); 71.9 (CH-3′); 65.5 (CH_2-5′). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{30}H_{29}O_5N_4$ = 525.21325. Found: 525.21332. *m/z* [M+Na$^+$] calcd for $C_{30}H_{28}O_5N_4Na$ = 547.19519. Found: 547.19525.

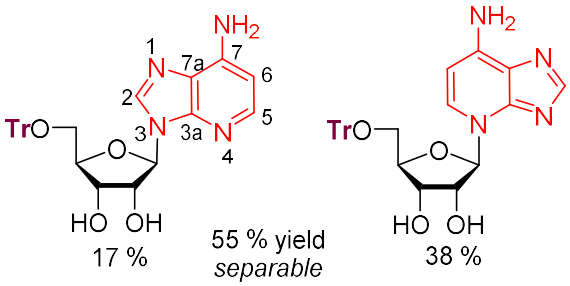
4-Amino-1-(5′-*O*-trityl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-d]pyrimidine (3.18j)



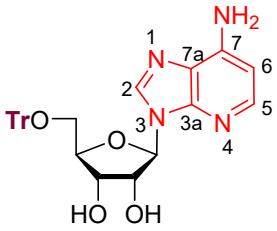
2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure 4-amino-7H-pyrrolo[2,3-d]pyrimidine (511 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 0% MeOH in H_2O to 100 % MeOH) furnished the unexpected product **3.18j** (463 mg, 36 %) exclusively as a pale red foam. R_f = 0.31 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: +27.1 (*c* 0.570, MeOH). ^{1}H NMR (400.0 MHz,CD_3OD): δ 8.57 (s, 1H, H-2); 7.50–7.10 (m, 16H, H-Ar, H-6); 6.61 (d, 1H, $J_{5,6}$ = 2.9 Hz, H-5); 6.25 (d, 1H, $J_{1',2'}$ = 2.7 Hz, H-1′); 4.43 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 3.2 Hz, H-2′); 4.40–4.31 (m, 2H, H-3′,4′); 4.54 (m, 1H, H-2′); 3.57–3.48 (m, 1H, H-5a′); 3.44 (1H, J_{gem} = 11.0, $J_{5b',4'}$ = 3.6 Hz, H-5b′). ^{13}C NMR (100 MHz, CD_3OD): δ 158.8 (C-4); 146.0 (C-7a); 145.2 (C-Ar); 140.6 (CH-2); 136.6 (CH-6); 130.1 (C-Ar); 129.2 (C-Ar); 128.5 (C-Ar); 106.7 (C-4a); 101.1 (CH-5); 94.7 (CH-1′); 88.8 (CPh_3); 85.5 (CH-4′); 77.7 (CH-2′); 71.4 (CH-

3′); 64.1 (CH_2-5′). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{30}H_{29}O_4N_4$ = 509.21833. Found: 509.21841.

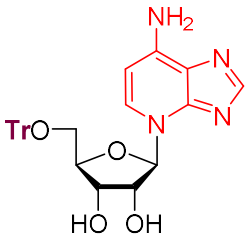
7-Amino-3-(5′-*O*-trityl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (3.18k) + 7-Amino-4-(5′-*O*-trityl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (3.18kx)



2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure 7-amino-3*H*-imidazo[4,5-*b*]pyrimidine (511 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 4:1 CH_2Cl_2:MeOH) furnished two products: the first eluting desired regioisomer **3.18k** (223 mg, 17 %) as a white powder and the second eluting undesired regioisomer **3.18kx** (486 mg, 38%) as a white powder.



Analytical data for 3.18k: R_f = 0.27 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: –22.3 (*c* 0.309, DMSO). ^{1}H NMR (400.0 MHz, DMSO-*d*6): δ 8.18 (s, 1H, H-2); 7.79 (d, 1H, $J_{5,6}$ = 5.5 Hz, H-5); 7.41–7.22 (m, 15H, H-Ar Tr); 6.41–6.36 (m, 3H, NH_2, H-5); 5.98 (d, 1H, $J_{1',2'}$ = 4.7 Hz, H-1′); 5.52 (bs, 1H, OH-2′); 5.20 (bs, 1H, OH-3′); 4.69 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.0 Hz, H-2′); 4.29 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 5.2 Hz, H-3′); 4.06 (td, 1H, $J_{4',3'} = J_{4',5b'}$ = 5.3, $J_{4',5a'}$ = 3.5 Hz, H-4′); 3.25 (dd, 1H, J_{gem} = 10.3, $J_{5a',4'}$ = 3.6 Hz, H-5a′); 3.21 (dd, 1H, J_{gem} = 10.3, $J_{5b',4'}$ = 5.5 Hz, H-5b′). ^{13}C NMR (100 MHz, DMSO-d_6): δ 147.5 (C-7); 147.4 (C-3a); 145.2 (CH-5); 144.1 (C-Ar Tr); 139.4 (CH-2); 128.7 (CH-Ar Tr); 128.3 (CH-Ar Tr); 127.5 (CH-Ar Tr); 123.7 (C-7a); 102.9 (CH-6); 88.2 (CH-1′); 86.5 (CPh_3); 83.0 (CH-4′); 73.4 (CH-2′); 70.8 (CH-3′); 64.5 (CH_2-5′). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{30}H_{29}O_4N_4$: 509.21833. Found: 509.21839.

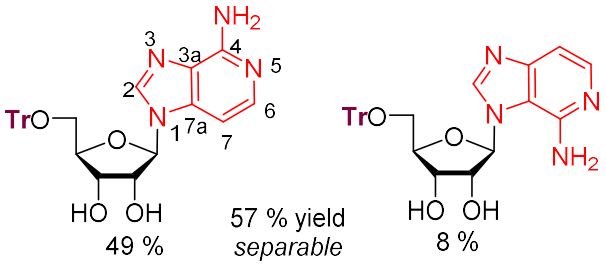


Analytical data for 3.18kx: R_f = 0.27 (4:1 CH_2Cl_2:MeOH) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: –3.5 (*c* 0.260, DMSO). ^{1}H NMR (400.0 MHz, DMSO-*d*6): δ 7.96 (d, 1H, $J_{5,6}$ = 7.2 Hz, H-5); 7.72 (s, 1H, H-2); 7.45–7.25 (m, 17H, NH_2 + H-Ar Tr); 6.24 (d, 1H, $J_{1',2'}$ = 2.9 Hz, H-1′); 6.18 (d, 1H, $J_{6,5}$ = 7.1 Hz, H-6); 5.92 (bs, 1H, OH-2′);

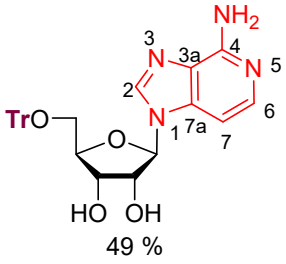
5.24 (bs, 1H, OH-3′); 4.33 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 4.0 Hz, H-2′); 4.26 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 5.8 Hz, H-3′); 4.14 (td, 1H, $J_{4',3'} = J_{4',5a'}$ = 6.4, $J_{4',5b'}$ = 3.4 Hz, H-4′); 3.40–3.30 (m, 2H, H-5a′, H-5b′). ^{13}C NMR (100 Hz, DMSO-d_6): δ 152.1 (CH-2); 149.7 (C-3a); 149.6 (C-7); 143.9 (C-Ar); 129.7 (CH-5); 128.8 (C-Ar); 128.4 (C-Ar); 127.6 (C-Ar); 125.8 (C-7a); 99.8 (CH-6); 93.4 (CH-1′); 86.8 (CPh_3); 83.0 (CH-4′); 75.0 (CH-2′); 70.2 (CH-3′); 63.5 (CH_2-5′).

HR ESIMS**:** *m/z* [M+H$^+$] calcd for $C_{30}H_{29}O_4N_4$: 509.21833. Found: 509.21834. *m/z* [M+Na$^+$] calcd for $C_{30}H_{28}O_4N_4Na$: 531.20028. Found: 531.20026.

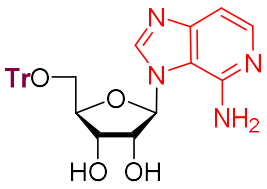
4-Amino-1-(5′-*O*-trityl-β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (3.18l) + 4-Amino-3-(5′-*O*-trityl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*c*]pyridine (3.18lx)



2.50 mmol scale: According to the general nucleobase epoxide ring opening procedure 4-amino-1*H*-imidazo[4,5-*b*]pyrimidine (511 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished two products: the first eluting undesired regioisomer **3.18lx** (101 mg, 8 %) as a pale orange foam and the second eluting desired regioisomer **3.18l** (633 mg, 49%) as a pale orange foam.



<u>Analytical data for 3.18l:</u> R_f = 0.40 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one black spot with p-anisaldehyde stain. $[\alpha]_D$: −13.6 (*c* 1.018, DMSO). ^{1}H NMR (400.0 MHz,CD_3OD): δ 8.21 (s, 1H, H-2); 7.48 (d, 1H, $J_{6,7}$ = 6.0 Hz, H-6); 7.42–7.07 (m, 15H, H-Ar Tr); 6.96 (d, 1H, $J_{7,6}$ = 6.1 Hz, H-6); 5.95 (d, 1H, $J_{1',2'}$ = 5.3 Hz, H-1′); 4.66 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.5 Hz, H-2′); 4.50–4.44 (m, 1H, H-3′); 4.31–4.26 (m, 1H, H-4′); 3.47–3.39 (m, 2H, H-5a′, H-5b′). ^{13}C NMR (100 MHz, CD_3OD): δ 151.9 (C-4); 143.5 (C-Ar Tr); 139.9 (CH-2); 139.8 (CH-6); 138.1 (C-7a); 128.5 (CH-Ar Tr); 127.6 (CH-Ar Tr); 127.0 (CH-Ar Tr); 126.8 (C-3a); 98.5 (CH-7); 89.8 (CH-1′); 87.1 (CPh_3); 84.4 (CH-4′); 74.5 (CH-2′); 70.6 (CH-3′); 63.4 (CH_2-5′). HR ESIMS: m/z [M+H$^+$] calcd for $C_{30}H_{29}O_4N_4$: 509.21833. Found: 509.21822.



Analytical data for 3.18lx: R_f = 0.56 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one black spot with p-anisaldehyde stain. $[\alpha]_D$: –57.6 (*c* 0.208, DMSO). ^{1}H NMR (400.0 MHz, CD_3OD): δ 8.40 (s, 1H, H-2); 7.77 (d, 1H, $J_{6,7}$ = 6.0 Hz, H-6); 7.34–7.18 (m, 15H, H-Ar Tr); 7.04 (d, 1H, $J_{7,6}$ = 5.9 Hz, H-6); 6.09 (d, 1H, $J_{1',2'}$ = 5.8 Hz, H-1′); 4.82 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.6 Hz, H-2′); 4.45 (dd, 1H, $J_{3',2'}$ = 5.4, $J_{3',4'}$ =3.8 Hz, H-3′); 4.32 (td, 1H, $J_{4',3'} = J_{4',5b'}$ = 3.7, $J_{4',5a'}$ = 2.6 Hz, H-4′); 3.48 (dd, 1H, J_{gem} = 10.9, $J_{5a',4'}$ = 2.8 Hz, H-5a′); 3.35–3.30 (m, 1H, H-5b′). ^{13}C NMR (100 MHz, CD_3OD): δ 151.5 (C-4); 148.8 (C-7a); 144.8 (C-Ar Tr); 143.6 (CH-2); 140.9 (CH-6); 129.7 (CH-Ar Tr); 128.9 (CH-Ar Tr); 128.3 (CH-Ar Tr); 119.9 (C-3a); 106.9 (CH-7); 90.8 (CH-1′); 88.4 (CPh_3); 86.5 (CH-4′); 75.8 (CH-2′); 72.0 (CH-3′); 64.3 (CH_2-5′).

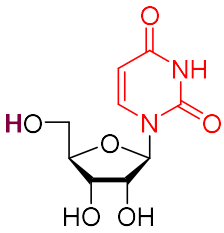
HR ESIMS: m/z [M+H$^+$] calcd for $C_{30}H_{29}O_4N_4$: 509.21833. Found: 509.21827.

5.2.5 Improved protocol for the two-step one-pot synthesis of ribofuranosyl nucleosides via anhydrose ring opening (1.00 g, 2.5 mmol scale)

An argon-purged, dried 250 mL round bottom flask containing a stir bar was charged with 5-*O*-trityl-D-ribose (1.00 g, 2.54 mmol, 1.0 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added MeCN (40 mL, 0.06 M with respect to the sugar) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 1.1 ml, 4.06 mmol, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (965 mg, 3.81 mmol, 1.5 equiv.) at room temperature. Over the course of ~15 minutes the reaction turned from a homogenous orange color to a heterogeneous white color. The precipitate formed was the reduced ADDP hydrazine product which indicated conversion to the 1,2-anhydrosugar. In parallel, an argon-purged, dried 50 mL round bottom or pear-shaped flask containing a stir bar was charged with the nucleobase (1.5 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added DMF (0.33 M with respect to the nucleobase) via syringe. To this stirring slurry or solution was added NaH (60 % in mineral oil, 1.5 or 3.0 equiv. as indicated) at r.t. Stirring was continued for 15 min before the contents of the flask were transferred via syringe or cannula to the epoxide. After stirring at r.t. for 12 h, 50 µL of crude reaction mixture was removed and set aside for TLC analysis to ensure complete cleavage of the trityl group. The reaction vessel was cooled to 0 °C and to it TFA/H_2O (9:1 v/v, 10 mL) was added dropwise over 5 min. The reaction was stirred again for 12 h at r.t. (or as indicated by consumption of the protected nucleoside by TLC) before increasing the pH to ~1 using

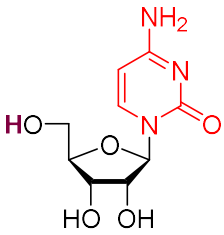
Dowex 1 x 2 resin ($^{-}$OH form). The resin was filtered off and the solvents removed *in vacuo* coevaporating with toluene several times. The crude oil was dissolved in a minimal volume MeOH and concentrated directly onto silica gel. Purification of the product using either normal phase (silica gel) or reverse phase (C-18) column chromatography was carried out as indicated.

Uridine (3.19a)



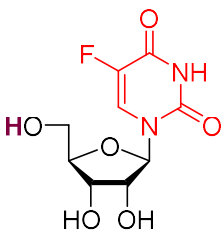
According to the general procedure, uracil (426 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 320 mg, 7.62 mmol, 3.0 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 4:1 CH_2Cl_2:MeOH) furnished the product **3.19a** (170 mg, 28 %) as a colorless foam that could be crystallized from EtOH. R_f = 0.33 (CH_2Cl_2: MeOH = 4:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. The analytical data were in accordance with a commercially available sample of uridine.

Cytidine (3.19b)



According to the general procedure cytosine (423 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 3:1 CH_2Cl_2:MeOH) furnished the product **3.19b** (221 mg, 36 %) as a white foam that could be crystallized from EtOH. R_f = 0.14 (CH_2Cl_2: MeOH = 4:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. The analytical data were in accordance with a commercially available sample of uridine.

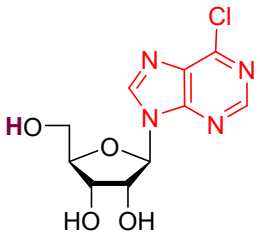
5-Fluorouridine (3.19c)



According to the general procedure 5-fluorouracil (500 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 320 mg, 7.62 mmol, 3.0 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 4:1 CH_2Cl_2:MeOH) furnished the product **3.19c** (210 mg, 31 %) as a foam that could be

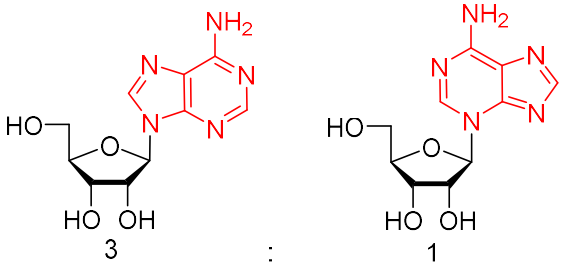
crystallized from EtOH. R_f = 0.12 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. The analytical data were in agreement with the literature.[187]

6-Chloro-9-(β-D-ribofuranosyl)purine (3.19d)



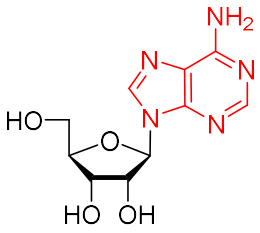
2.50 mmol scale: According to the general procedure 6-chloropurine (587 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the product **3.19d** (408 mg, 56 %) as a pale yellow foam that crystallized from water at 4 ºC. R_f = 0.32 (9:1 CH_2Cl_2:MeOH v/v) UV active, one black spot with *p*-anisaldehyde stain. Analytical data were in agreement with a commercially available sample.

Adenosine (3.19e) and 3-(β-D-ribofuranosyl)adenine (3.19ex)



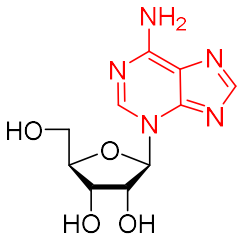
According to the general procedure adenine (515 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 4:1 CH_2Cl_2:MeOH) furnished the product **3.19e** and the N^3 glycosylated regioisomer **3.19ex** (~3:1 ratio) (451 mg, 66 %) as inseparable mixture. The desired product then could be crystallized from EtOH as a white solid (220 mg, 33 %). R_f = 0.54 (CH_2Cl_2: MeOH = 4:1, v/v) UV active, one black spot with *p*-anisaldehyde stain.

NMR data below are for the inseparable mixture of N^9 and N^3 regioisomers:



^{1}H NMR for the N^9 regioisomer **3.19e** (500.0 MHz, DMSO-d_6): δ 8.35 (s, 1H, H-2); 8.13 (s, 1H, H-8); 7.34 (s, 2H, H_2N); 5.87 (d, 1H, $J_{1',2'}$ = 6.2 Hz, H-1′); 5.47–4.40 (m, 2H, OH-2′, OH-5′); 5.20 (d, 1H, $J_{OH,3'}$ = 4.5 Hz, OH-3′); 4.61 (q, 1H, $J_{2',OH} = J_{2',3'} = J_{2',1'}$ = 5.7 Hz, H-2′); 4.14 (q, 1H, $J_{3',2'} = J_{3',4'} = J_{3',OH}$ = 4.2 Hz, H-3′); 3.96 (q, 1H, $J_{4',5'} = J_{4',3'}$ = 3.5 Hz, H-4′); 3.74–3.64 (m, 1H, H-5′a); 3.59–3.51 (m, 1H, H-5′b). ^{13}C NMR for the N^9

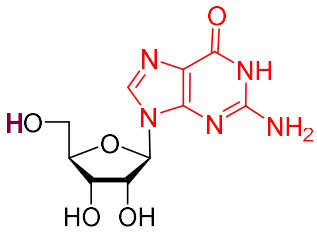
regioisomer **3.19e** (125.7 MHz, DMSO-d_6): δ 156.2 (C-6); 152.4 (CH-2); 149.1 (C-4); 139.9 (CH-8); 119.4 (C-5); 87.9 (CH-1′); 85.9 (CH-4′); 73.4 (CH-2′); 70.7 (CH-3′); 61.7 (CH_2-5′).



^{1}H NMR for the N^3 regioisomer **5ex** (500 MHz, DMSO-d_6): δ 8.56 (s, 1H, H-2); 8.27 (s, 1H, N**H**$_a$H$_b$); 8.18 (s, 1H, NH$_a$**H**$_b$); 7.78 (s, 1H, H-8); 5.85 (d, 1H, $J_{1',2'}$ = 7.0 Hz, H-1′); 5.51 (d, 1H, $J_{OH,2'}$ = 6.3 Hz, OH-2′); 5.47–4.40 (m, 1H, OH-5′); 5.23 (d, 1H, $J_{OH,3'}$ = 3.8 Hz, OH-3′); 4.82 (q, 1H, $J_{2',3'} = J_{2',OH} = J_{2',1'}$ = 5.9 Hz, H-2′); 4.12–4.08 (m, 2H, H-3′, H4′); 3.74–3.64 (m, 1H, H-5′a); 3.59–3.51 (m, 1H, H-5′b). ^{13}C NMR for the N^3 regioisomer **3.19ex** (125.7 MHz, DMSO-d_6): δ 155.7 (C-6); 151.2 (CH-8); 147.1 (C-4); 143.5 (CH-2); 120.7 (C-5); 94.9 (CH-1′); 87.8 (CH-4′); 72.1 (CH-2′); 71.0 (CH-3′); 61.8 (CH_2-5′).

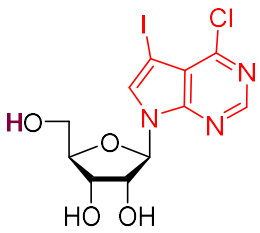
Analytical data for the crystallized desired product **3.19e** are in accordance with a commercially available sample of adenosine.

Guanosine (3.19f)



According to the general procedure **except that DMSO was used as solvent,** guanine (574 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMSO (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. After removal of the MeCN and H_2O *in vacuo* on a rotary evaporator, the crude reaction mixture was frozen and the DMSO removed on a lyophilizer. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 3:1 CH_2Cl_2:MeOH) furnished the desired product and the N7-glycosylated regioisomer (ratio ~3:1). A second purification using reverse phase chromatography (C-18, 5 % MeOH in H_2O to 100 % MeOH) provided pure desired product **3.19f** (106 mg, 15 %) as a white solid that crystallized readily from MeOH. TLC: R_f = 0.08 (4:1 CH_2Cl_2:MeOH v/v) UV active, one faint black spot with *p*-anisaldehyde stain. The analytical data were in agreement with a commercially available sample of guanosine.

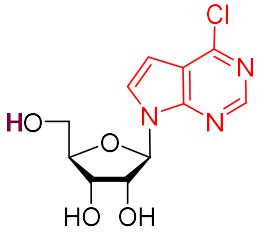
4-Chloro-7-(β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (3.19g)



According to the general procedure 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (580 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.19g** (354 mg, 49 %) as a foam that crystallized from MeOH/H_2O at –20 °C. R_f = 0.45 (CH_2Cl_2: MeOH = 9:1, v/v) UV active

Analytical data were in agreement with a previously synthesized sample from our laboratory and the literature.[145]

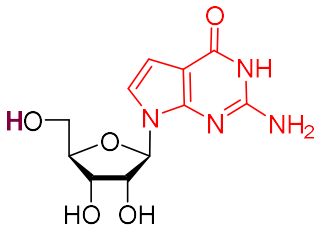
4-Chloro-5-iodo-7-(β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (3.19h)



According to the general procedure 4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine (1.06 g, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.19h** (51 mg, 51 %) as a colourless foam that crystallized from MeOH at –20 °C. R_f = 0.60 (CH_2Cl_2: MeOH = 9:1, v/v) UV active.

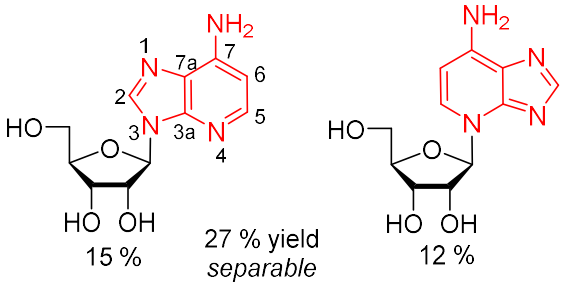
The analytical data were agreement with a previously synthesized sample from our laboratory.[153]

2-Amino-7-(β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4(3H)-one (3.19i)

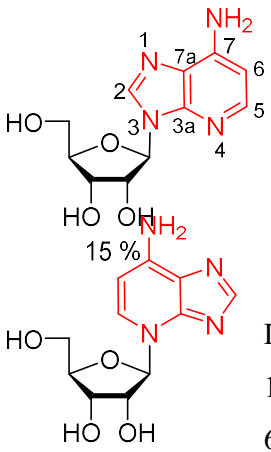


According to the general procedure 2-amino-7H-pyrrolo[2,3-d]pyrimidine-4(3H)-one (570 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.19i** (506 mg, 38 %) as a pale brown foam that crystallized from MeOH at –20 °C. R_f = 0.35 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. Analytical data were in agreement with the literature.[188]

7-Amino-3-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (3.19k) + 7-Amino-4-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (3.19kx)



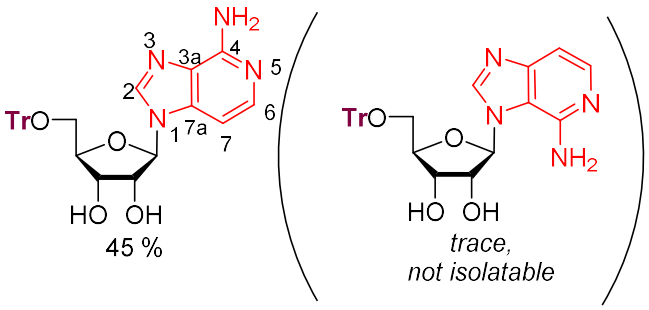
According to the general glycosylation–deprotection procedure described in Section 4 ***except that <u>50 %</u> of the quantities were used***, 7-amino-3*H*-imidazo[4,5-*b*]pyrimidine (255 mg, 1.90 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 80 mg, 1.90 mmol, 1.5 equiv.) were reacted in DMF (5 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 3:1 CH_2Cl_2:MeOH) furnished two products: the first eluting desired regioisomer **3.19k** (52 mg, 15 %) as a white powder and the second eluting undesired regioisomer **3.19kx** (41 mg, 12%) as a white powder.



<u>Analytical data for 3.19k:</u> R_f = 0.56 (CH_2Cl_2: MeOH = 4:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. The analytical data for **5k** were in agreement with the literature.[189]

<u>Analytical data for 3.19kx:</u> R_f = 0.08 (CH_2Cl_2: MeOH = 4:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: −15.6 (*c* 0.199, DMSO). ^{1}H NMR (500.0 MHz, CD_3OD+DMSO-d_6, 4:1, v/v): δ 8.47 (d, 1H, $J_{5,6}$ = 7.4 Hz, H-5); 8.36 (s, 1H, H-2); 6.69 (d, 1H, $J_{6,5}$ = 7.4 Hz, H-6); 6.23 (bd, 1H, $J_{1',2'}$ = 4.6 Hz, H-1′); 4.34 (dd, 1H, $J_{2',3'}$ = 5.0, $J_{2',1'}$ = 4.6 Hz, H-2′); 4.26 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 5.8 Hz, H-3′); 4.15 (dd, 1H, $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 4.5, H-3′); 4.09 (dt, 1H, $J_{4',3'}$ = 4.5, $J_{4',5'}$ = 2.7 Hz, H-4′); 3.82 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 2.7, H-5′a); 3.70 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 2.7, H-5′b). ^{13}C NMR (125.7 MHz, CD_3OD+DMSO-d_6, 4:1, v/v): δ 149.9 (C-7)*; 147.7 (C-3a)*; 145.0 (CH-2)*; 136.4 (CH-5); 115.8 (C-7a)*; 104.1 (CH-6); 95.0 (CH-1′); 87.5 (CH-4′); 76.5 (CH-2′); 71.1 (CH-3′); 62.0 (CH_2-5′). Asterisks indicate signals absent in the APT spectrum so were assigned by HSQC correlation. HR ESIMS**:** *m/z* [M+H$^+$] calcd for $C_{11}H_{15}O_4N_4$: 267.10878. Found: 267.10885. *m/z* [M+Na$^+$] calcd for $C_{11}H_{14}O_4N_4Na$: 289.09073. Found: 289.09078.

4-Amino-1-(β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (3.19l)



According to the general glycosylation–deprotection procedure described in Section 4, 4-amino-1*H*-imidazo[4,5-*b*]pyrimidine (511 mg, 3.81 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 160 mg, 3.81 mmol, 1.5 equiv.) were reacted in DMF (10 mL) and subsequently added to the pre-formed epoxide. The trityl group was then cleaved in one-pot. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished **3.19l** (302 mg, 45 %) as a white powder. The ^{1}H NMR of the crude sample indicated a very small amount (<5 %) of what can be presumed to be the regioisomer **3.19lx**, however, all attempts to isolate it purely failed (lost on the column or highly contaminated). R_f = 0.14 (CH_2Cl_2: MeOH = 4:1, v/v) UV active, one black spot with *p*-anisaldehyde stain.

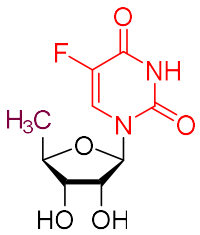
The analytical data for **3.19l** were in agreement with the literature.[165]

5.2.6 General procedure for the synthesis of 5*C*-modified ribofuranosyl nucleosides via epoxide ring opening.

****NOTE**** Due to the oily or foamy nature of the substrate, masses varied in each experiment.

An argon-purged, dried 50 mL round bottom or pear-shaped flask containing a stir bar was charged with 5-*O*-deoxy-D-ribose (**3.20**) (1.0 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added MeCN (0.06 M with respect to the sugar) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (1.5 equiv.) at room temperature. Over the course of ~15 minutes the reaction turned from a homogenous orange color to a heterogeneous white color (for compound **3.20** and **3.22**) or brown color (for compound **3.21**). The precipitate formed was the reduced ADDP hydrazine product which indicated conversion to the 1,2-anhydrosugar. At this time, to the flask was added the deprotonated nucleobase (as formed according to section 5.2.4) at room temperature as indicated for each compound. After addition of the nucleobase, the reaction was stirred at r.t. for 12 h. Reaction work-up and purification is reported individually for each compound.

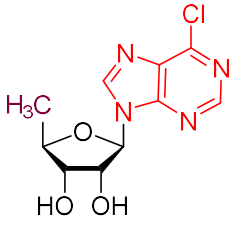
5′-Deoxy-5-fluorouridine (doxifluridine) (3.29a)



The epoxide was generated from 5-deoxy-D-ribose (**3.20**) (50 mg, 0.373 mmol, 1.0 equiv.), P(*n*Bu)$_3$ (93.5%, 190 µL, 0.600 mmol, 1.6 equiv.), and ADDP (141 mg, 0.560 mmol, 1.5 equiv) in MeCN (6 mL).

According to the general nucleobase epoxide ring opening procedure 5-fluorouracil (73 mg, 0.560 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 63 mg, 1.50 mmol, 3.0 equiv.) were reacted in DMF (2.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.29a** (50 mg, 54 %) as a colorless foam that crystallized from EtOAc/MeOH at -20 °C. This is a known compound. The analytical data are in accordance with the literature.[190] R_f = 0.55 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. m.p. 185–187 °C (lit. 189–190 °C). $[\alpha]_D$: –5.2 (*c* 0.326, DMSO). ^{1}H NMR (400.0 MHz, DMSO-d_6): δ 11.83 (bs, 1H, HN); 8.90 (d, 1H, $J_{6,F}$ = 7.0 Hz, H-6); 5.68 (dd, 1H, $J_{1',2'}$ = 5.1, $J_{1',F}$ = 1.7, H-1′); 5.34 (br. s, 1H, OH); 5.09 (br. s, 1H, OH); 4.09 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 5.1 Hz, H-2′); 3.83 (dd, $J_{4',5'}$ = 6.6, $J_{4',3'}$ = 5.1 Hz, H-4′); 3.69 (t, 1H $J_{3',2'}$ = $J_{3',4'}$ = 5.1, H-3′); 1.27 (d, 3H, $J_{5',4'}$ = 6.4, H-5′). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.5 (d, $J_{C4,F}$ = 26.1 Hz, C-4); 149.8 (C-2); 140.6 (d, $J_{C5,F}$ = 231.2 Hz, C-5); 125.3 ($J_{C6,F}$ = 33.7 Hz, C-6); 89.5 (CH-1′); 79.8 (CH-4′); 74.5 (CH-3′); 73.0 (CH-2′) 19.0 (CH_3-5′). HR ESIMS: *m/z* [M+Na$^+$] calcd for $C_9H_{11}O_5N_2FNa$: 269.05442. Found: 269.05447.

6-Chloro-9-(5′-deoxy-β-D-ribofuranosyl)purine (3.29b)

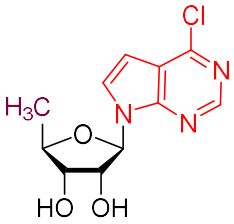


The epoxide was generated from 5-deoxy-D-ribose (**3.20**) (47 mg, 0.351 mmol, 1.0 equiv.), P(*n*Bu)$_3$ (93.5%, 150 µL, 0.562 mmol, 1.6 equiv.), and ADDP (132 mg, 0.560 mmol, 1.5 equiv) in MeCN (5.5 mL).

According to the general nucleobase epoxide ring opening procedure 6-chloropurine (81 mg, 0.526 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 22 mg, 0.526 mmol, 1.5 equiv.) were reacted in DMF (1.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.29b** (65 mg, 70 %) as a colorless foam that crystallized in a small volume of H_2O at 4 °C over several days to afford a pale yellow solid. This is a known compound. The optical rotation and melting point are in accordance with the literature.[191] R_f = 0.84 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. m.p. 152–155 °C (lit. 154–156 °C). $[\alpha]_D$: –40.5 (*c* 0.249, CH_3OH). ^{1}H NMR

(500.0 MHz, DMSO-d_6): δ 8.90 (s, 1H, H-8); 8.82 (s, 1H, H-2); 5.99 (d, 1H, $J_{1',2'}$ = 4.9); 5.54 (d, 1H, $J_{OH,2'}$ = 5.6, OH-2′); 5.25 (d, 1H, $J_{OH,3'}$ = 5.2, OH-3′); 4.69 (m, 1H, H-2′); 3.99 – 4.05 (m, 2H, H-3′,4′); 1.34 (d, 3H, $J_{5',4'}$ = 6.3, H-5′). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 152.0 (CH-2); 151.8 (C-4); 149.6 (C-6); 146.5 (CH-8); 131.7 (C-5); 88.7 (CH-1′); 80.52(CH-3′); 74.7 (CH-4′); 73.4 (CH-2′); 9.1 (CH_3-5′). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{10}H_{12}ClN_4O_3$: 271.05924. Found: 271.05941. *m/z* [M+Na$^+$] calcd for $C_{10}H_{11}ClN_4O_3Na$: 293.04119. Found: 293.04147.

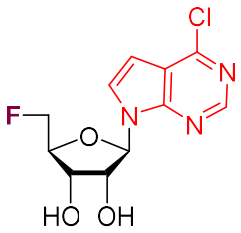
4-Chloro-7-(5′-deoxy-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (3.29c)



The epoxide was generated from 5-deoxy-D-ribose (**3.20**) (25.3 mg, 0.189 mmol, 1.0 equiv.), P(*n*Bu)$_3$ (93.5%, 80 µL, 0.302 mmol, 1.6 equiv.), and ADDP (72 mg, 0.560 mmol, 1.5 equiv) in MeCN (4 mL).

According to the general nucleobase epoxide ring opening procedure 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (44 mg, 0.378 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 12 mg, 0.378 mmol, 1.5 equiv.) were reacted in DMF (1 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.29c** (32 mg, 63 %) as a colorless foam. R_f = 0.69 (CH_2Cl_2: MeOH = 9:1, v/v) UV active. [α]$_D$: –46.4 (*c* 0.231, MeOH). ^{1}H NMR (400.0 MHz, DMSO-d_6): δ 8.67 (s, 1H, H-2); 7.93 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 6.75 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5); 6.15 (d, 1H, $J_{1',2'}$ = 5.2 Hz, H-1′); 5.51, 5.27 (2 × bs, 2 × 1H, OH-2′,3′); 4.53 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.0 Hz, H-2′); 3.98 (qd, 1H, $J_{4',5'}$ = 6.4, $J_{4',3'}$ = 5.0 Hz, H-4′); 3.91 (t, 1H, $J_{3',4'} = J_{3',2'}$ = 5.0 Hz, H-3′); 1.31 (d, 3H, $J_{5',4'}$ = 6.4 Hz, H-5′). ^{13}C NMR (100.0 MHz, DMSO-d_6): δ 151.1 (C-7a); 150.8 (C-4); 150.6 (CH-2); 128.9 (CH-6); 117.4 (C-4a); 99.8 (CH-5); 87.8 (CH-1′); 79.7 (CH-4′); 74.6 (CH-3′); 73.6 (CH-2′); 19.1 (CH_3-5′). HR ESIMS: *m/z* [M+Na$^+$] calcd for $C_{11}H_{12}O_3N_3ClNa$: 292.04594. Found: 292.04588.

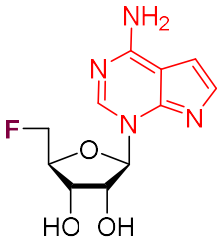
4-Chloro-7-(5′-deoxy-5′-fluoro-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-d]pyrimidine (3.31a)



The epoxide was generated from 5-deoxy-5-fluoro-D-ribose (**3.21**) (70.0 mg, 0.460 mmol, 1.0 equiv.), P(*n*Bu)$_3$ (93.5 %, 200 µL, 0.737 mmol, 1.6 equiv.), and ADDP (175 mg, 0.560 mmol, 1.5 equiv) in MeCN (7.5 mL). According to the general nucleobase epoxide ring opening procedure 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (106 mg, 0.690 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 29 mg, 0.740 mmol, 2.0 equiv.) were reacted in DMF (2 mL)

and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.31a** (68 mg, 51 %) as a colorless foam. R_f = 0.61 (CH_2Cl_2: MeOH = 19:1, v/v) UV active. $[\alpha]_D$: –33.5 (*c* 0.391, MeOH). ^{1}H NMR (400.0 MHz, CD_3OD): δ 8.59 (s, 1H, H-2); 7.70 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 6.72 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 6.37 (d, 1H, $J_{1',2'}$ = 5.0 Hz, H-1′); 4.71 (ddd, 1H, $J_{H,F}$ = 48.4, J_{gem} = 10.6, $J_{5a',4'}$ = 2.5 Hz, H-5a′); 4.66 71 (ddd, 1H, $J_{H,F}$ = 47.1, J_{gem} = 10.6, $J_{5b',4'}$ = 3.1 Hz, H-5b′); 4.47 (td, 1H, $J_{2',1'} = J_{2',3'}$ = 5.1, $J_{H,F}$ = 1.5 Hz, H-2′); 4.38 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 5.1 Hz); 4.22 (ddt, 1H, $J_{H,F}$ = 29.8, $J_{4',3'} = J_{4',5a'}$ = 4.5, $J_{4',5b'}$ = 2.8 Hz, H-4′). ^{13}C NMR (100 MHz, CD_3OD): δ 152.82 (C-7a); 152.76 (C-4); 151.6 (CH-2); 128.7 (CH-6); 119.4 (C-4a); 101.5 (CH-5); 89.6 (CH-1′); 84.4 (d, $J_{C,F}$ = 18.3 Hz, CH-4′); 83.9 (d, $J_{C,F}$ = 170.5 Hz, CH_2-5′); 76.1 (d, $J_{C,F}$ = 2.2 Hz, CH-2′); 71.4 (d, $J_{C,F}$ = 5.2 Hz, CH-3′). ^{19}F NMR (375 MHz, CD_3OD): δ -76.90 (s, CH_2F-5′). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{11}H_{12}O_3N_3ClF$: 288.05457. Found: 288.05480. *m/z* [M–H$^+$] calcd for $C_{11}H_{10}O_3N_3ClF$: 286.04002. Found: 286.03961.

4-Amino-1-(5′-deoxy-5′-fluoro-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-d]pyrimidine (3.31c)

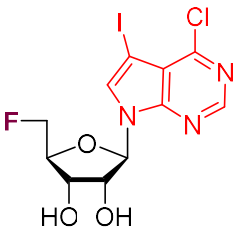


The epoxide was generated from 5-deoxy-5-fluoro-D-ribose (**3.21**) (194 mg, 1.26 mmol, 1.0 equiv.), P(*n*Bu)$_3$ (93.5 %, 540 µL, 2.04 mmol, 1.6 equiv.), and ADDP (479 mg, 1.89 mmol, 1.5 equiv) in MeCN (20 mL).

According to the general nucleobase epoxide ring opening procedure 4-amino-7H-pyrrolo[2,3-d]pyrimidine (201 mg, 1.89 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 80 mg, 1.89 mmol, 2.0 equiv.) were reacted in DMF (5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 0% MeOH in H_2O to 100 % MeOH) furnished solely the N-1 glycosylated product **3.31c** (151 mg, 46 %) as a colorless foam. R_f = 0.11 (CH_2Cl_2: MeOH = 4:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: –1.8 (*c* 0.333, MeOH). ^{1}H NMR (500.0 MHz, DMSO-*d*$_6$): δ 8.28 (s, 1H, H-2); 7.70 (bs, 2H, H_2N); 7.12 (d, 1H, $J_{6,5}$ = 2.5 Hz, H-6); 6.54 (d, 1H, $J_{5,6}$ = 2.5 Hz, H-5); 6.18 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1′); 4.77 (ddd, 1H, $J_{H,F}$ = 47.9, J_{gem} = 10.7, $J_{5'a,4'}$ = 2.8 Hz, H-5′a); 4.72 (ddd, 1H, $J_{H,F}$ = 47.5, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.8 Hz, H-5′b); 4.54 (m, 1H, H-2′); 4.16–4.26 (m, 2H, H-3′,4′). ^{13}C NMR (125.7 MHz, DMSO-*d*$_6$): δ 156.8 (C-4); 144.9 (C-7a); 140.1 (CH-2); 136.0 (CH-6); 104.0 (C-4a); 99.7 (CH-5); 93.2 (CH-1′); 83.1 (d, $J_{C,F}$ = 17.9 Hz, CH-4′); 82.9 (d, $J_{C,F}$ = 168.0 Hz, CH_2-5′); 73.9 (d, $J_{C,F}$ = 1.0

Hz, CH-2′); 69.4 (d, $J_{C,F}$ = 5.7 Hz, CH-3′). ^{19}F NMR (376 Hz, CD_3OD): δ -73.50 (s, CH_2F-5′). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{11}H_{14}O_3N_4F$: 269.10445. Found: 269.10448.

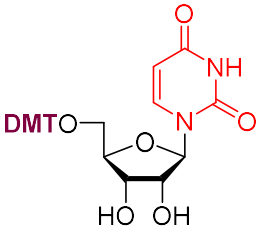
4-Chloro-5-iodo-7-(5′-deoxy-5′-fluoro-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (3.31c)



The epoxide was generated from 5-deoxy-5-fluoro-D-ribose (**3.21**) (75.0 mg, 0.493 mmol, 1.0 equiv.), P(*n*Bu)$_3$ (93.5%, 210 µL, 0.789 mmol, 1.6 equiv.), and ADDP (187 mg, 0.560 mmol, 1.5 equiv) in MeCN (8 mL).

According to the general nucleobase epoxide ring opening procedure 4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine (206 mg, 0.740 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 31 mg, 0.740 mmol, 2.0 equiv.) were reacted in DMF (2.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using reverse phase column chromatography (C-18, 5% MeOH in H_2O to 100 % MeOH) furnished the product **3.31c** (32 mg, 63 %) as a colorless foam that crystallized from MeOH at –20 °C to afford an off white solid. R_f = 0.60 (CH_2Cl_2: MeOH = 9:1, v/v) UV active. m.p. 140–141 °C (decomposes). [α]$_D$: –72.9 (*c* 0.203, DMSO). ^{1}H NMR (400.0 MHz, DMSO-d_6): δ 8.71 (s, 1H, H-2); 8.09 (s, 1H, H-6); 6.24 (d, 1H, $J_{1',2'}$ = 5.5 Hz, H-1′); 5.61 (d, 1H, *J* = 5.9 Hz, OH-2′), 5.45 (d, 1H, *J* = 5.1 Hz, OH-3′); 4.72–4.57 (m, 2H, H-5a′, H-5b′); 4.41 (q, 1H, $J_{2',1'}$ = 4.8, $J_{2',3'}$ = 4.2 Hz, H-2′ H-2′); 4.23–4.03 (m, 2H, H-3′, H-4′). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 151.7 (C-4); 151.5 (C-7a); 151.4 (CH-2); 133.7 (CH-6); 117.1 (C-4a); 87.8 (CH-1′); 83.4 (d, $J_{C5',F}$ = 168.0 Hz, CH-5′); 83.0 (d, $J_{C4',F}$ = 18.5 Hz, CH-4′); 74.0 (d, $J_{C2',F}$ = 2.3 Hz, CH-2′); 69.9 (d, $J_{C3',F}$ = 6.0 Hz, CH-3′); 54.5 (C-5). HR ESIMS: *m/z* [M+H^+] calcd for $C_{11}H_{11}O_3N_3ClFI$: 413.95122. Found: 413.95132. *m/z* [M+Na^+] calcd for $C_{11}H_{10}O_3N_3ClFINa$: 435.93316. Found: 435.93329.

5′-*O*-(4,4′-Dimethoxytrityl)uridine (3.33a)

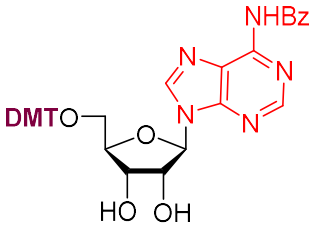


The epoxide was generated from 5-*O*-dimethoxytrityl-D-ribose (**3.22**) (400 mg, 0.885 mmol, 1.0 equiv.), P(*n*Bu)$_3$ (93.5%, 380 µL, 1.415 mmol, 1.6 equiv.), and ADDP (336 mg, 1.328 mmol, 1.5 equiv) in MeCN (15 mL).

According to the general nucleobase epoxide ring opening procedure uracil (149 mg, 1.33 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 55 mg, 1.328 mmol, 1.5 equiv.) were reacted in DMF (3.5 mL) and subsequently added to the pre-formed epoxide. Purification of the

product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the product **3.33a** (136 mg, 28 %) as a white solid. R_f = 0.17 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one bright orange spot with *p*-anisaldehyde stain. Analytical data were in agreement with S. Pitsch, P. A. Weiss, L. Jenny, A. Stutz, X. Wu, *Helv. Chim. Acta* **2001**, *84*, 3773.[192]

6-*N*-Benzoyl-5'-*O*-(4,4'-dimethoxytrityl)adenosine (3.33b)



The epoxide was generated from 5-*O*-dimethoxytrityl-D-ribose (**9**) (408 mg, 0.902 mmol, 1.0 equiv.), P(*n*Bu)$_3$ (93.5%, 385 µL, 1.44 mmol, 1.6 equiv.), and ADDP (342 mg, 1.35 mmol, 1.5 equiv) in MeCN (15 mL).

According to the general nucleobase epoxide ring opening procedure N^6-benzoyladenine (324 mg, 1.35 mmol, 1.5 equiv.) and NaH (60% in mineral oil, 56 mg, 1.35 mmol, 1.5 equiv.) were reacted in DMF (3.5 mL) and subsequently added to the pre-formed epoxide. Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the product **3.33b** (302 mg, 50 %) as a white solid. TLC: R_f = 0.24 (CH_2Cl_2: MeOH = 9:1, v/v) UV active, one bright orange spot with *p*-anisaldehyde stain. $[\alpha]_D$: −17.6 (*c* 0.529, DMSO). ^{1}H NMR (400.0 MHz, DMSO-d_6): δ 11.25 (N-H); 8.69 (s, 1H, H-2); 8.60 (s, 1H, H-8); 7.65–7.35 (m, 5H, H-Ar Bz); 7.30–6.80 (m, 13H, H-Ar Tr); 6.07 (d, 1H, $J_{1',2'}$ = 4.7 Hz, H-1′); 5.66 (bs, 1H, OH-2′); 5.30 (bs, 1H, OH-3′); 4.79 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.0 Hz, H-2′); 4.34 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 5.0 Hz, H-3′); 4.14 (q, 1H, $J_{4',3'} = J_{4',5a'} = J_{4',5b'}$ = 4.7 Hz, H-4′); 3.73 (s, 3H, H_3CO–); 3.72 (s, 3H, H_3CO–) 3.27–3.22 (m, 2H, H-5a′, H-5b′). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.2 (NCOPh); 158.5 (C-Ar DMT); 152.5 (C-4); 152.2 (CH-2) 150.9 (C-6); 145.3 (C-Ar DMT); 143.5 (CH-8) 136.0 (C-Ar DMT); 135.9 (C-Ar DMT); 133.8 (C-Ar Bz); 132.9 (CH-Ar Bz); 130.1 (CH-Ar DMT); 129.0 (CH-Ar Bz); 128.9 (CH-Ar Bz); 128.2 (CH-Ar DMT); 128.1 (CH-Ar DMT); 127.1 (CH-Ar Bz); 126.4 (C-5); 113.6 (CH-Ar DMT); 88.6 (CH-1′); 86.0 (C-DMT); 83.8 (CH-4′); 73.4 (CH-2′); 70.8 (CH-3′); 64.2 (CH_2-5′); 55.5 (2 x CH_3O). HR ESIMS: *m/z* [M+H$^+$] calcd for $C_{38}H_{36}O_7N_5$: 674.26092. Found: 674.26097. *m/z* [M+Na$^+$] calcd for $C_{38}H_3O_7N_5Na$: 696.24287. Found: 696.24295.

5.3 General procedure for the synthesis of non-nucleoside-based ribofuranosyl glycosides

5.3.1 Synthesis of 5-*O*-monoprotected β-ribofuranosyl glycosides

General procedure for the *in situ* formation of 1,2-anhydro-5-*O*-trityl-α-D-ribofuranose followed by nucleophilic ring opening (100 mg, 0.25 mmol scale).

An argon-purged, dried 25 mL round bottom or pear-shaped flask containing a stir bar was charged with 5-*O*-trityl-D-ribose (**1**) (100 mg, 0.254 mmol, 1.0 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added MeCN (4 mL, 0.06 M with respect to the sugar) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 110 µl, 0.406 mmol, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (96 mg, 0.381 mmol, 1.5 equiv.) at r.t. Over the course of ~15 minutes the reaction turned from a homogenous orange color to a heterogeneous white color. The precipitate formed was the reduced ADDP hydrazine product which indicated conversion to the 1,2-anhydrosugar. At this time, to the flask was added the nucleophile at room temperature as indicated for each compound. After addition of the nucleophile, the reaction was heated either stirred at room temperature or at 45 ^{o}C for 12 h as indicated. Reaction work-up and purification is reported individually for each compound.

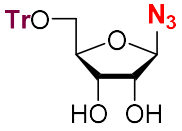
General procedure for the in situ formation of 1,2-anhydro-5-O-trityl-α-D-ribofuranose followed by nucleophilic ring opening (1.00 g, 2.5 mmol scale).

An argon-purged, dried 250 mL round bottom flask containing a stir bar was charged with 5-*O*-trityl-D-ribose (1.00 g, 2.54 mmol, 1.0 equiv.) and fitted with a rubber septum and argon-filled balloon. To this flask was added MeCN (40 mL, 0.06 M with respect to the sugar) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 1.1 ml, 4.06 mmol, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (965 mg, 3.81 mmol, 1.5 equiv.) at room temperature. Over the course of ~15 minutes the reaction turned from a homogenous orange color to a heterogeneous white color. The precipitate formed was the reduced ADDP hydrazine product which indicated conversion to the 1,2-anhydrosugar. At this time, to the flask was added the nucleophile at room temperature as indicated for each compound. After addition of the nucleophile, the reaction was stirred at either room

temperature or at 45 °C for 12 h as indicated. Reaction work-up and purification is reported individually for each compound.

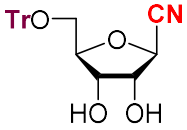
NOTE **Molar equivalents for the nucleophiles opening the epoxide are referenced against the sugar, which was always the limiting reagent in this study.**

5-*O*-Trityl-β-D-ribofuranosylazide (3.34a)



0.25 mmol scale: An argon-purged, dried 25 mL pear-shaped flask containing a stir bar was charged with 1,1,3,3-tetramethylguanidine (100 µL, 0.762, 3.0 equiv.) and MeOH (30 µL, 0.800 mmol, 3.15 equiv.) and fitted with a rubber septum and argon-filled balloon. MeCN (2 mL) was added and the stirring solution was cooled to 0 °C in an ice bath. To this cooled solution was added trimethylsilyl azide ($TMSN_3$) (100 µL, 0.762 mmol, 3.0 equiv.) dropwise via syringe. After stirring for 15 minutes at 0 °C, the reaction was transferred to the flask containing the epoxide using either a syringe or a cannula. After stirring at 45 °C for 12 h, the reaction was cooled to r.t. TLC analysis indicated that silylation of one or both of the hydroxyls occurred concomitantly during the epoxide ring opening, however, hydrolysis of the labile trimethylsilyl ether was easily effected by the addition of H_2O (0.5 mL) and further stirring of the reaction at r.t. for 6 h. The pH of the reaction was then decreased to ~7 using 1 M HCl(aq) if necessary. To the reaction mixture was added Dowex 50W×8 (Na^+ form, 2 g) and the flask stirred for 15 min to remove some of the guanidinium salt. The resin was filtered off and the filtrate transferred to a separatory funnel. CH_2Cl_2 (20 mL) and H_2O (10 mL) was added. The organic layer was collected, dried over Na_2SO_4, and concentrated *in vacuo* directly onto silica gel. Purification using column chromatography (silica gel, 4:1 to 1:1 EtOAc:Pet. ether) furnished **3.34a** (80 mg, 75 %) as a colorless oil. R_f = 0.54 (EtOAc: petroleum ether = 1:1, v/v) slightly UV active, one black with spot *p*-anisaldehyde stain. $[\alpha]_D$: −62.6 (*c* 0.436, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.56–7.16 (m, 15H, H-Ar); 5.33 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1); 4.25 (dd, 1H, $J_{3,4}$ = 7.2, $J_{3,2}$ = 4.6 Hz, H-3); 4.11 (ddd, $J_{4,3}$ = 7.2, $J_{4,5b}$ = 4.8, $J_{4,5a}$ = 2.7 Hz, 1H, H-4); 3.87 (dd, 1H, $J_{2,3}$ = 4.6, $J_{2,1}$ = 1.6 Hz, H-2), 3.35–3.32 (m, 1H, H-5a); 3.40 (dd, 1H, J_{gem} = 10.3, $J_{5b,4}$ = 4.9 Hz, H-5b). ^{13}C NMR (100 MHz, CD_3OD): δ 145.3 (C-Ar); 129.9 (C-Ar); 128.8 (C-Ar); 128.1 (C-Ar); 96.7 (CH-1); 87.8 (CPh_3); 84.1 (CH-4); 76.3 (CH-2); 71.9 (CH-3); 65.0 (CH_2-5). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{24}H_{23}O_4N_3Na$: 440.15808. Found: 440.15818.

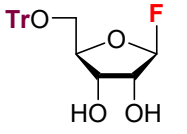
5-*O*-Trityl-β-D-ribofuranosylcyanide (3.34b)



0.25 mmol scale: DMF (1 mL) followed by tetraethylammonium cyanide (120 mg, 0.762 mmol, 3.0 equiv) were added directly to the stirring epoxide at r.t. After stirring at 45 °C for 12 h, the reaction was cooled to r.t. and the reaction quenched by decreasing the pH to ~7 using 1 M HCl(aq). To the reaction mixture was added Dowex 50W×8 (Na^+ form, 2 g) and the flask stirred for 15 min to remove some of the tetraethylammonium salt. The resin was filtered off and the filtrate transferred to a 100 mL separatory funnel. CH_2Cl_2 (20 mL) and H_2O (10 mL) was added. The organic layer was collected, dried over Na_2SO_4, and concentrated *in vacuo* directly onto silica gel. Purification using column chromatography (silica gel, 4:1 to 1:1 EtOAc:Pet. ether) furnished **3.34b** (76 mg, 75 %) as a colorless oil.

2.5 mmol scale: DMF (10 mL) followed by tetraethylammonium cyanide (1.20 g, 7.62 mmol, 3.0 equiv) were added directly to the stirring epoxide at r.t. After stirring at 45 °C for 12 h, the reaction was cooled to r.t. and the reaction quenched by decreasing the pH to ~7 using 1 M HCl(aq). To the reaction mixture was added Dowex 50W×8 (Na^+ form, 20 g) and the flask stirred for 15 min to remove some of the tetraethylammonium salt. The resin was filtered off and the filtrate transferred to a 500 mL separatory funnel. CH_2Cl_2 (200 mL) and H_2O (100 mL) was added. The organic layer was collected, dried over Na_2SO_4, and concentrated *in vacuo* directly onto silica gel. Purification using column chromatography (silica gel, 4:1 to 1:1 EtOAc:Pet. ether) furnished **3.34b** (670 mg, 66 %) as a colorless oil. R_f = 0.65 (EtOAc: petroleum ether = 1:1, v/v) very slightly UV active, one brown spot with *p*-anisaldehyde stain. $[\alpha]_D$: +10.0 (*c* 0.220, $CHCl_3$). ^1H NMR (400 MHz, CD_3OD): δ 7.50–7.20 (m, 15H, H-Ar); 4.59 (d, 1H, $J_{1,2}$ = 5.6 Hz, H-1); 4.54 (dd, 1H, $J_{2,1}$ = 5.7, $J_{2,3}$ = 4.6 Hz, H-2); 4.17 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 4.5 Hz, H-3); 4.04 (td, 1H, $J_{4,3}$ = $J_{4,5b}$ =4.1, $J_{4,5a}$ = 3.0 Hz, H-4), 3.37 (dd, 1H, J_{gem} = 10.6, $J_{5a,4}$ = 3.0 Hz, H-5a), 3.25 (dd, 1H, J_{gem} = 10.6, $J_{5b,4}$ = 3.9 Hz, H-5b). ^{13}C NMR (100 MHz, CD_3OD): δ 145.1 (C-Ar); 129.9 (C-Ar); 128.8 (C-Ar); 128.2 (C-Ar); 119.5 (C≡N); 88.1 (CPh_3); 86.0 (CH-4); 76.8 (CH-2); 73.1 (CH-3); 71.7 (CH-1); 64.7 (CH_2-5). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{25}H_{23}O_4NNa$: 424.15193. Found: 424.15189.

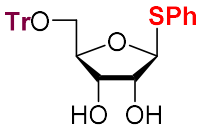
5-*O*-Trityl-β-D-ribofuranosylfluoride (3.34c)



0.25 mmol scale: Tetrabutylammonium fluoride (TBAF) (1 M in THF, 1.0 mL, 1.00 mmol, 4.0 equiv.) was added directly to the stirring epoxide at r.t. via syringe. After stirring at 45 °C for 12 h, the reaction was cooled to r.t. and the reaction quenched by decreasing the pH to ~7 using 1 M HCl(aq). To reaction mixture was

added Dowex 50W×8 (Na^+ form, 2 g) and the flask stirred for 15 min to remove some of the tetrabutylammonium salt. The resin was filtered off and the filtrate transferred to a separatory funnel. CH_2Cl_2 (20 mL) and H_2O (10 mL) was added. The organic layer was collected, dried over Na_2SO_4, and concentrated *in vacuo* directly onto silica gel. Purification using column chromatography (silica gel, 4:1 to 1:1 EtOAc:Pet. ether) furnished **3.34c** (70 mg, 70 %) as a white, waxy solid that was stored, but stable indefinitely, at −20 °C. R_f = 0.51 (EtOAc: petroleum ether = 1:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: +29.3 (*c* 0.259, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.52–7.23 (m, 15H, H-Ar); 5.63 (d, 1H, $J_{1,F}$ = 64.1 Hz, H-1); 4.25 (dddd, 1H, $J_{4,F}$ = 48.8, $J_{4,3}$ = 8.2, $J_{4,5b}$ = 5.0, $J_{4,5a}$ = 2.6 Hz, H-4); 4.18–4.07 (m, 2H, H-2, H-3); 3.37 (dd, 1H, J_{gem} = 10.3, $J_{5a,4}$ = 3.0 Hz, H-5a); 3.14 (dd, 1H, J_{gem} = 10.3, $J_{5b,4}$ = 5.3 Hz, H-5b). ^{13}C NMR (100 MHz, CD_3OD): δ 145.4 (C-Ar); 129.9 (C-Ar); 128.8 (C-Ar); 128.0 (C-Ar); 115.9 (d, $J_{1,F}$ = 222.1 Hz, C-1); 87.8 (CPh_3) 84.9 (C-4); 75.6 (d, $J_{2,F}$ = 31.7 Hz, C-2); 71.3 (d, $J_{3,F}$ = 2.5 Hz, C-3); 65.3 (C-5). ^{19}F NMR (376 Hz, CD_3OD): δ -117.13 – -117.33 (m, CHF). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{24}H_{23}O_4FNa$: 417.14726. Found: 417.14761.

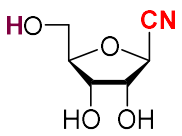
Phenyl 1-thio-5-*O*-trityl-β-D-ribofuranoside (3.34d)



0.25 mmol scale: DMF (1 mL) followed by NaSPh (101 mg, 0.762 mmol, 3.0 equiv) was added directly to the flask containing the epoxide with stirring at r.t. After stirring at 45 °C for 12 h, the reaction was cooled to r.t. and the reaction quenched by decreasing the pH to ~7 using 1 M HCl(aq). The reaction mixture was concentrated *in vacuo* directly onto silica gel. Purification using column chromatography (silica gel, 4:1 to 1:1 EtOAc:Pet. ether) furnished **3.34d** (74 mg, 61 %) as a colorless oil. R_f = 0.52 (EtOAc: petroleum ether 1:1, v/v) UV active, one black spot with *p*-anisaldehyde stain. $[\alpha]_D$: −61.1 (*c* 0.480, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57–7.20 (m, 20H, H-Ar); 5.34 (d, 1H, $J_{1,2}$ = 4.8 Hz, H-1); 4.22 (q, 1H, $J_{2,1}$ = $J_{2,3}$ = 4.9 Hz); 4.16 (q, 1H, $J_{3,2}$ = 5.3, $J_{3,4}$ = 4.8 Hz, H-3), 4.12 (1H, q, $J_{4,3}$ = $J_{4,5}$ = 4.4 Hz, H-4), 3.34 (dd, 1H, J_{gem} = 10.0, $J_{5a,4}$ = 4.6 Hz, H-5a), 3.25 (dd, 1H, J_{gem} = 10.1, $J_{5b,4}$ = 4.3 Hz, H-5b); 2.80 (d, $J_{OH,2}$ = 4.7 Hz, OH-2); 2.55 (d, $J_{OH,3}$ = 4.2 Hz, OH-3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.8 (C-Ar); 133.7 (C-Ar); 132.0 (C-Ar); 129.0 (C-Ar); 128.8 (C-Ar); 127.9 (C-Ar); 127.6 (C-Ar); 127.2 (C-Ar); 90.3 (C-1); 87.0 (CPh_3); 83.6 (C-4); 75.4 (C-2); 72.4 (C-3); 64.2 (C-5). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{30}H_{28}O_4SNa$: 507.16005. Found: 507.16009.

5.3.2 Synthesis of deprotected β-ribofuranosylcyanide.

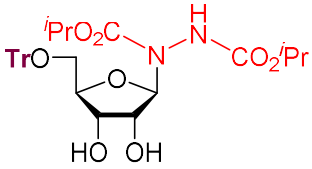
β-D-Ribofuranosylcyanide (6)



DMF (10 mL) followed by tetraethylammonium cyanide (1.20 g, 7.62 mmol, 3.0 equiv) were added directly to the stirring epoxide at r.t. After stirring at 45 °C for 12 h, the trityl group was cleaved in one-pot as according to general procedure for the preparation of nucleosides (Procedure in Section 5.2.5). Purification of the product using normal phase column chromatography (silica gel, 25:1 to 9:1 CH_2Cl_2:MeOH) furnished the product **6** (260 mg, 64 %) as a colourless oil. R_f = 0.32 (CH_2Cl_2: MeOH = 9:1, v/v) one white spot with $KMnO_4$ stain. $[\alpha]_D$: –3.6 (*c* 0.193, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.50 (d, 1H, $J_{1,2}$ = 5.4 Hz, H-1); 4.35 (t, 1H, $J_{2,1} = J_{2,3}$ = 5.2 Hz, H-2); 4.14 (t, 1H $J_{3,2} = J_{3,4}$ = 4.9 Hz, H-3); 3.93 (td, 1H, $J_{4,3} = J_{4,5b}$ = 4.5, $J_{4,5a}$ = 3.4 Hz, H-4), 3.70 (dd, 1H, J_{gem} = 12.3, $J_{5a,4}$ = 3.4 Hz, H-5a), 3.60 (dd, 1H, J_{gem} = 11.3, $J_{5b,4}$ = 4.4 Hz, H-5b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 119.2 (C≡N); 87.3 (CH-4); 76.7 (CH-2); 72.6 (CH-3); 71.7 (CH-1); 62.8 (CH_2-5). HR ESIMS: *m/z* [M+Na^+] calcd for $C_6H_9O_4NNa$: 182.04238. Found: 182.04237. *m/z* [M–H^+] calcd for $C_6H_8O_4N$: 158.04588. Found: 158.04603.

5.4 Optimization and confirmation of the *in situ* formation of the anhydroses.

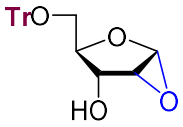
5.4.1 Isolation of diisopropyl 1-(5′-*O*-trityl-β-D-ribofuranosyl)hydrazine-1,2-dicarboxylate (3.15)



These conditions are similar to those described above in Section 5.2.3, just in the absence of nucleobase: An argon-purged, dried 25 mL pear-shaped flask containing a stir bar was charged with MeCN (4 mL) and fitted with a rubber septum and argon-filled balloon. To this flask was added diisopropyl azodicarboxylate (DIAD) (105 µL, 0.533 mmol, 2.1 equiv.) followed by P(*n*Bu)$_3$ (93.5 %, 135 µl, 0.508 mmol, 2.0 equiv.) both via syringe at r.t. 5-*O*-trityl-D-ribose (**3.13**) (100 mg, 0.254 mmol, 1.0 equiv.) was subsequently added and the reaction stirred at r.t. for 12 h. After 12 h, TLC analysis (1:1 EtOAc: petroleum ether v/v) indicated two spots. The top spot was found to be unwanted side-product **3.15** (R_f = 0.42) and the lower spot starting material **3.13**. The reaction mixture was concentrated directly onto silica gel and isolation of the two spots by column chromatography (silica gel, 4:1 to 1:1 EtOAc:Pet. ether) provided side-product **3.15** contaminated with O=P(*n*Bu)$_3$ (55 mg, 38 % contaminated,

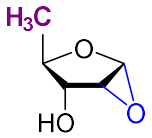
actual yield between 20–30 %) as a colorless oil and **3.13** (60 mg, 60 %). Unfortunately, all attempts to isolate anhydrose **3.14** failed. R_f = 0.42 (EtOAc: petroleum ether = 1:1, v/v) slightly UV active, one black spot using *p*-anisaldehyde stain. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50–7.22 (m, 15H, H-Ar); 5.98–5.68 (bs, 1H, HN); 5.05–4.85 (m, 3H, 2 x ($CH(CH_3)_2$), H-1′); 4.22–4.16 (m, 2H, H-2′,H-3′); 4.06 (d, 1H, $J_{4',3'} = {}_{4',5a'} = {}_{4',5b'}$ = 4.4 Hz, H-4′); 3.37 (dd, 1H, J_{gem} = 10.4, $J_{5'a,4'}$ = 3.8, H-5′a); 3.21 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.1, H-5′b); 1.31–1.20 (m, 9H, 2 x ($CH(CH_3)_2$)).^{13}C NMR (100 MHz, $CDCl_3$): δ 143.8 (C-Ar); 128.7 (C-Ar); 127.9 (C-Ar); 127.1 (C-Ar); 111.8 (-$CO_2{}^i$Pr); 111.7 (-$CO_2{}^i$Pr); 95.1 (CH-1′); 86.9 (CPh_3); 82.3 (CH-4′); 71.3, 71.1 (CH-2′,CH-3′); 70.9, 70.8 (2 x ($CH(CH_3)_2$); 63.9 (CH_2-5′); 31.5 (2 x ($CH(CH_3)_2$)). HR ESIMS: *m/z* [M+Na^+] calcd for $C_{32}H_{38}O_8N_2Na$: 601.25204. Found: 601.25194.

5.4.2 Confirmation of 1,2-anhydro-5-*O*-trityl-α-D-ribofuranose (3.14) formed in situ.



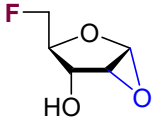
An argon-purged, dried 10 mL pear-shaped flask containing a stir bar was charged with 5-*O*-trityl-D-ribose **3.13** (25 mg, 0.064 mmol, 1.0 equiv.) and fitted with a rubber septum and an argon-filled balloon. To this flask was added CD_3CN (1.5 mL) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 27 µl, 0.102 mmol, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (24 mg, 0.096 mmol, 1.5 equiv.) at r.t. After 15 minutes, 250 µL was removed via syringe and placed in a dried NMR tube. The reaction mixture in the NMR tube was further diluted with 250 µL CD_3CN which mostly dissolved the ADDP-based hydrazine byproduct. The NMR tube was quickly capped and sealed with Parafilm. If the NMR tube remains capped and sealed with Parafilm, the epoxide shows no observable signs of decomposition after one month. ^{1}H NMR (400 MHz, CD_3CN): δ 7.50–7.24 (m, 15H, H-Ar); 5.26 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1); 4.19 (d, 1H, $J_{3,4}$ = 6.7 Hz, H-3); 3.65 (td, 1H, $J_{4,3} = J_{4,5b}$ = 6.3, $J_{4,5a}$ = 2.6 Hz, H-4); 3.58 (s, 1H, H-2), 3.22 (dd, J_{gem} = 10.6, $J_{5a,4}$ = 2.6 Hz, H-5a), 3.08 (dd, 1H, J_{gem} = 10.5, $J_{5b,4}$ = 5.8 Hz, H-5b). ^{13}C NMR (100 MHz, CD_3CN): δ 144.9 (C-Ar); 129.4 (C-Ar); 128.8 (C-Ar); 128.0 (C-Ar); 87.2 (CPh_3) 81.4 (CH-1); 81.0 (CH-4); 72.8 (CH-3); 64.0 (CH_2-5); 58.8 (CH-2).

5.4.3 Confirmation of 1,2-anhydro-5-*O*-deoxy-α-D-ribofuranose (3.28) formed in situ



An argon-purged, dried 25 mL pear-shaped flask containing a stir bar was charged with 5-*O*-deoxy-D-ribose (**3.20**) (15.8 mg, 0.118 mmol, 1.0 equiv.) and fitted with a rubber septum and an argon-filled balloon. To this flask was added CD_3CN (2 mL) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 50 µl, 0.189 mmol, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (45 mg, 0.189 mmol, 1.5 equiv.) at room temperature. After 15 minutes, 250 µL was removed via syringe and placed in a dried NMR tube. The reaction mixture in the NMR tube was further diluted with 250 µL CD_3CN which mostly dissolved the ADDP-based hydrazine byproduct. The NMR tube was quickly capped and sealed with Parafilm. ^{1}H NMR (400 MHz, CD_3CN): δ 5.10 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1); 3.82 (dd, 1H, $J_{3,4}$ = 6.6, $J_{3,2}$ = 1.8 Hz, H-3); 3.54 (q, 1H, $J_{4,5}$ = $J_{4,3}$ = 6.5 Hz, H-4); 3.49 (d, 1H, $J_{2,1}$ = $J_{2,3}$ = 1.8 Hz, H-2), 1.19 (d, 3H, $J_{5,4}$ = 6.5 Hz, H-5). ^{13}C NMR (100 MHz, CD_3CN): δ 81.1 (CH-1, absent in in the APT spectrum, assigned by HSQC analysis); 78.0 (CH-3); 77.7 (CH-4); 58.9 (CH-2); 18.1 (CH_3-5).

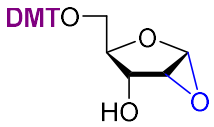
5.4.4 Confirmation of 1,2-anhydro-5-*O*-deoxy-5-fluoro-α-D-ribofuranose (3.30) formed in situ



An argon-purged, dried 25 mL pear-shaped flask containing a stir bar was charged with 5-*O*-deoxy-5-fluoro-D-ribose (**3.21**) (20.4 mg, 0.134 mmol, 1.0 equiv.) and fitted with a rubber septum and an argon-filled balloon. To this flask was added CD_3CN (2 mL) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 60 µl, 0.215 mmol, 1.6 equiv.) via syringe followed by 1,1′-(azodicarbonyl)dipiperidine (ADDP) (45 mg, 0.201 mmol, 1.5 equiv.) at room temperature. After 15 minutes, 250 µL was removed via syringe and placed in a dried NMR tube. The reaction mixture in the NMR tube was further diluted with 250 µL CD_3CN which mostly dissolved the ADDP-based hydrazine byproduct. The NMR tube was quickly capped and sealed with Parafilm. ^{1}H NMR (500.0 MHz, CD_3CN): δ 5.21 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1); 4.54 (ddd, 1H, $J_{H,F}$ = 48.0, J_{gem} = 10.9, $J_{5a,4}$ = 2.0 Hz, H-5a); 4.44 (ddd, 1H, $J_{H,F}$ = 48.0, J_{gem} = 10.9, $J_{5b,4}$ = 4.4 Hz, H-5b); 4.19 (bm, 1H, H-3); 3.63 (dddd, 1H, $J_{H,F}$ = 27.4, $J_{4,3}$ = 7.1, $J_{4,5}$ = 4.4, 2.0, H-4); 3.56 (m, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3CN): δ 82.5 (d, $J_{C,F}$ = 170.2 Hz, CH_2-5);

81.6 (CH-1); 80.6 (d, $J_{C,F}$ = 17.7 Hz, CH-4); 71.3 (d, $J_{C,F}$ = 7.6, CH-3); 58.5 (d, $J_{C,F}$ = 1.3, CH-2).

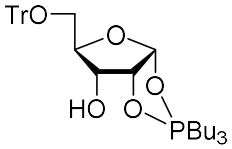
5.4.5 Confirmation of 1,2-anhydro-5-*O*-(4,4′-dimethoxytrityl)-α-D-ribofuranose (3.32) formed *in situ*



An argon-purged, dried 25 mL pear-shaped flask containing a stir bar was charged with 5-*O*-dimethoxytrityl-D-ribose (**3.22**) (25.0 mg, 0.0552 mmol, 1.0 equiv.) and fitted with a rubber septum and an argon-filled balloon. To this flask was added CD_3CN (1.5 mL) via syringe. To this stirring solution was added P(*n*Bu)$_3$ (93.5 %, 24 µl, 0.0885 mmol, 1.6 equiv.) via syringe followed by ADDP (21 mg, 0.0828 mmol, 1.5 equiv.) at room temperature. After 15 minutes, 500 µL was removed via syringe and placed in a dried NMR tube. The NMR tube was quickly capped and sealed with Parafilm. ^{1}H NMR (400 MHz, CD_3CN): δ 7.51–6.87 (m, 13H, H-Ar); 5.22 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1); 4.14 (dd, 1H, $J_{3,4}$ = 6.8, $J_{3,2}$ = 1.9 Hz, H-3); ~3.76 (s, 6H, H_3CO); 3.64 (td, 1H, $J_{4,3}$ = $J_{4,5b}$ = 6.4, $J_{4,5a}$ = 3.0 Hz, H-4); 3.54 (d, 1H, $J_{2,1}$ = $J_{2,3}$ = 2.0 Hz, H-2); 3.18 (dd, J_{gem} = 10.4, $J_{5a,4}$ = 2.7 Hz, H-5a), 3.08 (dd, 1H, J_{gem} = 10.5, $J_{5b,4}$ = 5.8 Hz, H-5b). ^{13}C NMR (100 MHz, CD_3CN): δ Signals in the aromatic region were obscured so omitted for clarity. 86.8 (C-DMT); 82.2 (CH-1, absent in the APT spectrum, assigned by HSQC); 81.5 (CH-4); 72.9 (CH-3); 63.9 (CH_2-5); 58.9 (CH-2); 55.9 (CH_3O).

5.5 Mechanism-related experiments.

5.5.1 Confirmation of the formation of 1,3-dioxaphospholane intermediate



To a standard 5 mm NMR tube was added CD_3CN (500 µL) and P(*n*Bu)$_3$ (93.5 %, 8 µL, 0.031 mmol, 1.6 equiv.) and the tube sealed and cooled to –40 °C in a 500 MHz NMR machine. After the ^{31}P NMR spectrum was recorded (Fig. S1a, bottom) the NMR tube was removed and ADDP (7.5 mg, 0.028 mmol, 1.5 equiv.) was added to the top of the tube as fast as possible and the tube vigorously shaken for 2–3 seconds before quickly adding it back into the NMR machine. Once again the ^{31}P NMR spectrum was recorded (Fig. S1a, middle) and the expected phosphonium betaine clearly present. The NMR tube was again removed and to it **3.13** (7.5 mg, 0.019 mmol, 1.0 equiv.) was added as quickly as possible and the tube shaken vigorously for 2–3 seconds the NMR tube was once again very quickly cooled to –40 °C and to confirm the presence of the *cis*-1,3-

dioxaphospholane, 2D COSY (Figure 5.1) and 2D ROESY spectra (Figure 5.2) was recorded which proved the existence of and stereochemical position of H-1 on the ribosyl ring.

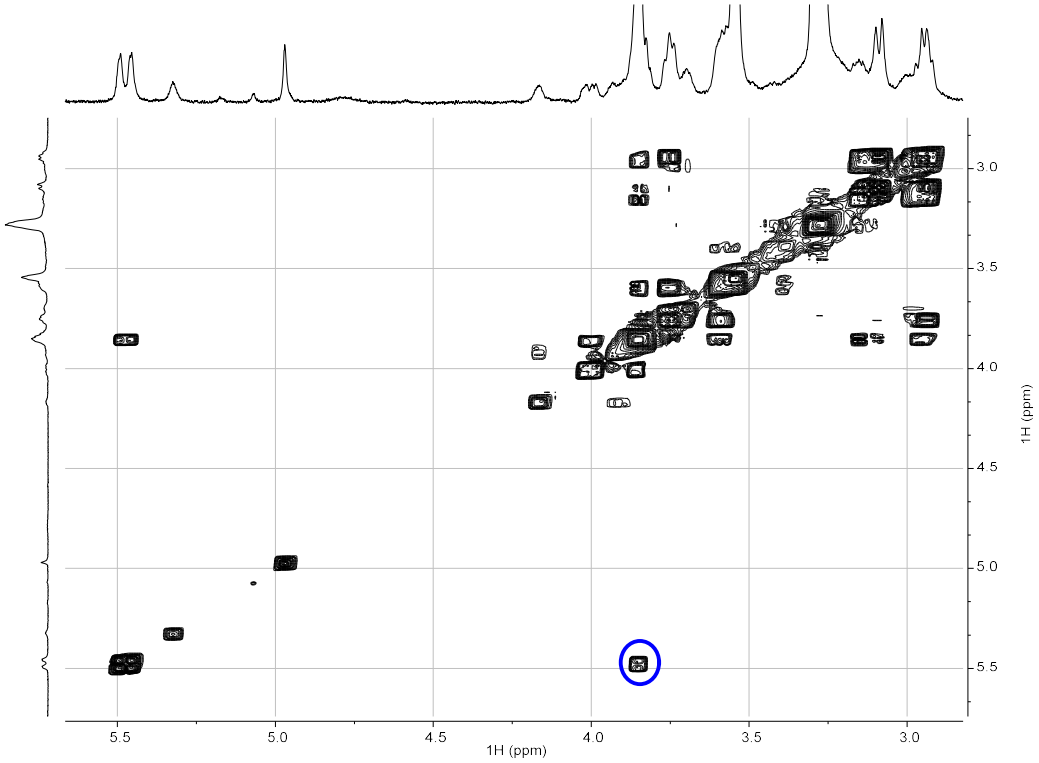


Figure 5.1: COSY spectrum of the *in situ* presence of compound **3.18** confirming it is indeed H-1 that is coupling to the P atom of the phospholane.

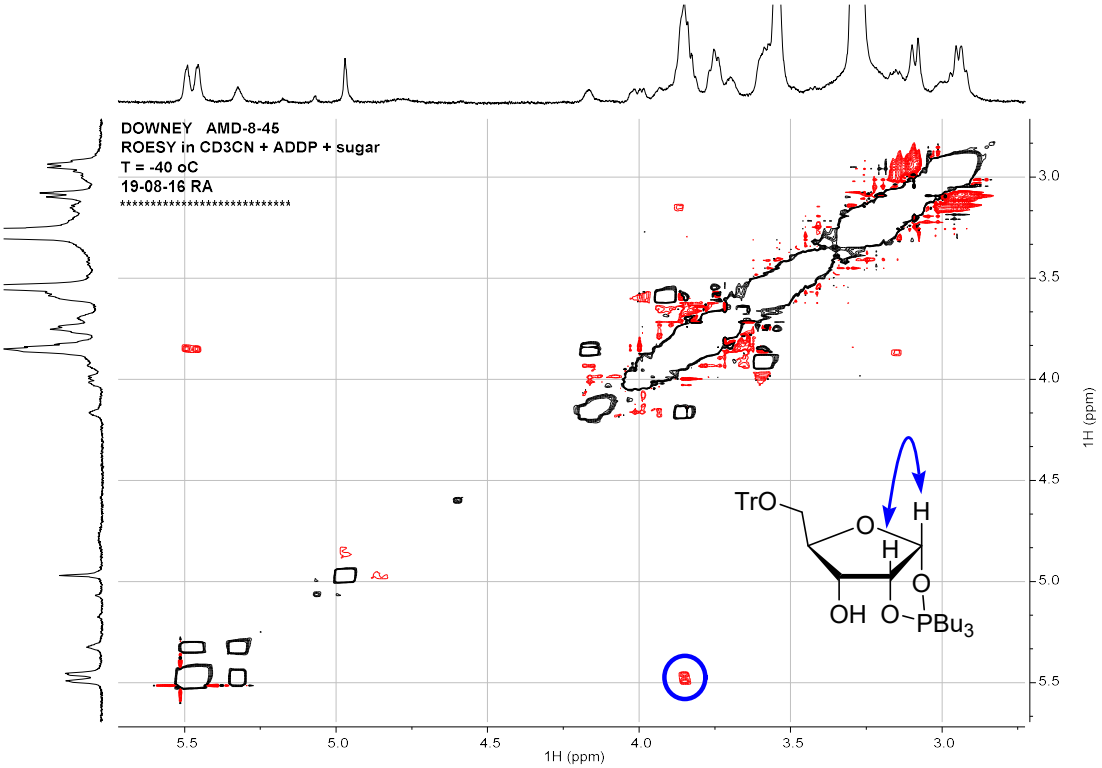
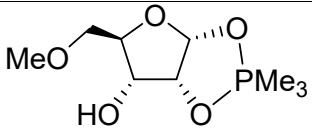


Figure 5.2: ROESY spectrum confirming the *cis* conformation of compound **3.18**.

5.5.2 Theoretical calculations

I am indebted to Prof. J. Roithová (Charles University in Prague) for her assistance in carrying out these calculations. The calculations were performed with B3LYP functional[193-195] including the D3BJ empirical correction[196] for dispersion interactions and the 6-311+G** basis set as implemented in Gaussian 09.[197] The solvent effect of MeCN was modelled using the SMD method.[198] All minima and transition structures were optimized and verified by computation of the Hessian matrices. The transition structures were also characterized by intrinsic reaction coordinate calculations.[199] For all minima and transition structures a detailed conformational analysis was performed. The energies refer to Gibbs free energies in MeCN solution at 298 K; the change in the number of moles (n) in the dissociation reaction was accounted for by a correction of (1.9 Δn) kcal mol^{-1}. The favoured conformation of the methoxymethyl group in the transition structures and the products is with the oxygen atom above the five-membered ring. For the phospholane, a more favoured conformation with the oxygen of the methoxymethyl group in the other gauche position with respect to the ring oxygen atom exists. It is favoured by 0.4 kcal mol^{-1}. The energy difference is very small, we, therefore, considered the same orientation of the methoxymethyl group (above the five-membered ring) for all structures for discussion and for the figure in the main document. The corresponding geometries are given in Table 5.1. The energies in the main document refer to Gibbs free energies in acetonitrile solution at 298 K; the change in the number of moles (n) in the dissociation reaction was accounted for by a correction of (1.9 Δn) kcal mol^{-1}.

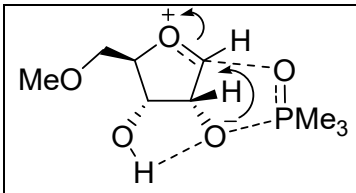
Table 5.1: Geometries and energies of the structures discussed in the main document.

Structure on the potential energy surface	# B3LYP/6-311+g** opt/opt=(TS,NoEigenTest,RCFC) *Note: either opt or opt=TS was used* freq EmpiricalDispersion=GD3BJ scrf=(SMD,solvent=acetonitrile)
	Full mass-weighted force constant matrix: Low frequencies --- -19.2731 -15.1104 -0.0021 -0.0021 0.0009 10.7325 Low frequencies --- 39.8213 45.4148 61.2146 Zero-point correction= 0.284129 (Hartree/Particle) Thermal correction to Energy= 0.301496

Thermal correction to Enthalpy= 0.302440
Thermal correction to Gibbs Free Energy= 0.239377
Sum of electronic and zero-point Energies= -1071.896024
Sum of electronic and thermal Energies= -1071.878658
Sum of electronic and thermal Enthalpies= -1071.877713
Sum of electronic and thermal Free Energies= -1071.940776

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	0.037995	-1.051627	-1.055860
2	6	0	0.093752	0.470125	-1.290254
3	6	0	-1.189343	1.007601	-0.658777
4	6	0	-1.454221	-0.013939	0.458815
5	8	0	-1.027875	-1.263085	-0.092804
6	8	0	1.204337	0.960172	-0.545661
7	15	0	2.308017	-0.095591	0.189012
8	6	0	2.002567	-0.834822	1.834181
9	8	0	-1.095993	2.348322	-0.208050
10	6	0	-2.887987	-0.102722	0.930656
11	8	0	-3.767682	-0.279355	-0.168066
12	6	0	-5.120730	-0.392085	0.242426
13	8	0	1.262537	-1.440918	-0.604883
14	6	0	3.732834	-0.839510	-0.682617
15	6	0	3.194897	1.430906	0.800055
16	1	0	-0.243070	-1.621808	-1.944529
17	1	0	0.222680	0.741477	-2.337753
18	1	0	-1.999147	0.966177	-1.387349
19	1	0	-0.842156	0.248515	1.334119
20	1	0	-2.987938	-0.938848	1.636658
21	1	0	-3.135250	0.822316	1.471221
22	1	0	-0.220222	2.442951	0.194636
23	1	0	-5.724356	-0.525375	-0.656489
24	1	0	-5.266201	-1.256775	0.903842
25	1	0	-5.455397	0.512390	0.768493
26	1	0	4.064456	1.135163	1.391909
27	1	0	3.534835	2.040435	-0.040608
28	1	0	2.536270	2.037902	1.425972
29	1	0	4.663738	-0.555452	-0.190826
30	1	0	3.635175	-1.922047	-0.727010
31	1	0	3.738753	-0.446314	-1.703179
32	1	0	1.114534	-0.363135	2.262646
33	1	0	1.832194	-1.905984	1.758570
34	1	0	2.849676	-0.622388	2.488463



Concerted process

Low frequencies --- -104.2190 -32.7421 -12.6988 -8.7612 -0.0005 0.0012

Low frequencies --- 0.0020 25.9173 51.5603

****** 1 imaginary frequencies (negative Signs) ******

Zero-point correction= 0.278840 (Hartree/Particle)

Thermal correction to Energy= 0.297236

Thermal correction to Enthalpy= 0.298180

Thermal correction to Gibbs Free Energy= 0.231259

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Sum of electronic and thermal Energies= -1071.846903

Sum of electronic and thermal Enthalpies= -1071.845958

Sum of electronic and thermal Free Energies= -1071.912879

Standard orientation:

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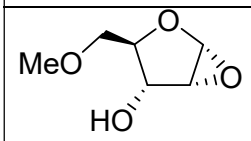
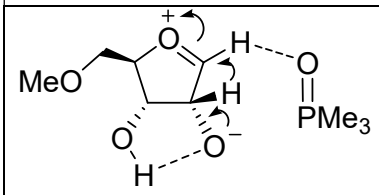
4	6	0	1.715423	1.373839	0.013050
5	6	0	2.144163	0.385487	-1.061479
6	8	0	-0.286339	1.114491	1.389898
7	8	0	0.831775	2.348491	-0.489176
8	6	0	3.535664	-0.181288	-0.932948
9	8	0	3.710358	-0.647863	0.391536
10	6	0	4.982116	-1.251201	0.598528
11	8	0	-1.803955	-0.966450	-0.853011
12	15	0	-3.006548	-0.373548	-0.130126
13	6	0	-2.964776	-0.652301	1.660477
14	6	0	-3.158876	1.419625	-0.350450
15	6	0	-4.574934	-1.080278	-0.718001
16	1	0	-0.020729	-1.558305	0.511939
17	1	0	1.539077	0.215342	1.897113
18	1	0	2.590649	1.830890	0.479655
19	1	0	1.956776	0.735123	-2.073953
20	1	0	3.681604	-0.994497	-1.655443
21	1	0	4.247837	0.620036	-1.172554
22	1	0	0.056835	2.217566	0.129440
23	1	0	5.009880	-1.594004	1.633072
24	1	0	5.124657	-2.108292	-0.071270
25	1	0	5.792982	-0.530782	0.433953
26	1	0	-3.861836	-0.247755	2.135550
27	1	0	-2.905285	-1.725524	1.857661
28	1	0	-2.072825	-0.147646	2.040524
29	1	0	-5.419877	-0.645700	-0.178614
30	1	0	-4.686571	-0.877473	-1.785733
31	1	0	-4.564236	-2.161163	-0.558884
32	1	0	-2.264611	1.881681	0.070615

	33 1 0 -3.226971 1.650224 -1.416125 34 1 0 -4.047710 1.797651 0.160144 ---
	Full mass-weighted force constant matrix: Low frequencies --- -16.7053 -0.0008 -0.0006 0.0006 9.5876 24.6796 Low frequencies --- 63.6731 78.5784 107.3592 Zero-point correction= 0.163006 (Hartree/Particle) Thermal correction to Energy= 0.172788 Thermal correction to Enthalpy= 0.173732 Thermal correction to Gibbs Free Energy= 0.127452 Sum of electronic and zero-point Energies= -535.519666 Sum of electronic and thermal Energies= -535.509884 Sum of electronic and thermal Enthalpies= -535.508940 Sum of electronic and thermal Free Energies= -535.555220 Standard orientation: --- Center Atomic Atomic Coordinates (Angstroms) Number Number Type X Y Z --- 1 8 0 2.598332 -0.510975 0.168714 2 6 0 1.502008 -1.323806 -0.281272 3 8 0 0.396997 -1.333194 0.544807 4 6 0 -0.017582 0.042543 0.753803 5 6 0 0.646677 0.888330 -0.372391 6 6 0 1.721237 -0.028583 -0.902982 7 8 0 1.110119 2.148985 0.093111 8 6 0 -1.524615 0.120101 0.807952 9 8 0 -2.074622 -0.193748 -0.459906 10 6 0 -3.492163 -0.111946 -0.466604 11 1 0 2.189335 0.134247 -1.866166 12 1 0 -0.067190 1.101751 -1.168329 13 1 0 1.752535 -2.296182 -0.684924 14 1 0 0.375037 0.377943 1.718458 15 1 0 -1.902371 -0.571340 1.573234 16 1 0 -1.805378 1.140233 1.108026 17 1 0 -3.826368 -0.356389 -1.475747 18 1 0 -3.935176 -0.824902 0.241285 19 1 0 -3.833799 0.899328 -0.208904 20 1 0 1.793392 1.986933 0.758222 ---
	Full mass-weighted force constant matrix: Low frequencies --- -700.5643 -23.0603 -14.9983 -11.2477 -0.0008 0.0013 Low frequencies --- 0.0014 20.2558 40.5315 ****** 1 imaginary frequencies (negative Signs) ****** Zero-point correction= 0.276121 (Hartree/Particle) Thermal correction to Energy= 0.295124 Thermal correction to Enthalpy= 0.296068

Thermal correction to Gibbs Free Energy= 0.227146

Sum of electronic and zero-point Energies= -1071.860252

Sum of electronic and thermal Energies= -1071.841249

Sum of electronic and thermal Enthalpies= -1071.840305

Sum of electronic and thermal Free Energies= -1071.909227

Standard orientation:

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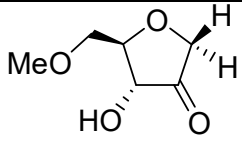
3	6	0	-0.541029	-0.195053	-1.134273
4	6	0	-0.769662	1.162587	-0.692889
5	6	0	-1.464990	0.998083	0.687001
6	8	0	-0.149666	2.243233	-0.983474
7	8	0	-0.544650	1.418181	1.689694
8	6	0	-3.202570	-0.885689	0.749270
9	8	0	-3.797379	-0.280575	-0.385079
10	6	0	-5.174588	-0.605967	-0.519931
11	8	0	2.073678	-1.063665	-1.173367
12	15	0	2.928484	-0.399561	-0.105084
13	6	0	4.629247	-1.036912	-0.070724
14	6	0	3.067200	1.394032	-0.337013
15	6	0	2.282221	-0.637517	1.575273
16	1	0	-0.056908	-0.501071	-2.050096
17	1	0	-1.665823	0.814829	-1.515596
18	1	0	-2.384063	1.584515	0.742709
19	1	0	-1.269441	-0.938259	1.673486
20	1	0	-3.296377	-1.978167	0.700785
21	1	0	-3.681391	-0.542106	1.676818
22	1	0	-0.087755	2.188650	1.314276
23	1	0	-5.542444	-0.086989	-1.405507
24	1	0	-5.315338	-1.686392	-0.650279
25	1	0	-5.746219	-0.275642	0.356667
26	1	0	3.605720	1.853224	0.495447
27	1	0	3.605768	1.592677	-1.267092
28	1	0	2.060294	1.817036	-0.414855
29	1	0	5.218540	-0.523127	0.692405
30	1	0	4.609632	-2.107009	0.148981
31	1	0	5.091659	-0.882326	-1.048343
32	1	0	1.332988	-0.108910	1.681944
33	1	0	2.127417	-1.704577	1.751558
34	1	0	2.990079	-0.250147	2.312216



Full mass-weighted force constant matrix:

Low frequencies --- -20.3148 -6.6338 -0.0007 -0.0003 -0.0002 25.2864

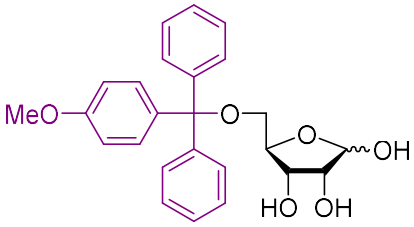
Low frequencies --- 60.5397 77.7562 107.7424

	Zero-point correction= 0.161457 (Hartree/Particle) Thermal correction to Energy= 0.172033 Thermal correction to Enthalpy= 0.172977 Thermal correction to Gibbs Free Energy= 0.124869 Sum of electronic and zero-point Energies= -535.556856 Sum of electronic and thermal Energies= -535.546280 Sum of electronic and thermal Enthalpies= -535.545336 Sum of electronic and thermal Free Energies= -535.593444 Standard orientation: --- Center Atomic Atomic Coordinates (Angstroms) Number Number Type X Y Z --- 1 6 0 1.941087 -0.067082 -0.350802 2 6 0 0.661471 0.720296 -0.107736 3 6 0 -0.128979 -0.247811 0.775415 4 8 0 0.229518 -1.543265 0.252204 5 6 0 1.618773 -1.519661 -0.075268 6 8 0 0.869163 1.975243 0.501116 7 6 0 -1.629341 -0.083506 0.766649 8 8 0 -2.111272 -0.004884 -0.563035 9 6 0 -3.521082 0.155627 -0.614313 10 8 0 3.012231 0.410034 -0.645689 11 1 0 0.146513 0.814526 -1.074333 12 1 0 1.805712 -2.159463 -0.940137 13 1 0 0.221243 -0.152955 1.812551 14 1 0 -2.085991 -0.932343 1.293966 15 1 0 -1.882046 0.831148 1.321104 16 1 0 -3.802814 0.217231 -1.666285 17 1 0 -4.036521 -0.697800 -0.154371 18 1 0 -3.835565 1.074801 -0.102300 19 1 0 1.582144 2.425035 0.024796 20 1 0 2.238640 -1.864380 0.764571 ---
$P(O)Me_3$	Full mass-weighted force constant matrix: Low frequencies --- 0.0008 0.0023 0.0024 24.0557 34.3421 46.6414 Low frequencies --- 196.9277 226.0215 231.6537 Zero-point correction= 0.116939 (Hartree/Particle) Thermal correction to Energy= 0.124246 Thermal correction to Enthalpy= 0.125190 Thermal correction to Gibbs Free Energy= 0.086734 Sum of electronic and zero-point Energies= -536.366017 Sum of electronic and thermal Energies= -536.358710 Sum of electronic and thermal Enthalpies= -536.357766 Sum of electronic and thermal Free Energies= -536.396222 Standard orientation: ---

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	8	0	0.000345	0.003359	1.684798
2	15	0	0.000077	0.000271	0.167086
3	6	0	-0.427639	-1.616375	-0.543685
4	6	0	-1.185760	1.176735	-0.547851
5	6	0	1.613027	0.436429	-0.547219
6	1	0	1.572714	0.420480	-1.638855
7	1	0	1.896502	1.436743	-0.211538
8	1	0	2.363368	-0.279563	-0.203580
9	1	0	-0.415353	-1.576557	-1.635402
10	1	0	0.293694	-2.362305	-0.201795
11	1	0	-1.425320	-1.905368	-0.204784
12	1	0	-1.157114	1.144155	-1.639506
13	1	0	-2.192413	0.924830	-0.206061
14	1	0	-0.937760	2.185920	-0.210630

5.6 Synthesis of the starting sugars used

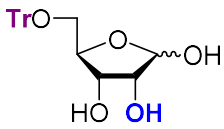
5-*O*-(4-methoxytrityl)-D-ribofuranose (3.4)



A solution of D-ribose (2.00 g, 13.34 mmol, 2.0 equiv.) in DMF (15 mL) was cooled to 0 °C in an ice bath and to it was added 4-dimethylaminopyridine (DMAP) (45 mg, 0.37 mmol, 5 mol%), NEt_3 (3 mL) and 4-methoxytrityl chloride (2.05 g, 6.66 mmol, 1.0 equiv.) with stirring. The reaction was slowly allowed to come to room temperature over 12 h. After stirring at room temperature for a further 12 h, the reaction was poured into ice water (100 mL) and extracted with CH_2Cl_2 (100 mL) . The organic layer was washed with concentrated aq. NH_4Cl (100 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude yellow oil was purified using flash column chromatography (1:1 EtOAc–Petroleum ether to elute the multiply-tritylated side products then 19:1 CH_2Cl_2–MeOH to elute the product) to afford the product **3.4** (1.74 g, 62%, α:β ~1:6) as a white foam. R_f = 0.25 (3:2 EtOAc–Petroleum ether) (α and β anomers co-elute). $[\alpha]_D$: +33.3 (*c* 0.561, CH_2Cl_2). ^{1}H NMR (500.0 MHz, DMSO-d_6): Data for the major β-anomer: δ 2.98 (dd, 1H, J_{gem} = 9.6, $J_{5'b,4'}$ = 6.2, H-5′b); 3.10 (dd, 1H, J_{gem} = 9.6, $J_{5'a,4'}$ = 2.9, H-5′a); 3.61 (td, 1H, $J_{2',3'} = J_{2',OH}$ = 4.3, $J_{2',1'}$ = 1.3, H-2′); 3.74 (s, 3H, CH_3O); 3.85-3.89 (m, 2H, H-3′,4′); 4.73 (d, 1H, $J_{OH,3'}$ = 7.0, OH-3′); 4.92 (d, 1H, $J_{OH,2'}$ = 4.3, OH-2′); 5.00 (dd, 1H, $J_{1',OH}$ = 5.3, $J_{1',2'}$ = 1.3, H-1′); 6.29 (d, 1H, $J_{OH,1'}$ = 5.3, OH-1′); 6.90 (m, 2H, H-*m*-C_6H_4OMe-MMTr); 7.23

(m, 2H, H-*p*-C_6H_5-MMTr); 7.27 (m, 2H, H-*o*-C_6H_4OMe-MMTr); 7.32 (m, 4H, H-*m*-C_6H_5-MMTr); 7.42-7.45 (m, 4H, H-*o*-C_6H_5-MMTr). Data for the minor α-anomer: δ 2.93 (dd, 1H, J_{gem} = 10.0, $J_{5'b,4'}$ = 5.6, H-5′b); 3.09 (dd, 1H, J_{gem} = 10.0, $J_{5'a,4'}$ = 3.1, H-5′a); 3.73 (m, 1H, H-3′); 3.75 (s, 3H, CH_3O); 3.82 (ddd, 1H, $J_{2',OH}$ = 6.9, $J_{2',3'}$ = 5.8, $J_{2',1'}$ = 4.0, H-2′); 3.97 (td, 1H, $J_{4',5'}$ = 5.6, 3.1, $J_{4',3'}$ = 5.6, H-4′); 4.725 (d, 1H, $J_{OH,2'}$ = 6.9, OH-2′); 4.76 (d, 1H, $J_{OH,3'}$ = 6.8, OH-3′); 5.19 (dd, 1H, $J_{1',OH}$ = 7.8, $J_{1',2'}$ = 4.0, H-1′); 5.88 (d, 1H, $J_{OH,1'}$ = 7.8, OH-1′); 6.90 (m, 2H, H-*m*-C_6H_4OMe-MMTr); 7.23 (m, 2H, H-*p*-C_6H_5-MMTr); 7.27 (m, 2H, H-*o*-C_6H_4OMe-MMTr); 7.32 (m, 4H, H-*m*-C_6H_5-MMTr); 7.42-7.45 (m, 4H, H-*o*-C_6H_5-MMTr). ^{13}C NMR (125.7 MHz, DMSO-d_6): Data for the major β-anomer: δ 55.22 (CH_3O); 65.98 (CH_2-5′); 71.37 (CH-3′); 75.46 (CH-2′); 80.59 (CH-4′); 85.63 (C-MMTr); 101.78 (CH-1′); 113.35 (CH-*m*-C_6H_4OMe-MMTr); 126.93, 126.94 (CH-*p*-C_6H_5-MMTr); 128.01 (CH-*m*-C_6H_5-MMTr); 128.28 (CH-*o*-C_6H_5-MMTr); 130.29 (CH-*o*-C_6H_4OMe-MMTr); 135.41 (C-*i*-C_6H_4OMe-MMTr); 144.71 (C-*i*-C_6H_5-MMTr); 158.29 (C-*p*-C_6H_4OMe-MMTr). Data for the minor α-anomer: δ 55.23 (CH_3O); 64.51 (CH_2-5′); 70.81 (CH-3′); 70.87 (CH-2′); 81.02 (CH-4′); 85.63 (C-MMTr); 96.57 (CH-1′); 113.40 (CH-*m*-C_6H_4OMe-MMTr); 127.02 (CH-*p*-C_6H_5-MMTr); 128.06 (CH-*m*-C_6H_5-MMTr); 128.16 (CH-*o*-C_6H_5-MMTr); 130.21 (CH-*o*-C_6H_4OMe-MMTr); 135.28 (C-*i*-C_6H_4OMe-MMTr); 144.56, 144.63 (C-*i*-C_6H_5-MMTr); 158.34 (C-*p*-C_6H_4OMe-MMTr). IR: (KBr) Data for the β anomer: ν_{max} 3418, 3033, 3000, 2931, 2836, 1584, 1575, 1463, 1447, 1414, 1300, 1252, 1153, 1117, 1034, 832, 639, 632, 595. Data for the α anomer: ν_{max} 3418, 3085, 3058, 2931, 2836, 1608, 1510, 1491, 1463, 1447, 1252, 1180, 1078, 1034, 595. HR ESIMS: *m/z* [M+Na^+] calcd for $C_{25}H_{26}O_6Na$: 445.16216. Found: 445.16223.

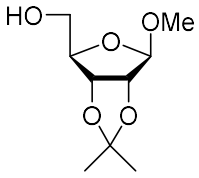
5-*O*-Trityl-β-D-ribofuranose (3.13)



This compound was prepared similar to the literature procedure.[154] To a stirring slurry of D-ribose (155.0 g, 1.05 mol, 1.05 equiv.) in pyridine (500 mL) in a 2 L round bottom flask was added trityl chloride (278.0 g, 1.00 mol, 1.00 equiv.) and the reaction heated to 50 °C for 6 h. After this time, the flask was cooled to r.t. and the pyridine removed *in vacuo* at 50 °C. The resulting dark brown sludge was redissolved in CH_2Cl_2 (1.5 L) the organic layer washed with H_2O (1 × 600 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 400 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The brown sludge was once again redissolved in CH_2Cl_2 (300 mL) and then added dropwise to a mixture of cyclohexane (1 L) and CH_2Cl_2 (100 mL) with rigorous stirring after which a solid crashed out of solution. After stirring at r.t. for a

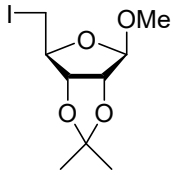
further 1 h, the precipitate was filtered off washing with hexane (~500 mL) and H_2O (~500 mL) to afford the pure product **3.13** (203.9 g, 52 %) as a slightly off white solid. Analytical data were in accordance with the literature.[154] R_f = 0.57 (CH_2Cl_2: MeOH = 19:1, v/v) one black spot using acidic *p*-anisaldehyde stain.

Methyl 2,3-*O*- Isopropylidene-β-D-ribofuranoside (3.23)



This compound was prepared similar to the literature procedure.[171] To a stirring slurry of D-ribose (50.00 g, 0.300 mol, 1.0 equiv.) in acetone (400 mL) in a 2 L round bottom flask was added 2,2-dimethoxypropane (100 mL, 0.815 mol, 2.4 equiv.) and the reaction was subsequently cooled to 0 °C in an ice water bath. To the cooled reaction was added $HClO_4$ (70 % in H_2O, 20 mL, 0.230 mol, 0.69 equiv.) dropwise via syringe. The reaction homogenized immediately and after 15 min, the cooling bath was removed. After stirring at r.t. for a further 1.5 h, MeOH (70 mL, 1.73 mol, 5.2 equiv) and stirring was continued for 3 h. After 3 h, the reaction was neutralized with concentrated Na_2CO_3(aq) (~100 mL) which resulted in the formation of a precipitate that was filtered off rinsing with Et_2O (3 × 100 mL). The filtrate was concentrated *in vacuo*. The crude oil was subsequently extrated with Et_2O (2 × 300 mL) and the organic layer washed with brine (2 × 300 mL), dried over Na_2SO_4 and concentrated *in vacuo* to provide a faint yellow oil **3.23** (61.26 g, 90 %, β/α >20:1) that was sufficiently pure to be used without further purification R_f = 0.43 (hexane:EtOAc = 1:1, v/v) one black spot using acidic *p*-anisaldehyde stain. The analytical data were in agreement with the literature.[171]

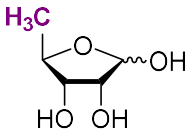
Methyl 5-Deoxy-5-iodo-2,3-*O*- isopropylidene-β-D-ribofuranoside (3.24).



To a stirring solution of compound **3.23** (31.06 g, 0.152 mol, 1.0 equiv.) in toluene (700 mL) in a 2 L round bottom flask was added imidazole (22.80 g, 0.335 mol, 2.2 equiv.), PPh_3 (43.91 g, 0.167 mol, 1.1 equiv.), and I_2 (42.49 g, 0.167 mol, 1.1 equiv.) in three portions at r.t. The reaction was subsequently heated to 80 °C and stirred for 30 min after which time TLC analysis indicated complete consumption of the starting material. The reaction was cooled to r.t. and quenched with 1 M $Na_2S_2O_3$(aq) (100 mL). The reaction mixture was transferred to a separatory funnel and diluted with EtOAc (1 L) and the organic layer washed with 1 M $Na_2S_2O_3$(aq) (500 mL), concentrated Na_2CO_3(aq) (500 mL), and brine (500 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude brown oil was purified using column chromatography (SiO_2, 20:1 hexane:EtOAc to 5:1 hexane:EtOAc) to provide the product **3.24** (41.10 g, 86 %) as a colourless oil. R_f = 0.82

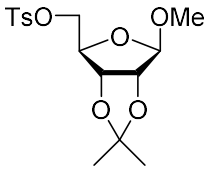
(hexane:EtOAc = 7:1, v/v) one black spot using acidic *p*-anisaldehyde stain. The analytical data were in agreement with the literature.[172]

5-*O*-Deoxy-D-ribofuranose (3.20)



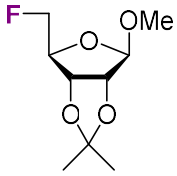
This is a known compound.[170] To a solution of **S2** (10.00 g, 31.8 mmol, 1.0 equiv.) in MeOH (150 mL) in a 500 mL was added NEt_3 (44 mL, 318 mmol, 10.0 equiv.) and the flask purged with argon several times. To the flask was added Pd/C (1.00 g) carefully **(<u>warning:</u> fire hazard)** in five portions. An H_2-filled balloon was fitted to the top of the flask and the reaction was stirred at r.t. for 16 h. TLC analysis (10:1 hexane:EtOAc) indicated complete conversion to the product. The reaction was filtered through a bed of Celite and ~50 % of the MeOH removed using a rotary evaporator with the pressure no lower than 220 mBar and the water bath at a temperature no greater than 35 °C as the product is a liquid. The crude reaction mixture was transferred to a separatory funnel and the mixture diluted with CH_2Cl_2 (300 mL). The organic layer was washed with 1 M HCl(aq) (2 x 100 mL), dried over Na_2SO_4, and concentrated under the same conditions as described above. This provided a colourless liquid, compound **3.25**, that was used without further purification. To this colourless liquid in 250 mL round bottom flask was added H_2O:THF (4:1 v/v, 80 mL) and Dowex 50W resin (H^+ form, 15.0 g). The suspension was stirred at 70 °C for 12 h over which time the reaction compound fully dissolved. The resin was filtered off, rinsing with H_2O (~50 mL) and the filtrate neutralized with NEt_3. The solvent was removed *in vacuo* coevaporating with EtOH (3 x 50 mL). Purification using column chromatography (SiO_2, 25:1 CH_2Cl_2:MeOH to 9:1 CH_2Cl_2:MeOH) afford the product **3.20** (3.82 g, 89 % over two steps) as a colourless oil. R_f = 0.35 (CH_2Cl_2: MeOH = 9:1, v/v) one black spot with *p*-anisaldehyde stain. ^{1}H NMR (500.0 MHz, D_2O, ext. ref(dioxane) = 3.75 ppm): δ 5.47 (d, 1H, $J_{1,2}$ = 4.3, H-1α); 5.30 (d, 1H, $J_{1,2}$ = 2.0, H-1β); 4.26 (dd, 1H, $J_{2,3}$ = 5.7, $J_{2,1}$ = 4.3, H-2α); 4.24 (qd, 1H, $J_{4,5}$ = 6.4, $J_{4,3}$ = 5.7, H-4α); 4.05–4.16 (m, 3H, H-2,3,4β); 3.92 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 5.7, H-3α); 3.92 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 5.7, H-3α); 1.44 (d, 3H, $J_{5,4}$ = 6.2, H-5β); 1.36 (d, 3H, $J_{5,4}$ = 6.4, H-5α). ^{13}C NMR (125.7 MHz, D_2O, ext. ref(dioxane) = 69.3 ppm): δ 103.7 (C-1β); 98.6 (C-1α); 81.1 (C-4β); 81.0 (C-4α); 78.0, 78.3 (C-2,3β); 77.7 (C-3α); 73.3 (C-2α); 22.0 (C-5β); 20.7 (C-5α). HR ESIMS: *m/z* [M+Na^+] calcd for $C_5H_{10}O_4Na$: 157.04713. Found: 157.04716.

Methyl 5-*p*-toluenesulfonyl-2,3-*O*- isopropylidene-β-D-ribofuranoside (3.26)



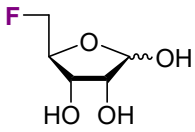
This compound was prepared similar to the literature procedure.[173]To a stirring solution of **3.26** (5.315 g, 26.0 mmol, 1.0 equiv.) in pyridine (20 mL) was added *p*-toluenesulfonyl chloride (7.454 g, 39.1 mmol, 1.5 equiv.) at r.t. After stirring for 2 h, the reaction was poured into a stirring slurry ice water (200 mL). The resulting precipitate was filtered off and rinsed with cold water. The pale brown solid **S3** (8.36 g, 90 %) was dried on a high vacuum and was sufficiently pure to be used directly in the next step. R_f = 0.23 (hexane:EtOAc = 9:1, v/v) one black spot using acidic *p*-anisaldehyde stain.[173]

Methyl 5-Deoxy-5-fluoro-2,3-*O*- isopropylidene-β-D-ribofuranoside (3.27)



This compound was prepared using a modified literature procedure.[173] To a solution of **3.26** (1.80 g, 5.03 mmol, 1.0 equiv.) in MeCN (25 mL) in a 250 mL round bottom flask was added tetrabutylammonium fluoride (1 M solution in THF, 10.0 mL, 10.00 mmol, 2.0 equiv.) and the reaction stirred at reflux for 24 h. After 24 h, the solution was cooled to r.t. and concentrated directly onto silica gel. Purification using column chromatography (SiO_2, 15:1 hexane:EtOAc to 9:1 hexane:EtOAc) provided the product **3.27** (753 mg, 73 %) as a colourless high boiling liquid. **NOTE**: As the product is a liquid the pressure of the rotary evaporator should not fall below 200 mBar and the heating bath above 45 °C. The analytical data were accordance with the literature.[173]

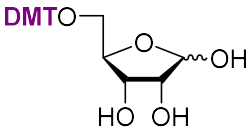
5-*O*-Deoxy 5-fluoro-D-ribofuranose (3.21)



This is a known compound.[173] To a suspension of **3.27** (630 mg, 3.06 mmol, 1.0 equiv.) in H_2O:THF (4:1 v/v, 80 mL) in a 50 mL round bottom flask was added added Dowex 50W resin (H^+ form, 1.50 g). The suspension was stirred at 70 °C for 12 h over which time the reaction compound fully dissolved. The resin was filtered off, rinsing with H_2O (~10 mL) and the filtrate neutralized with NEt_3. The solvent was removed *in vacuo* coevaporating with EtOH (3 × 10 mL). Purification using column chromatography (SiO_2, 25:1 CH_2Cl_2:MeOH to 9:1 CH_2Cl_2:MeOH) afford the product **3.21** (396 mg, 85 %) as a colourless oil. R_f = 0.32 (CH_2Cl_2: MeOH = 9:1, v/v) one black spot using *p*-anisaldehyde stain. $[\alpha]_D$: +4.0 (*c* 0.498, MeOH). ^{1}H NMR (500.0 MHz, D_2O): δ 4.00 (dt, 1H, $J_{2,3}$ = 4.7, $J_{2,1}$ = $J_{H,F}$ = 1.7 Hz, H-2β); 4.11 (ddd, 1H, $J_{2,3}$ = 5.5, $J_{2,1}$ = 4.0, $J_{H,F}$ = 1.2 Hz, H-2α); 4.12 (dddd, 1H, $J_{H,F}$ = 25.9, $J_{4,3}$ = 7.0, $J_{4,5}$ = 4.7, 2.3 Hz, H-4β); 4.17 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 5.5 Hz, H-3α); 4.25 (dddd, 1H, $J_{H,F}$ = 27.3, $J_{4,3}$ = 5.5, $J_{4,5}$ = 4.2, 2.4 Hz, H-4α); 4.30 (dd, 1H, $J_{3,4}$ = 7.0, $J_{3,2}$ = 4.7 Hz, H-3β); 4.55 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.7, $J_{5b,4}$ = 4.2 Hz, H-5bα); 4.57 (ddd,

1H, $J_{H,F}$ = 47.3, J_{gem} = 10.6, $J_{5b,4}$ = 4.7 Hz, H-5bβ); 4.62 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.7, $J_{5a,4}$ = 2.4 Hz, H-5aα); 4.67 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.6, $J_{5a,4}$ = 2.3 Hz, H-5aβ); 5.27 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1β); 5.41 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1α). ^{13}C NMR (125.7 MHz, D_2O): δ 72.0 (d, $J_{C,F}$ = 6.6 Hz, CH-3α); 72.1 (d, $J_{C,F}$ = 6.9 Hz, CH-3β); 73.4 (d, $J_{C,F}$ = 1.7 Hz, CH-2α); 77.8 (d, $J_{C,F}$ = 1.7 Hz, CH-2β); 83.2 (d, $J_{C,F}$ = 18.1 Hz, CH-4β); 83.7 (d, $J_{C,F}$ = 17.7 Hz, CH-4α); 85.6 (d, $J_{C,F}$ = 167.6 Hz, CH_2-5α); 86.1 (d, $J_{C,F}$ = 168.5 Hz, CH_2-5β); 99.2 (CH-1α); 103.8 (CH-1β). ^{19}F NMR (470.4 MHz, D_2O): –227.41 (td, $J_{F,H}$ = 47.2, 27.3 Hz, Fα); –225.09 (td, $J_{F,H}$ = 47.3, 25.9 Hz, Fβ).

5-*O*-(4,4′-dimethoxytrityl)-D-ribofuranose (3.22)



This is a known compound,[174] however, a detailed experimental procedure has not been published. To a solution of D-ribose (0.750 g, 5.00 mmol, 1.1 equiv.) in pyridine (100 mL) in 500 mL round bottom flask at r.t. was added and 4,4′-dimethoxytrityl chloride (1.54 g, 4.55 mmol, 1.0 equiv.) with stirring. The reaction was stirred at r.t for 24 h. After 24 h, the reaction was poured into ice water (100 mL) and extracted with CH_2Cl_2 (100 mL) . The organic layer was washed with concentrated aq. NH_4Cl (100 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude yellow oil was purified using flash column chromatography (silica gel, 3:2 EtOAc–Petroleum ether to elute the multiply-tritylated side products then 19:1 CH_2Cl_2–MeOH to elute the product) to afford 9 (1.13 g, 55%, α/β ~4:1) as a white foam. R_f = 0.38 (CH_2Cl_2: MeOH = 19:1, v/v) UV active, one bright orange spot with *p*-anisaldehyde stain (α and β anomers co-elute). $[\alpha]_D$: +7.1 (*c* 0.296, DMSO). ^{1}H NMR (400.0 MHz, CD_2Cl_2): Data for the major α-anomer: δ 7.50–6.80 (m, 13H, H-Ar DMT); 5.37 (d, 1H, $J_{1,2}$ = 4.3 Hz, H-1) 4.20–4.15 (m, 2H, H-2, H-4); 4.02 (dd, 1H, *J* = 5.7, 3.6 Hz, H-3); 3.77 (s, 6H, 2 x H_3CO); 3.26 (dd, 1H, J_{gem} = 10.2, $J_{5a,4}$ = 3.8 Hz, H-5a); 3.18 (dd, 1H, J_{gem} = 10.1, $J_{5b,4}$ = 4.3 Hz, H-5b). Data for the minor β-anomer: 7.50–6.80 (m, 13H, H-Ar DMT); 5.23 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1) 4.36 (dd, 1H, *J* = 6.2, 4.9 Hz, H-3); 4.05–4.00 (m, 2H, H-2, H-4); 3.77 (s, 6H, 2 x H_3CO); 3.26 (dd, 1H, J_{gem} = 10.2, $J_{5a,4}$ = 3.8 Hz, H-5a); 3.18 (dd, 1H, J_{gem} = 10.1, $J_{5b,4}$ = 4.3 Hz, H-5b)). ^{13}C NMR (100 MHz, CD_2Cl_2): Data for the major α-anomer: δ 159.2 (C-Ar DMT); 145.5 (C-Ar DMT) 136.3 (C-Ar DMT); 130.6 (CH-Ar DMT); 128.6 (CH-Ar DMT); 128.4 (CH-Ar DMT); 127.3 (CH-Ar); 113.7 (CH-Ar DMT); 97.4 (CH-1); 86.8 (C-DMT); 83.3 (CH-4); 72.5 (CH-3); 72.1 (CH-2); 64.5 (CH_2-5); 55.8 (CH_3O). Data for the minor β-anomer: δ 159.2 (C-Ar DMT); 145.3 (C-Ar DMT) 136.3 (C-Ar DMT); 130.6 (CH-Ar DMT); 128.7 (CH-Ar DMT); 128.4 (CH-Ar DMT); 127.4 (CH-

Ar); 113.7 (CH-Ar DMT); 102.6 (CH-1); 87.1 (C-DMT); 83.1 (CH-4); 76.6 (CH-2); 72.4 (CH-3); 64.9 (CH_2-5); 55.8 (CH_3O). HR ESIMS: *m/z* [$M+Na^+$] calcd for $C_{26}H_{28}O_7Na$: 475.17272. Found: 475.17280.

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