Nucleoside Chemistry

Fionn O'Hara

Nucleoside Chemistry (Selected) Timeline:

1869	Miescher isolates a phosphorus rich substance he calls nuclein
1889	Altman isolates protein free nucleic acid
1905-1940	Levene identifies the components of DNA, structure remains unclear
1928	Griffith publishes on "bacterial transformation"
1935	Klein and Thannhauser cleave DNA and isolate crystalline nucleotides
1944	Avery identifies that DNA is responsible for the transforming activity
1950s	Todd establishes structure of nucleosides by chemical synthesis
1953	Crick and Watson publish on the DNA double helix structure
1959	Discovery of idoxuridine, first anti-viral nucleoside drug marketed
1960s	Discovery of antibiotic/antiviral activity of nucleoside natural products
1964	AZT (zidovudine) first synthesized as a potential anti-cancer medicine
1969	Ara-C (cytarabine) approved as anti-cancer medicine (leukaemias)
1970s	Acyclovir discovered, first really successful nucleoside antiviral
1980s	HIV identified as cause of AIDS, search begins to find treatments
1985	AZT identified as having anti-HIV activity
1987	AZT approved as a drug for AIDS and HIV
1990s+	Numerous nucleoside drugs approved as anti-cancer, anti-viral immunosuppressive, cardiovascular and antiprotozoal medicines

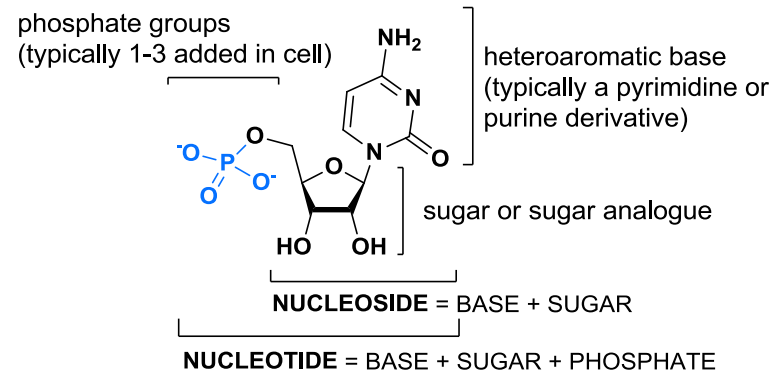
Nucleosides or their derivatives are also used in agrochemistry (herbicides, fungicides and insecticides), biotechnology (e.g. DNA sequencing) and biology. The challenging structures of many antibiotic nucleoside natural products has also made them interesting targets for total synthesis.

Topics covered: nucleoside structure, types of nucleosides, synthesis of complete nucleosides, synthesis of nucleosides as medicines
Briefly mentioned: sugar syntheses, phosphate chemistry, natural products
Topics not covered: nucleic acid structure, oligonucleotide synthesis, DNA synthesis or sequencing, biosynthesis, drug mechanisms, synthesis of functionalized purine or pyridine bases, prebiotic chemistry

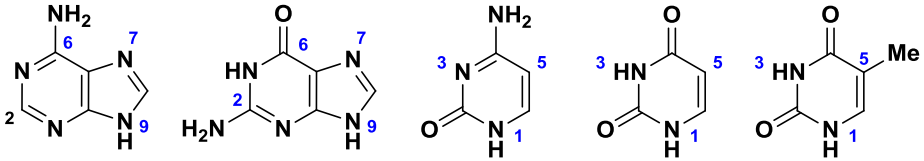
Useful books/reviews:

Simons, C. *Nucleoside Mimetics: Their Chemistry and Biological Properties.*
Blackburn, M.G. and Gait, M. *Nucleic Acids in Chemistry and Biology*.
Vaghefi, M. *Nucleoside Triphosphates and their Analogs: Chemistry, Biotechnology, and Biological Applications.*
AIDS-Driven Nucleoside Chemistry. Huryn, D.M. and Okabe, M. *Chem. Rev.* **1992**, *92*, 1745-1768.
Palladium-Assisted Routes to Nucleosides. Agrofoglio, L.A.; Gillaizeau, I. and Saito, Y. *Chem. Rev.* **2003**, *103*, 1875-1916.
Synthesis of Complex Nucleoside Antibiotics. Knapp, S. *Chem. Rev.* **1995**, *95*, 1859-1876.

Nucleoside Structure and Conformation:



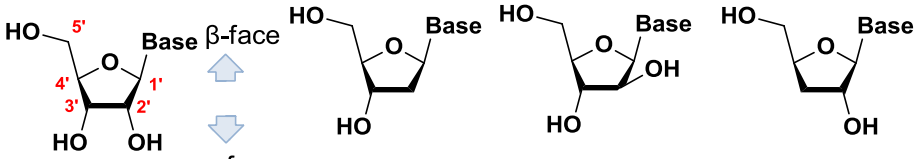
Bases commonly occuring in DNA and RNA:



adenine (A) | guanine (G) | cytosine (C) | uracil (U) | thymine (T)

purines | **pyrimidines**

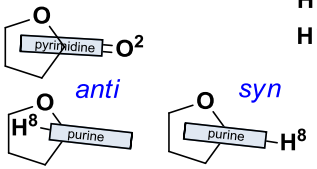
Commonly occuring sugars:



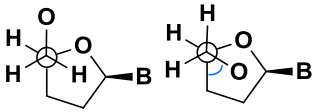
ribose | 2-deoxyribose | β-D-arabinofuranose | 3-deoxy-β-D-ribofuranose

Conformational nomenclature:

Glycosidic bond:

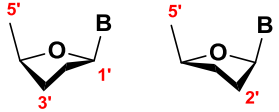


mostly *anti*-preferred
GMP prefers *syn*



C4'-C5' geometry
pyrimidine: *+sc*
purine: equal *ap* and *+sc*

Sugar pucker:

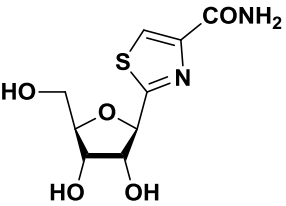


$C^{2'}$-*endo* (S) $C^{3'}$-*endo* (N)

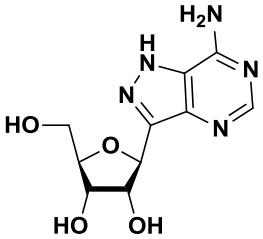
Nucleoside Chemistry

Fionn O'Hara

Nucleosides and Nucleoside Analogues

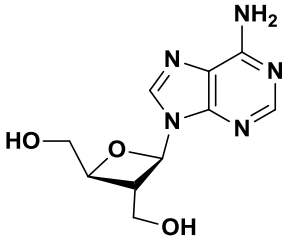


tiazofurin
anti-cancer

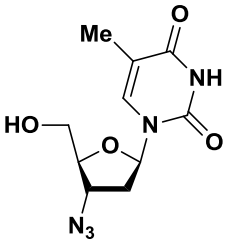


formycin

C-nucleosides

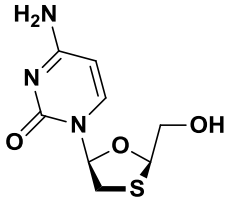


oxetanocin

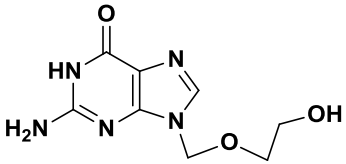


AZT (Zidovudine)
anti-viral (HIV)

modified sugars

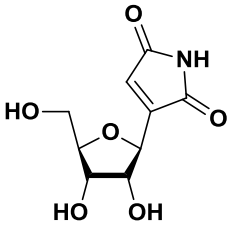


lamivudine (Epivir)
anti-viral (HIV)

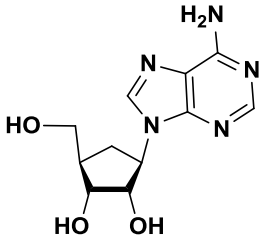


Acyclovir (Zovirax)
anti-viral (HSV)

acyclic nucleosides

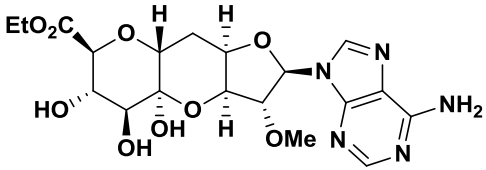


showdomycin

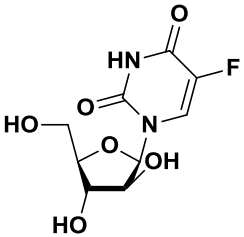


aristeromycin

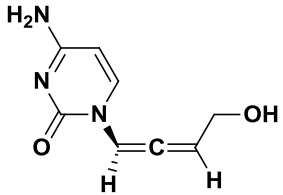
natural products



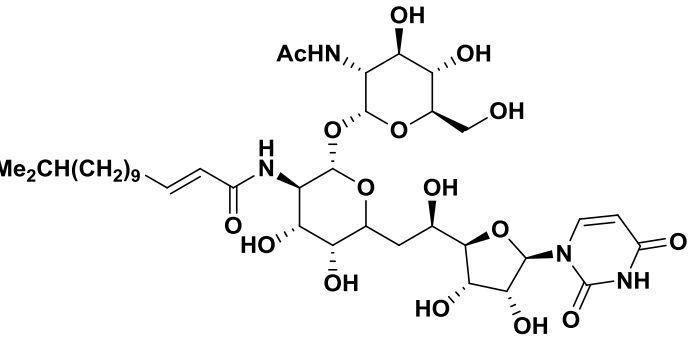
herbicidin B
herbicidal natural product



Ara-FU
anti-cancer

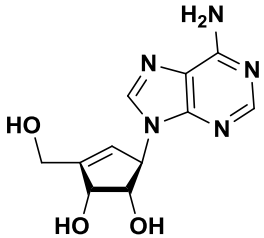


(*R*)-(-)-cytallene
anti-viral

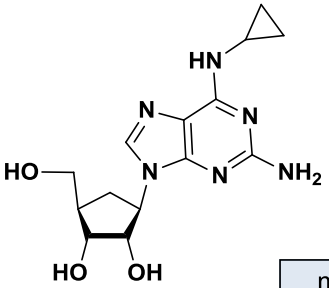


tunicamycin V

carbocyclic nucleosides

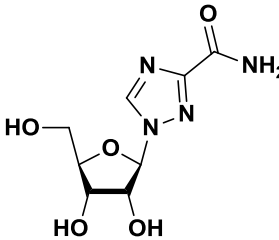


Neplanocin A
antibiotic, anti-cancer



Abacavir
anti-viral (HIV/AIDS)

modified bases

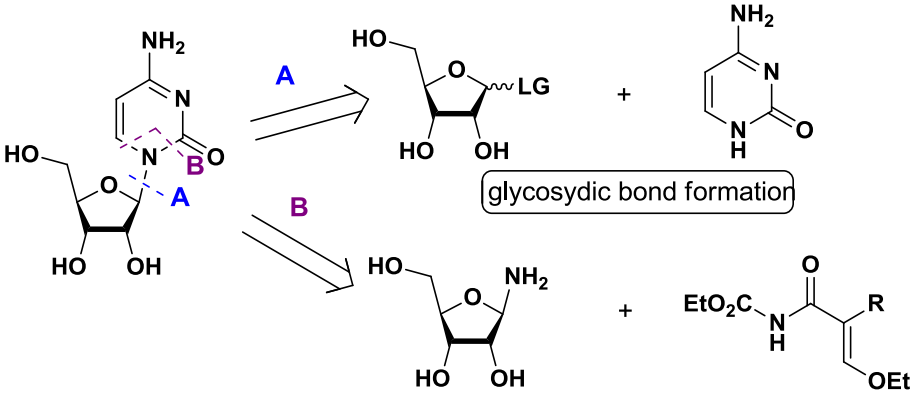


Ribavirin
anti-viral (RSV)

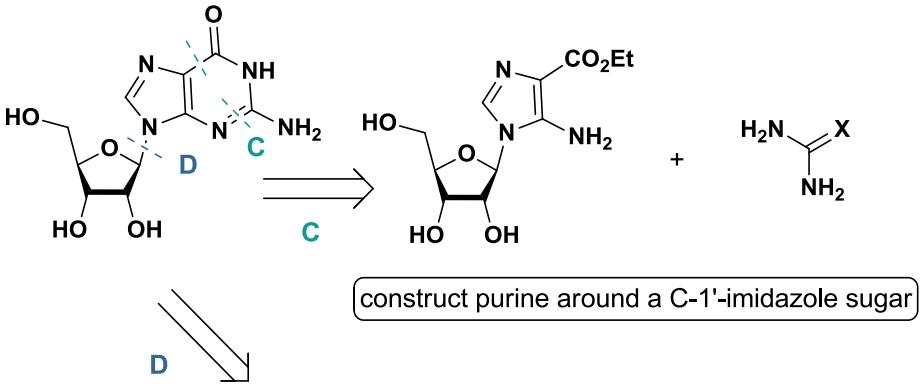
Nucleoside Chemistry

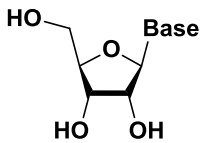
Fionn O'Hara

Common disconnections towards nucleosides:



useful for purines and analogues:

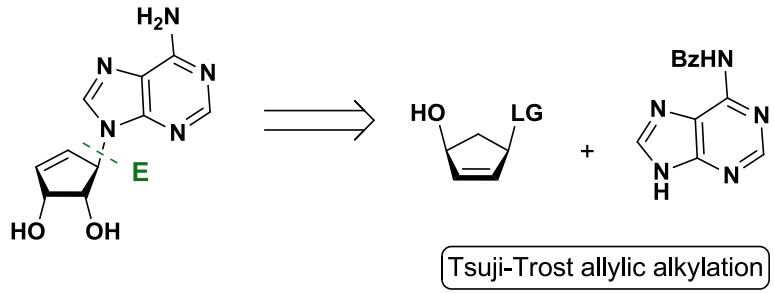




typically transfers sugars from pyrimidines to more basic purines

transglycosylation: either chemically or enzymatically

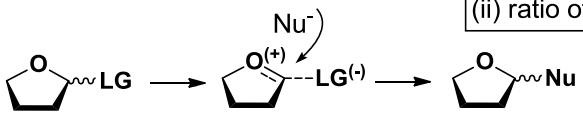
useful for unusual sugars and carbocyclic derivatives:



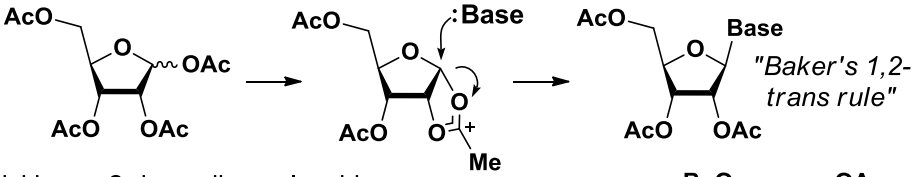
Major methods for the construction of nucleosides:

A. Formation of the glycosidic bond

Key issues:
(i) regioselectivity of base substitution
(ii) ratio of α:β anomers

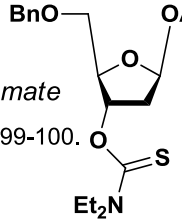


importance of neighbouring group participation for stereochemical control:



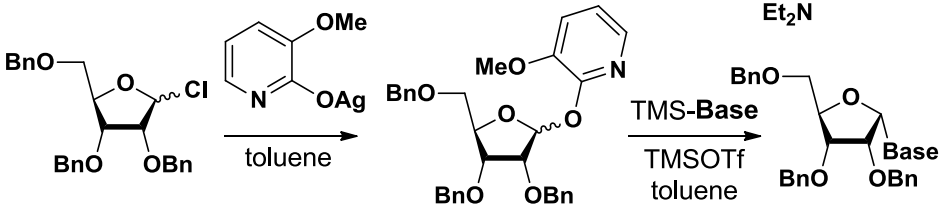
trickier on 2-deoxyribonucleosides:

(i) use a ribonucleoside then deoxygenate
(ii) use an alternative directing group, e.g. 3'-thiocarbamate



Mukaiyama. *Chem. Lett.* **1996**, *25*, 99-100.

to favour α-substitution on ribonucleosides:



Hanessian. *Tet. Lett.* **1995**, *36*, 5865-5868.

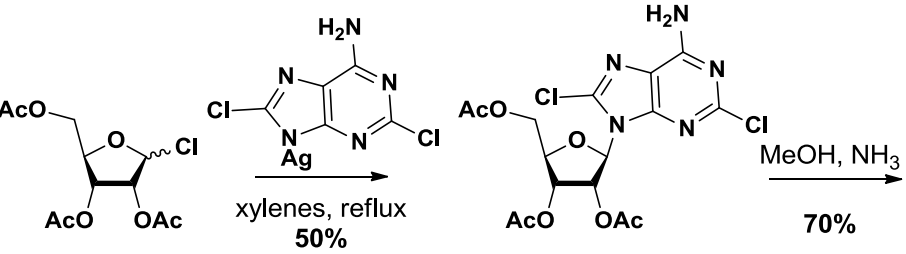
Nucleoside Chemistry

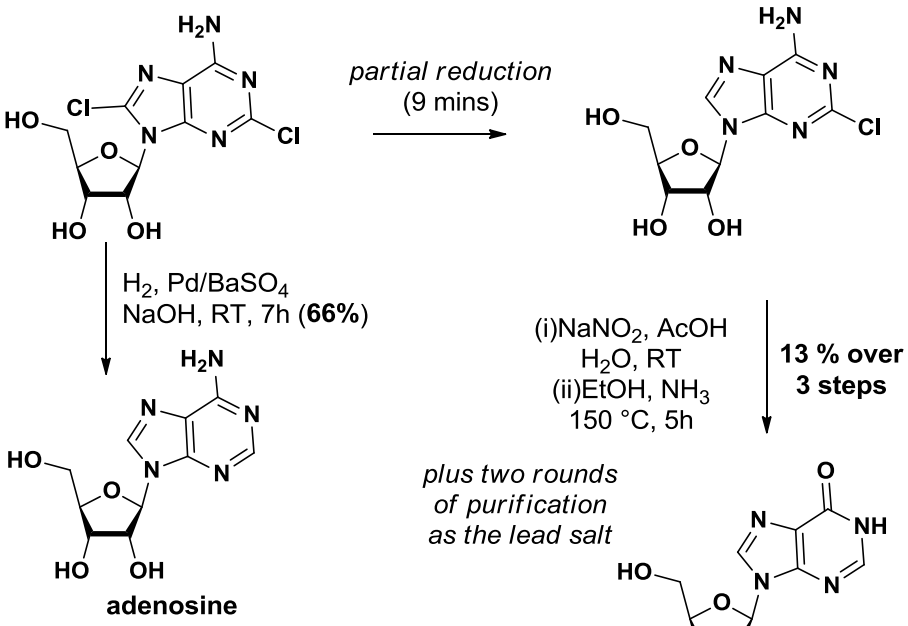
Fionn O'Hara

A. Formation of the glycosidic bond

(i) Coupling metal salts to a C1' halogenated sugar

Fischer-Helferich procedure: first syntheses of adenosine and guanosine

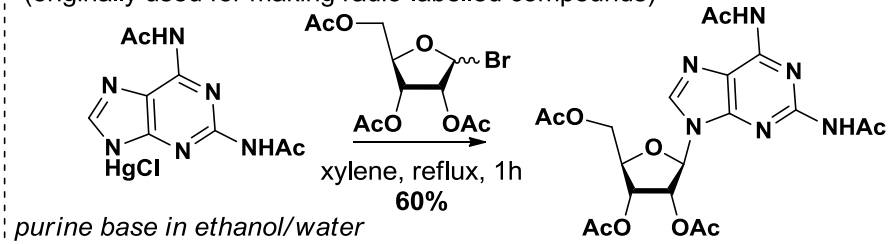




Todd awarded 1957 Nobel Prize in Chemistry for work on nucleotides and nucleotide coenzymes.

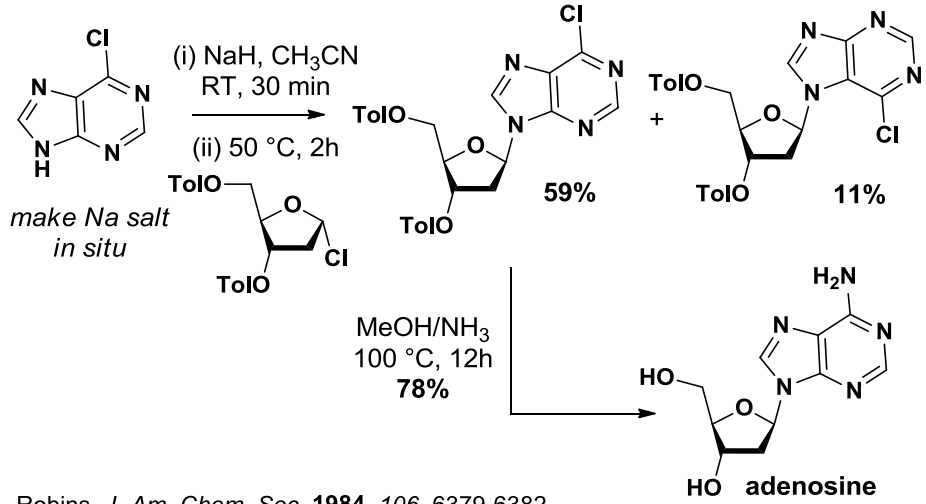
Todd. *J. Chem. Soc.* **1948**, 967-969 and 1685-1687.
Fischer and Helfrich. *Ber*. **1914**, *47*, 210.

Chloromercuri procedure: using Hg(II) salts increased the yields (originally used for making radio-labelled compounds)



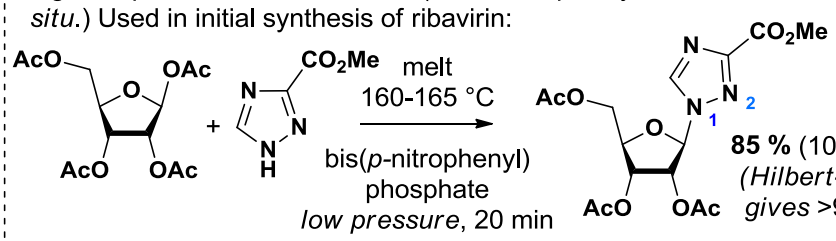
Davoll. *J. Am. Chem. Soc.* **1951**, *73*, 1650-1655.

Sodium salt derivatives: less expensive and toxic, and gives good yields but can have more regioselectivity issues than the heavy metal salts



Robins. *J. Am. Chem. Soc.* **1984**, *106*, 6379-6382.

(ii) "Fusion synthesis": melt of a C-1' acetoxysugar, the base, a Lewis acid at high temperature under vaccum. (Makes frequently unstable C-1' halo-sugar *in situ*.) Used in initial synthesis of ribavirin:



Witkowski. *J. Med. Chem.* **1972**, *15*, 1150-1154.

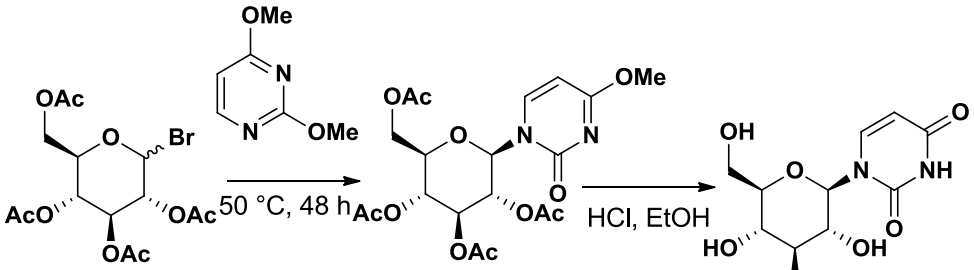
Nucleoside Chemistry

Fionn O'Hara

A. Formation of the glycosidic bond

(iii) Hilbert-Johnson Reaction (Quaternization procedure)

effectively an S_N2-type reaction with nucleophilic substituted pyrimidines



usually get mixtures of anomers
improve β selectivity with $HgBr_2$

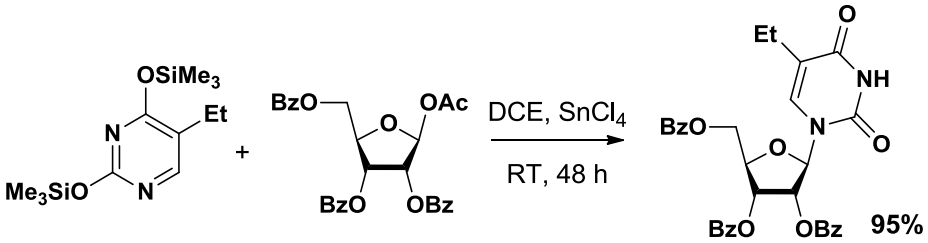
Hilbert and Johnson. *Science.* **1929**, *69*, 579-580.
Hilbert and Johnson. *J. Am. Chem. Soc.* **1930**, *52*, 4489-4494.

alkylation of the base with the alkyl iodide formed during the reaction is a problem; the use of TMS-protected derivatives improves this

Nishimura *et. al*. *Chem. Pharm. Bull.* **1963**, *11*, 1470-1472.
Birkofer. *Chem. Ber.* **1960**, *93*, 2804.

(iv) Vorbrüggen procedure (silyl Hilbert-Johnson reaction)

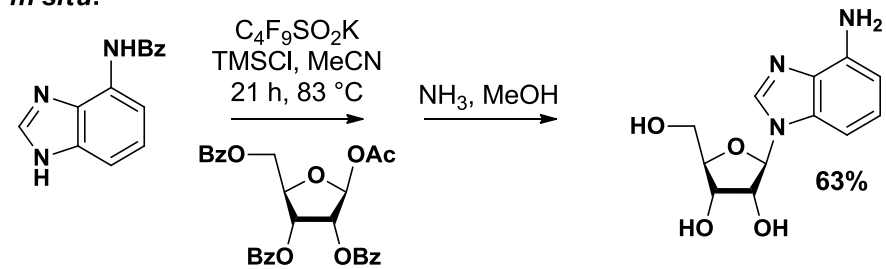
use a Friedel-Crafts catalyst e.g. $SnCl_4$, $ZnCl_2$ or TMSOTf with the silyl protected derivatives, mild conditions (often RT), allows the use of -OR and -OAc sugars which are easier to make and more stable than halo sugars



Vorbrüggen. *Angew. Chem. Int. Ed.* **1970**, *9*, 461-462.

"...because of the high yields and the simplicity of the procedure pyrimidine nucleosides have thus become readily accessible on an industrial scale."

one-pot procedure that makes the (moisture sensitive) TMS-heterocycle *in situ*:

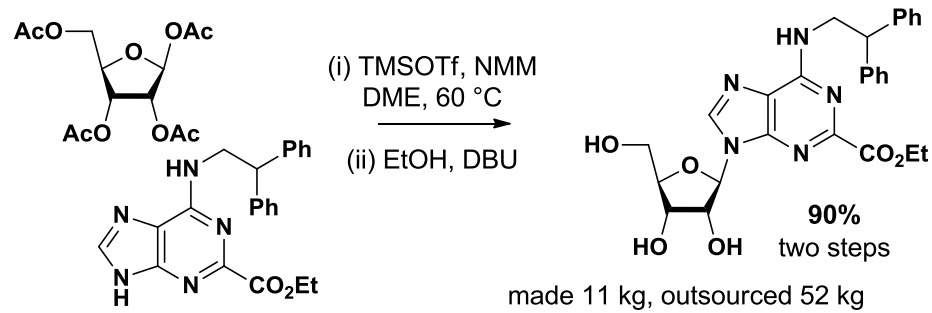


Vorbrüggen. *Tet. Lett.* **1978**, *19*, 1339-1342.

using TMSOTf gives better control over N-9:N-7 selectivity than $SnCl_4$

use of a modified procedure at much larger scale:

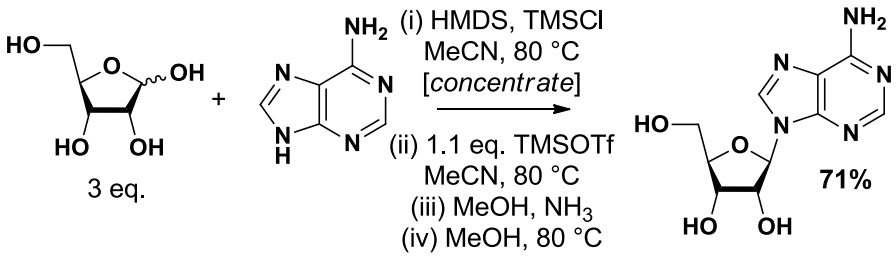
from a synthesis of a Pfizer compound as potential inhaled COPD treatment



made 11 kg, outsourced 52 kg

Entwistle and Smith. *Org. Process Res. Dev.* **2012**, *16*, 470-483.

extension to *in situ* silyl protection of the sugars;
(useful if the protected sugar isn't commercially available - multistep synthesis)



doesn't work for 2'-deoxyribose

Vorbrüggen. *Tet. Lett.* **1995**, *36*, 7845-7848.

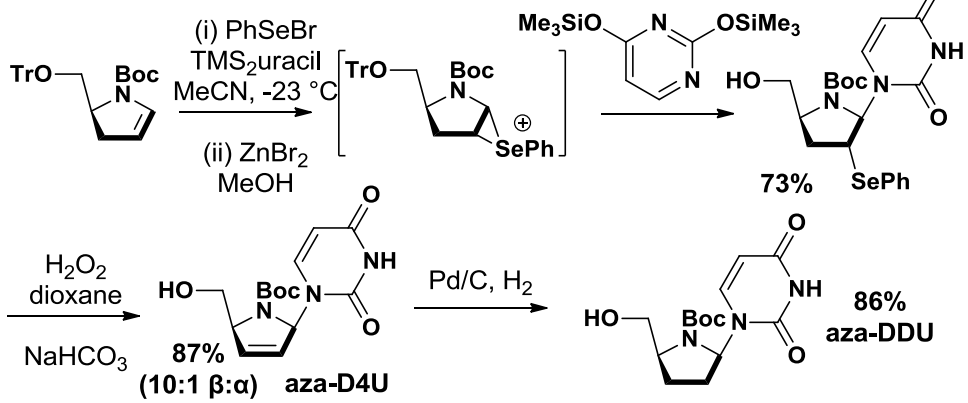
Nucleoside Chemistry

Fionn O'Hara

A. Formation of the glycosidic bond

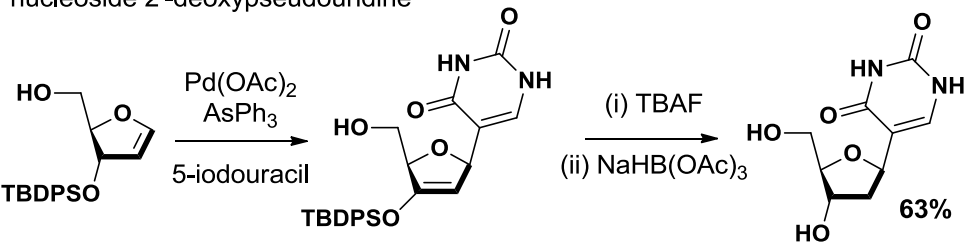
(v) attack onto a glycal derivative

traditionally this involved activating an alkene with e.g. I, S, Se, then attacking with the base as the nucleophile: e.g. synthesis of an aza-nucleoside



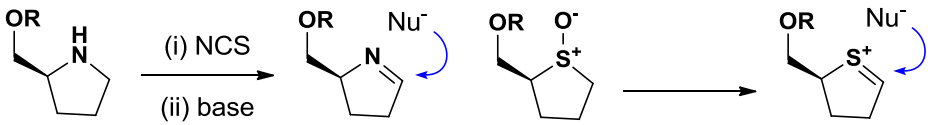
Correia. *Tetrahedron Lett.* **2001**, *42*, 1599-1602.

Pd-catalyzed cross-coupling provides a similar transformation: synthesis of C-nucleoside 2'-deoxypseudouridine



Daves. *J. Org. Chem.* **1992**, *57*, 4690-4696.

Useful tricks for glycosidic bond formation on heterocyclic sugar analogues:

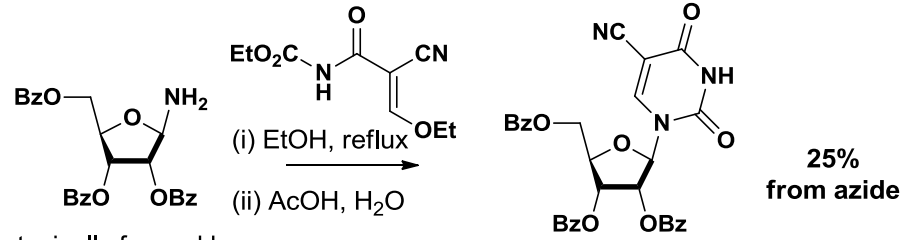


Pummerer-type with Ac_2O or TMS-base
TMSOTf and Et_3N

Review on heterocyclic analogues: Merino. *Chem. Rev.* **2010**, *110*, 3337-3370.

B. Building the heterocycle around the C-1' nitrogen of an aminosugar

see also use for construction of carbocyclic nucleosides and iso-nucleosides
i.e. particularly useful when the base is not adjacent to a heteroatom

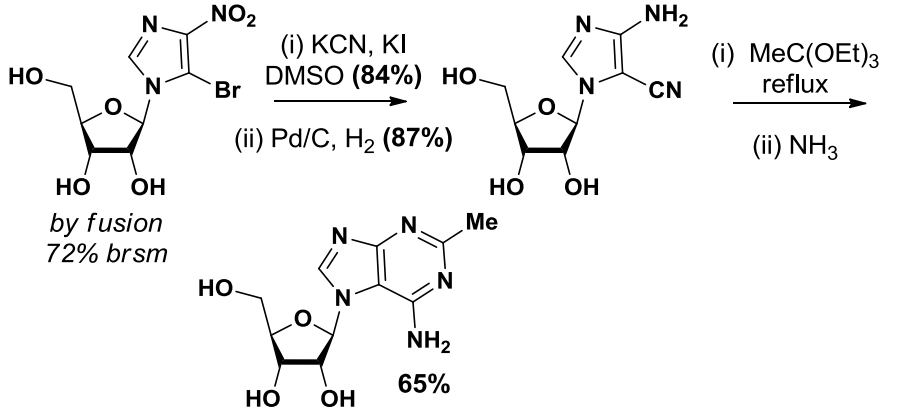


typically formed by reduction of an azide installed by S_N2

Shaw. *J. Chem. Soc.* **1958**, *42*, 2294-2299.

C. Construct purine around a C-1' imidazole sugar

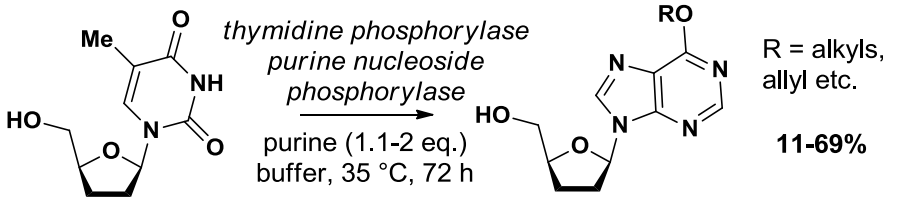
in this case used to get regioselectivity for an unusual *N*-7 purine nucleoside



Rousseau. *J. Am. Chem. Soc.* **1968**, *90*, 2661-2668.

D. Transglycosylation

enzymatic transfer of sugar between bases - useful if you want to transfer a modified sugar to a different base (see also enzymatic synthesis of ribavarin)



Burns. *J. Med. Chem.* **1983**, *36*, 378-384.

Nucleoside Chemistry

Fionn O'Hara

C-Nucleosides: nucleosides with a C-1'-Base C-C bond

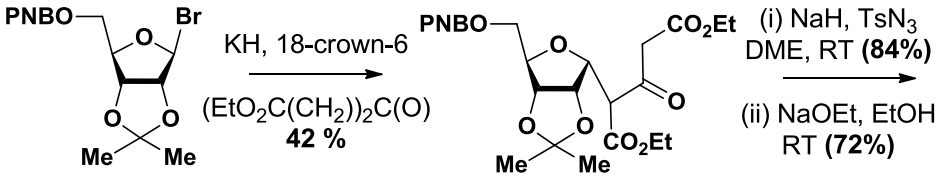
can be naturally occuring (e.g. showdomycin, pyrazofurin) or made synthetically

stable to enzymatic hydrolysis due to C-C glycosidic link; useful for medicines

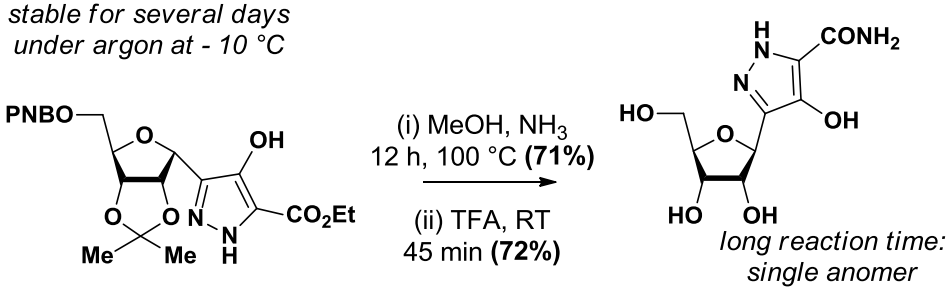
Typical ways of making the C-1'-C glycosidic link:

(i) make a C-1' carbonyl derivative and make your heterocycle

e.g. synthesis of pyrazomycin - anti-cancer, anti-viral natural product



stable for several days under argon at - 10 °C

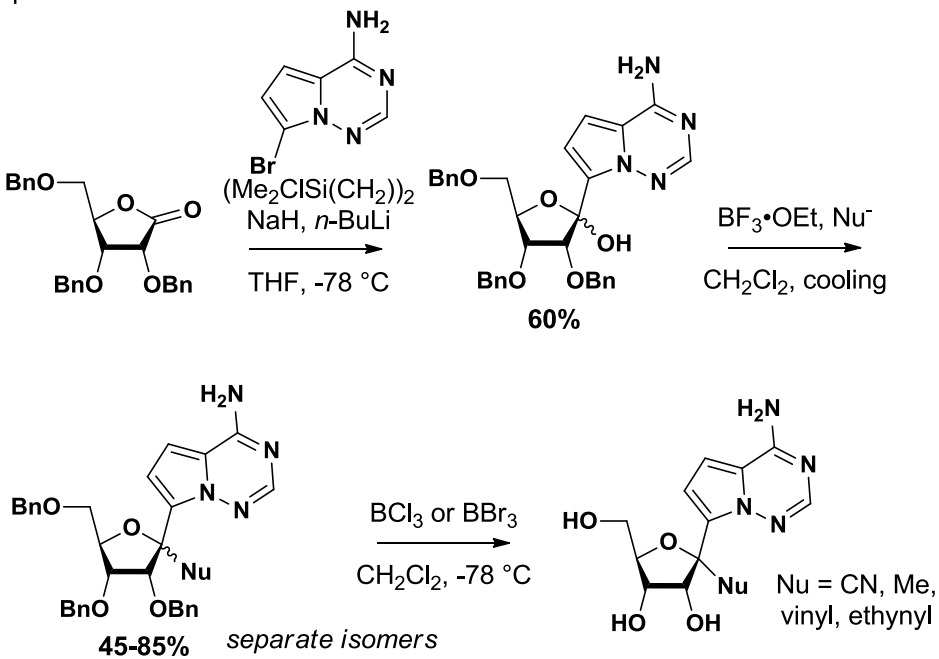


long reaction time: single anomer

Weigele. *J. Org. Chem.* **1976**, *41*, 287-290.

(ii) attack a lactone derivative of the sugar with a heterocycle anion

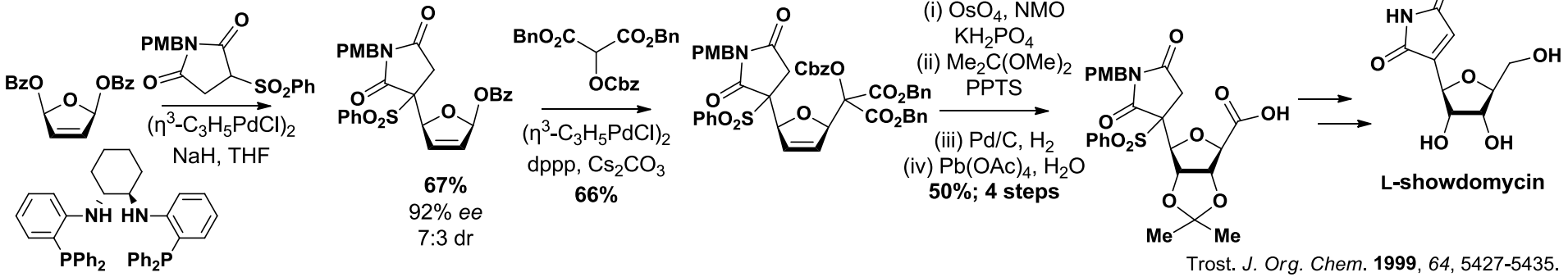
e.g. Gilead Sciences: series of 4-aza-7,9-dideazaadenosine C-nucleosides as potential anti-virals



Cho. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2705-2707.

A more modern approach: Trost asymmetric allylic alkylation chemistry

older methods tend to go from sugars to get the stereocentres, but it is possible to build these in

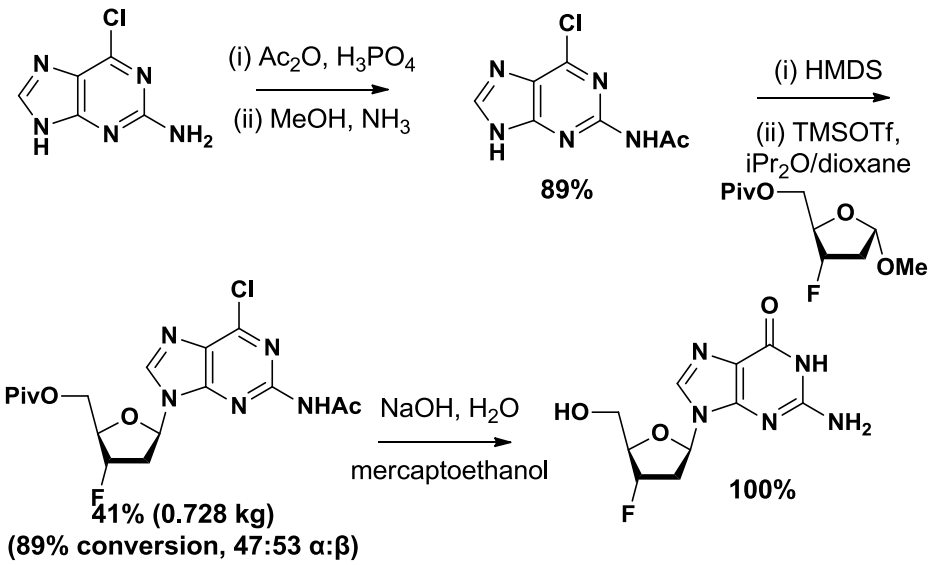


Trost. *J. Org. Chem.* **1999**, *64*, 5427-5435.

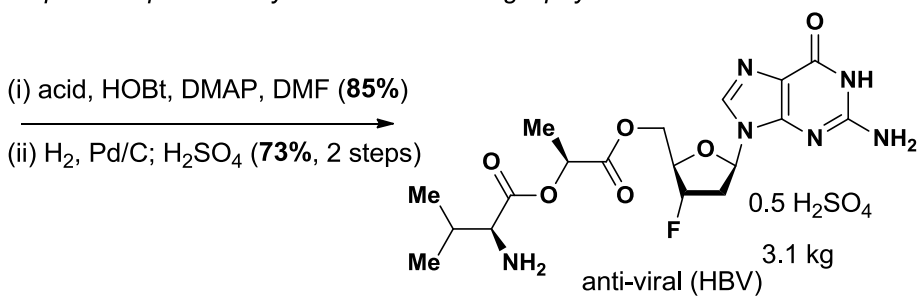
Nucleoside Chemistry

Fionn O'Hara

Synthesis of Lagociclovir Valactate: anomeric ratio is a big problem



extensive screening of solvent and Lewis acid requires separation by column chromatography



Wennerberg. *Org. Process Res. Dev.* **2011**, *15*, 1027-1032.

Adjusting anomeric ratio of glycosidic bond formation:

some evidence that more polar solvents increase levels of the α-anomer on these systems, presumably by favouring a more S_N1 than S_N2-like process

Howell. *J. Org. Chem.* **1985**, *53*, 85-88.

substitution of halosugars thought to be S_N2-like, so can get good ratios in chloroform where anomerisation of C-1' halide is slow

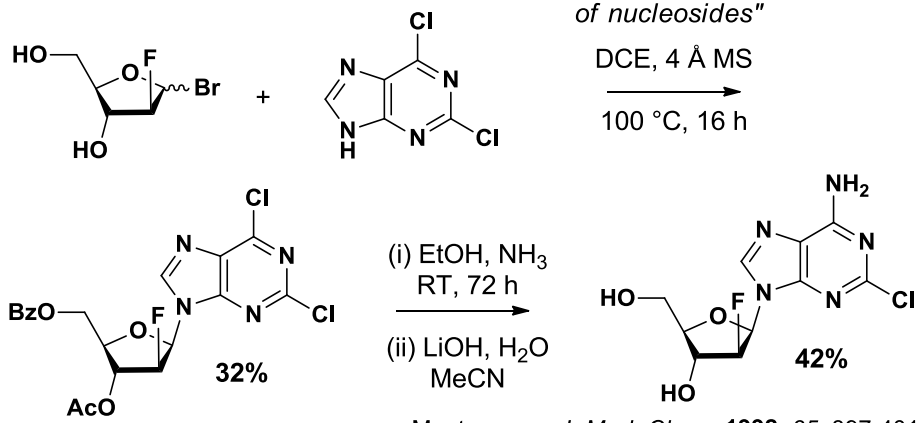
Walker. *Nucl. Acids Res.* **1984**, *12*, 6827-6837.

Synthesis of Clofarabine: extensive screening to improve anomeric ratio

DNA polymerase inhibitor, treat resistant leukaemias:

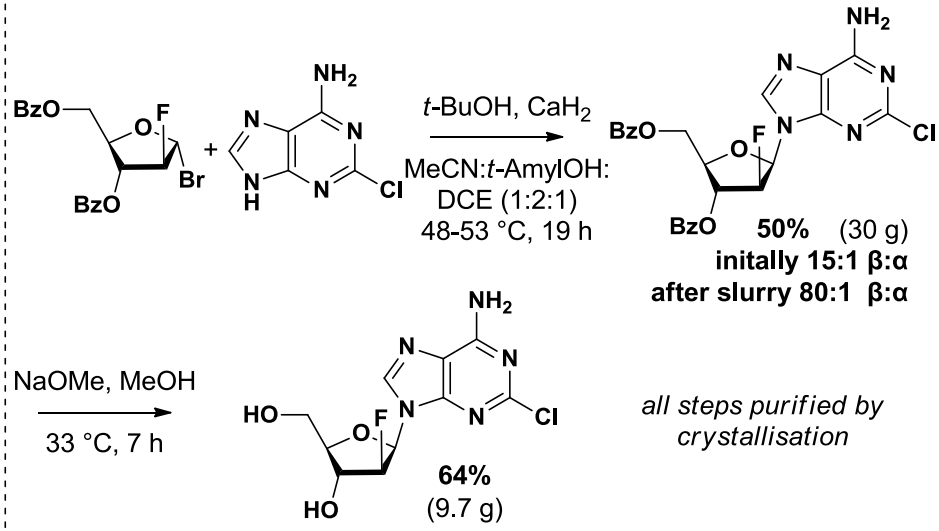
Initial route:

selectivity an issue? "production of a series of nucleosides"



Montgomery. *J. Med. Chem.* **1992**, *35*, 397-401.
Montgomery. *J. Med. Chem.* **1986**, *29*, 2389-2392.

Improvements on larger scale (particularly focussing on anomeric ratio):



all steps purified by crystallisation

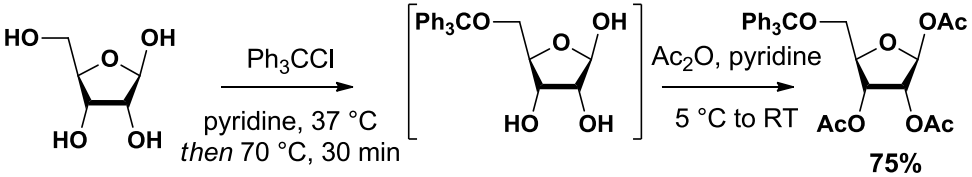
Bauta. *Org. Process Res. Dev.* **2004**, *8*, 889-896.

Nucleoside Chemistry

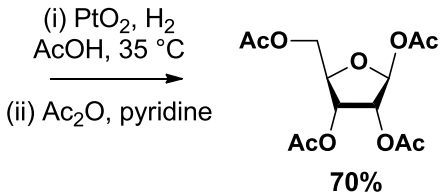
Fionn O'Hara

A brief look at some relevant sugar chemisty...

Todd's synthesis of 1,2,3,5-tetraacetyl D-ribofuranose (on the way to the first synthesis of cytidine):



shown is Barker's improved procedure for the trityl/acetyl ribose (approx. 3x yield)

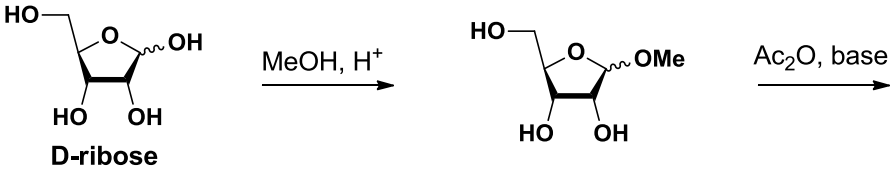


Todd. *J. Chem. Soc.* **1947**, 1052-1054.
Bredereck. *Chem. Ber.* **1940**, *73*, 956.
Barker. *J. Chem. Soc.* **1950**, 23-26.

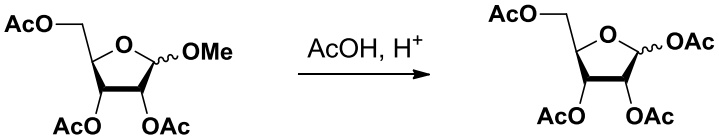
compounds primarily characterized by m.p., elemental analysis and $[\alpha]_D$... to make things even more difficult "a classic case of a disappearing polymorph"

Kálmán. *Angew. Chem. Int. Ed.* **2003**, *115*, 2001-2004.

A more practical approach for scale up...



methanolysis of most pentoses give methyl furanosides as kinetic product, which gradually convert to thermodynamic methyl pyranosides

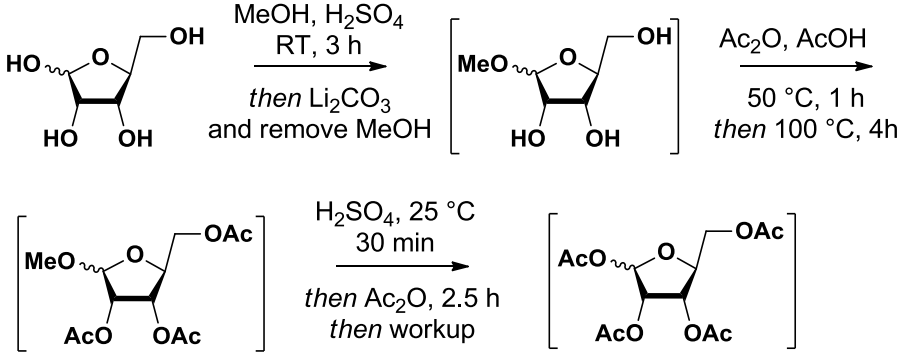


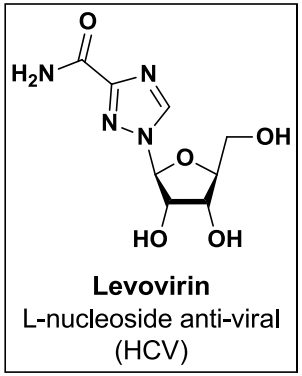
Guthrie. *Chem. Ind.* **1968**, 547.
Mowery. *J. Am. Chem. Soc.* **1955**, *77*, 1667-1669.

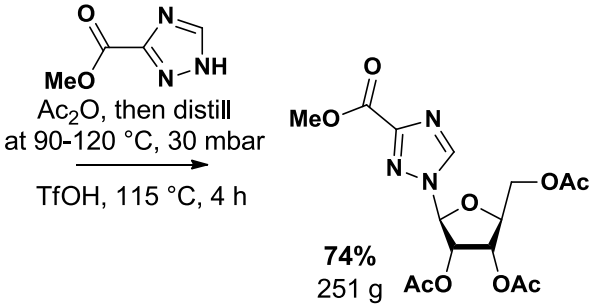
ribose in solution is an equilibrating mixture of the straight chain, ribofuranose and ribopyranose rings (both α and β anomers) so can't simply add Ac_2O

A similar concept for making the L-ribose analogue on large scale:

one-pot manufacturing procedure for a key intermediate of Levovirin (Roche)

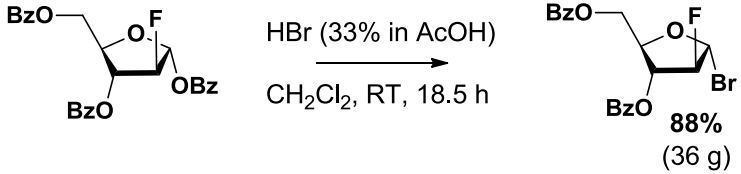






Zhang. *Org. Process Res. Dev.* **2005**, *9*, 583-592.

A scalable example of making a halosugar:



Bauta. *Org. Process Res. Dev.* **2004**, *8*, 889-896.

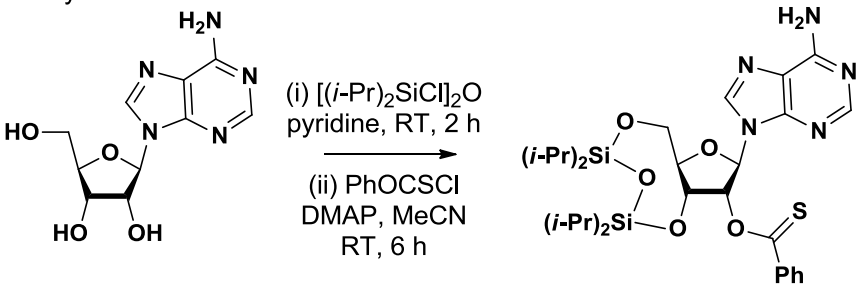
Nucleoside Chemistry

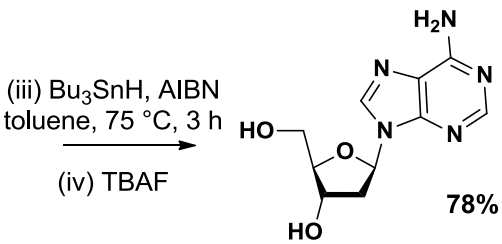
Fionn O'Hara

A brief look at some (more) relevant sugar chemisty...

making 2'-deoxyribonucleosides from ribonucleosides

useful if anchimeric assistance from the 2'-position was necessary to control anomeric ratio during glycosylation, or to convert available nucleosides into 2'-deoxynucleosides



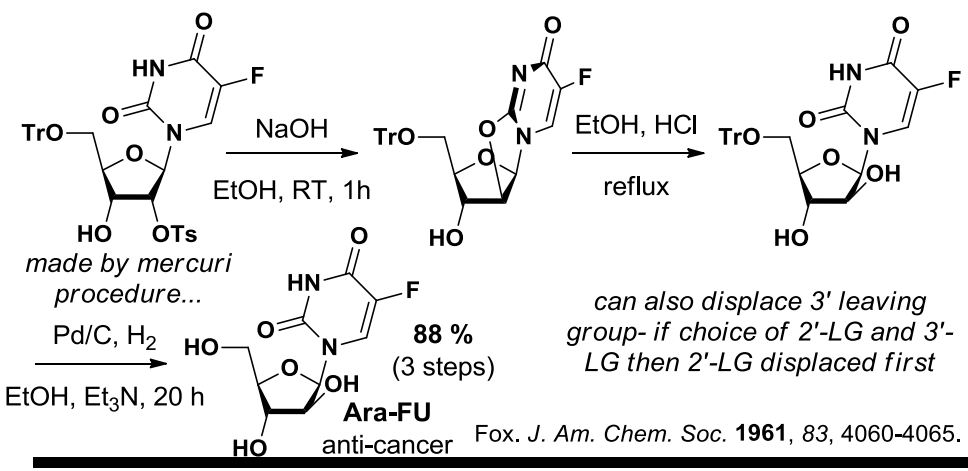


"This is superior to yields obtained in prior chemical syntheses of 2'-deoxyadenosine by any route."

Robins. *J. Am. Chem. Soc.* **1981**, *103*, 932-933.

exploiting anhydronucleosides (pyrimidines) to access modified sugars:

inverting hydroxyl groups at the C-2' and C-3' positions to make different sugars

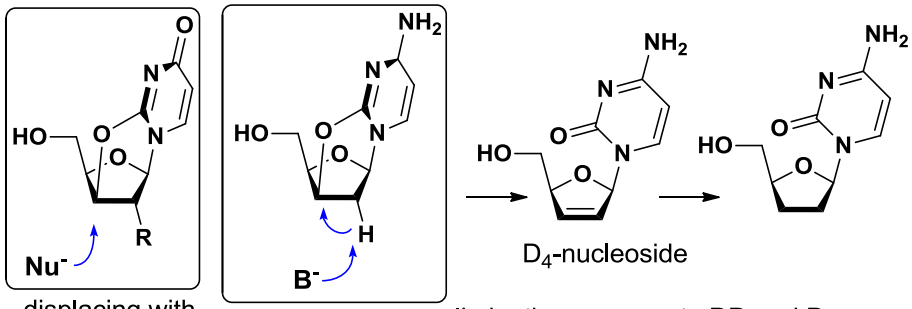


made by mercuri procedure...

can also displace 3' leaving group- if choice of 2'-LG and 3'-LG then 2'-LG displaced first

Fox. *J. Am. Chem. Soc.* **1961**, *83*, 4060-4065.

anhydronucleosides are also very useful for:

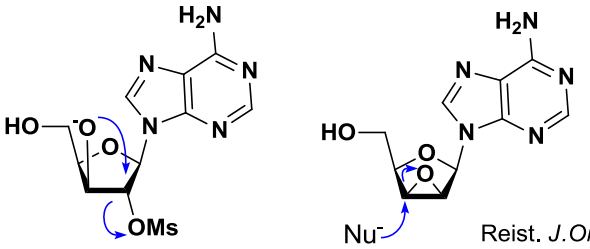


displacing with nucleophiles
Nu = halide, thiol hydride, azide, NH_3...

eliminating - access to DD and D_4 nucleosides e.g. DDC (early HIV drug)

Fox. *Pure Appl. Chem.* **1969**, *18*, 223-256.
Horwitz. *J. Org. Chem.* **1967**, *32*, 817-818.

if you have a purine, then epoxides can allow similar manipulations:



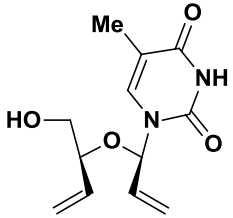
nucleophiles predominantly attack at C-3' on 2,3-anhydrofuranosides

Reist. *J.Org. Chem.* **1962**, *27*, 3274-3280.
Davoll. *J.Chem. Soc.* **1951**, 2230-2233.

Other ways of making 2',3'-didehydro-2',3'dideoxyribonucleosides:

from ribose derivatives: (i) reaction with α-acetoxyisobutyryl bromide and reductive elimination;
(ii) Corey-Winter olefination

more modern alternative:
ring closing metathesis



examples of sugar-modified nucleosides:

isonucleosides (2' or 3')

X= NR aza-nucleosides
X= S thionucleosides

rings with two heteroatoms (see Lamivudine)
changes in sugar ring size (see Oxetanocin)
carbocyclic nucleosides (see Abcavir)
L-nucleosides (see Levovirin)
acyclic nucleosides (see Acyclovir)

Nucleoside Chemistry

Fionn O'Hara

Carbocyclic nucleosides: all carbon cyclopentane "sugar" ring

stable to hydrolysis, enhanced biostability

natural products include aristeromycin and neplanocin A, useful for many drugs

Some typical ways of making carbocyclic nucleosides:

traditionally considered one of the most difficult types of nucleoside analogues to make (stereochemistry) but organometallic chemistry has given new options

(i) convert a sugar into a carbocycle, retaining stereoinformation (many steps)

(ii) derivatize a cyclopentadiene intermediate (but need to install stereochemistry)

(iii) form a cyclopentene intermediate by ring closing metathesis

Attaching the nucleoside:

(i) Alkylations: S_N2, Mitsunobu etc.

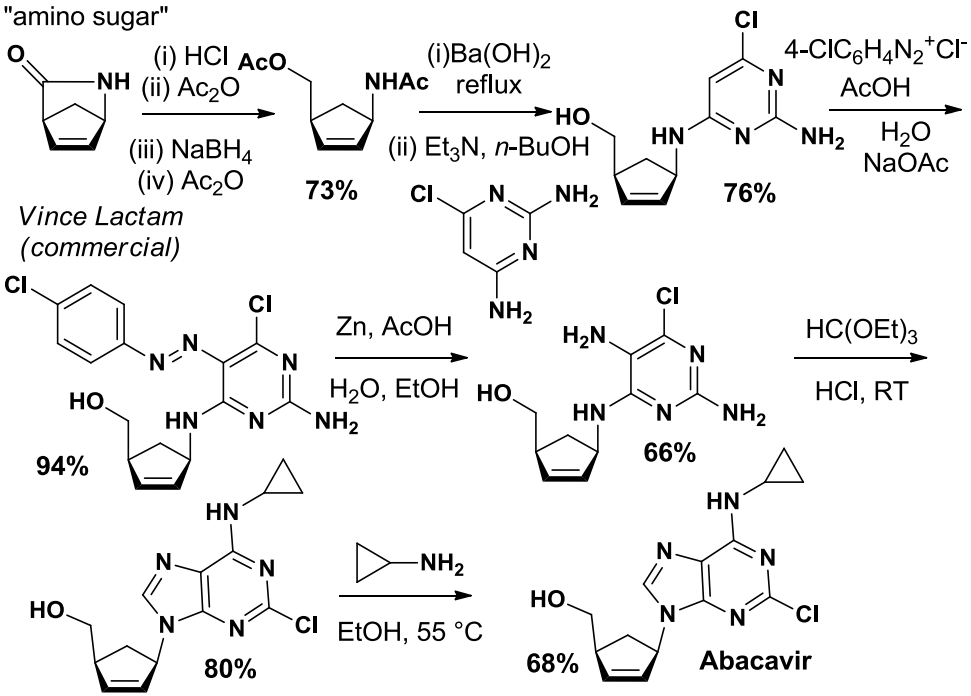
(ii) Building up base from a cyclopentyl amine derivative

(iii) Trost Pd-catalysed allylic alkylation

Trost. *J. Am. Chem. Soc.* **2000**, *122*, 5947-5956.

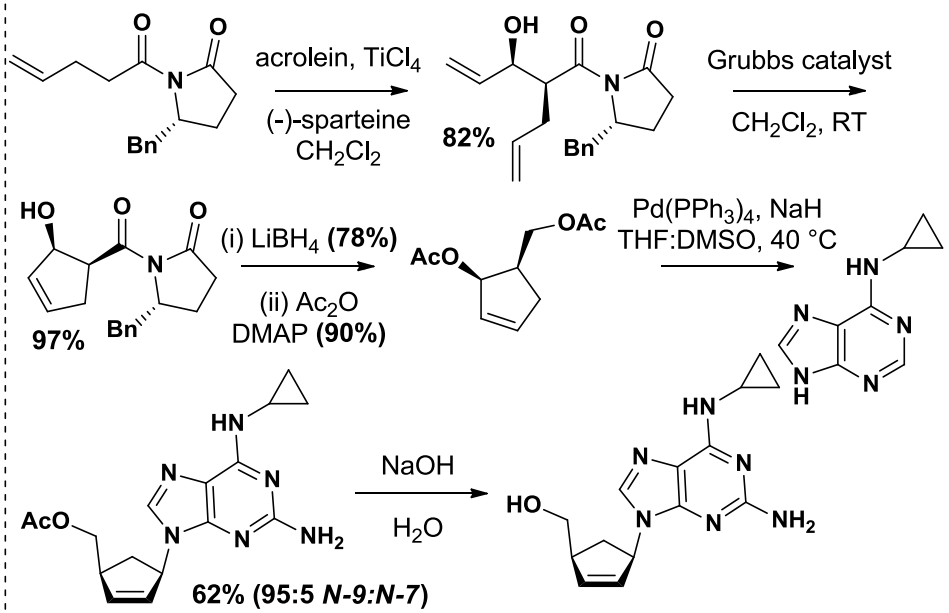
Abacavir: anti-viral medicine (HIV and AIDS)

Synthesis of Abcavir from Vince Lactarm: example of building base up from an "amino sugar"



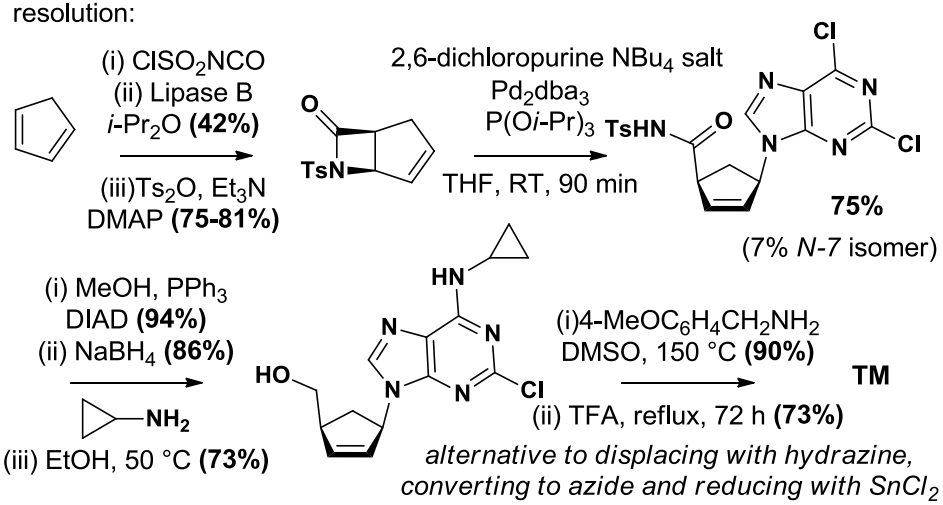
Vince. *Current Protocols in Nucleic Acid Chemistry*. **2006**, 14.41-14.48.

Crimmins synthesis: example of asymmetric aldol, ring closing metathesis and Trost-type allylic substitution approach to making carbocyclic nucleosides



Crimmins. *J. Org. Chem.* **2000**, *65*, 8499-8509.

Recent enantioselective synthesis of Abacavir (anti-HIV) uses enzymatic resolution:



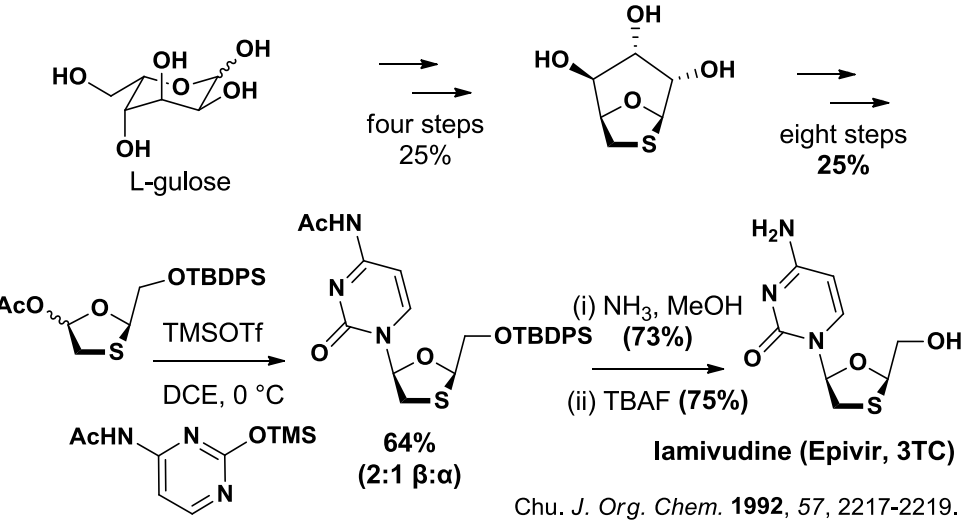
Morgans. *Org. Biomol. Chem.* **2012**, *10*, 1870-1876.

Nucleoside Chemistry

Fionn O'Hara

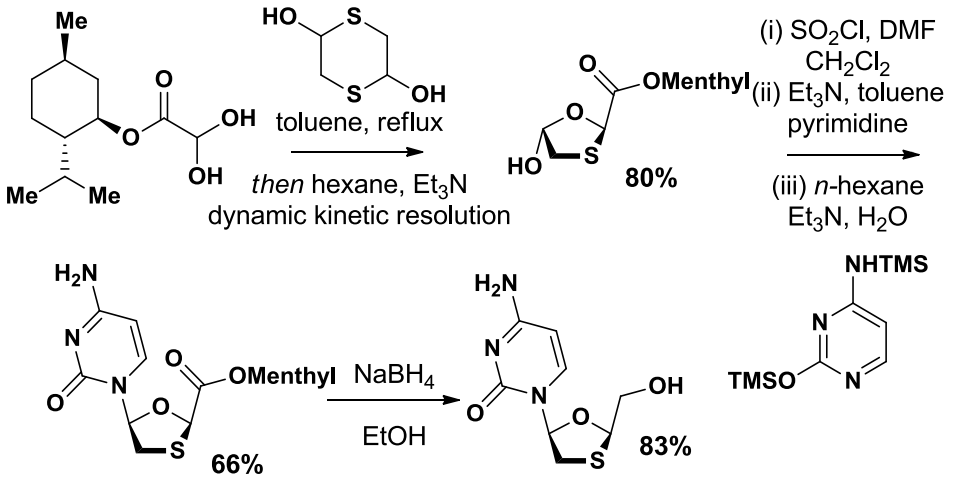
Lamvudine (Epivir, 3TC):L-nucleoside drug

anti HIV nucleoside analog reverse transcriptase inhibitor (nRTI), also HBV
first example of the L-nucleoside being more potent than the D-nucleoside



Chu. *J. Org. Chem.* **1992**, *57*, 2217-2219.

Glaxo's route suitable for large scale synthesis to support development:



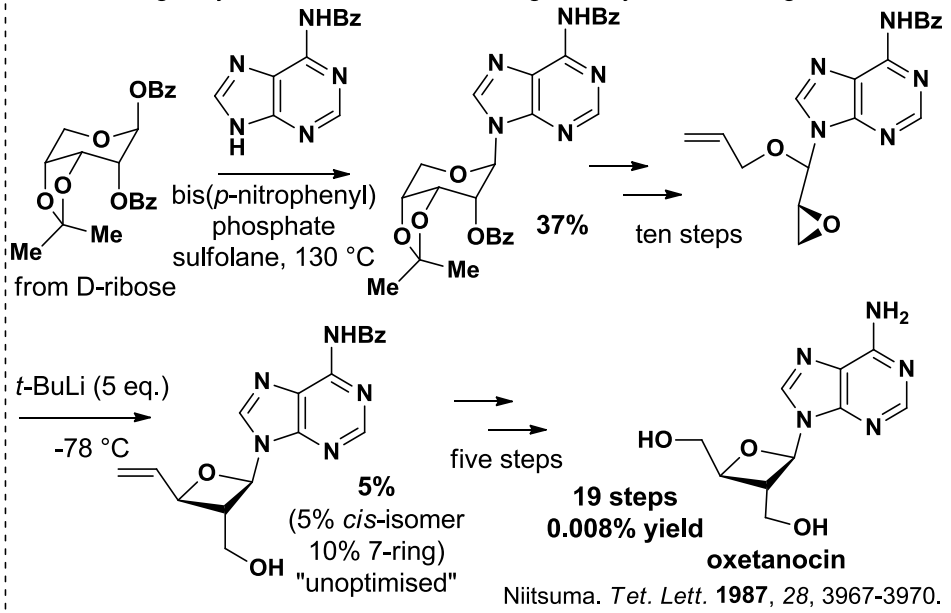
Whitehead. *Tet. Lett.* **2005**, *46*, 8538-8538.

large scale synthesis uses cocrystal formation with (S)-BINOL to purify

Roy. *Org. Process Res. Dev.* **2009**, *13*, 450-455.

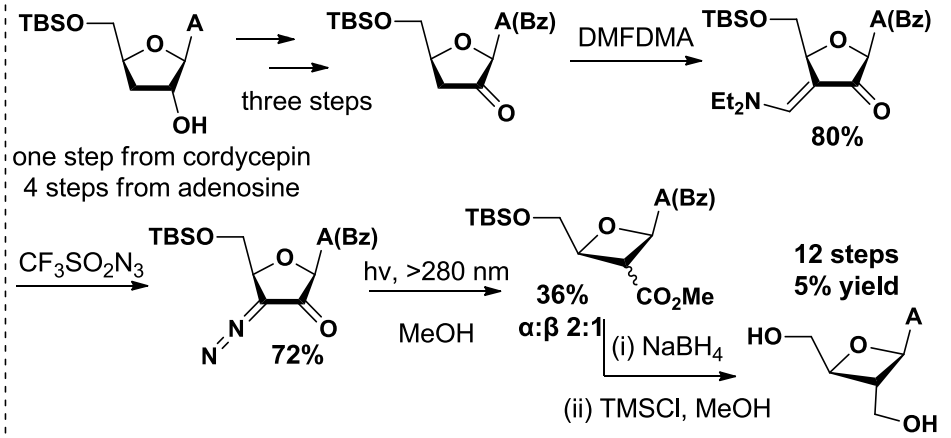
Oxetanocin: natural product with anti-HIV and anti-bacterial properties

as with most unusual sugar derivatives, both approaches make the unusual oxetane sugar by modification of an existing, readily available sugar



Niitsuma. *Tet. Lett.* **1987**, *28*, 3967-3970.
Niitsuma. *Tet. Lett.* **1987**, *28*,4713-4714.

Norbeck identifies oxetanocin as a structural isomer of cordycepin, accessible by ring contraction.



Norbeck. *J. Am. Chem. Soc.* **1988**, *110*, 7217-7218.

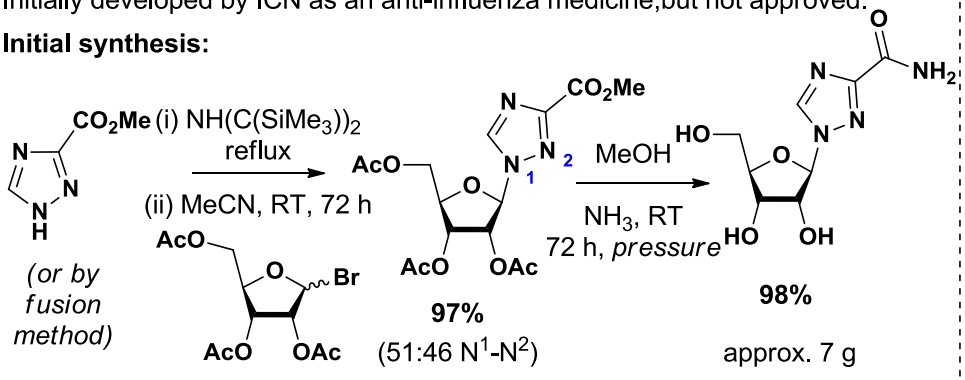
Nucleoside Chemistry

Fionn O'Hara

Ribavirin (Virazole): anti-viral for severe RSV and hepatitis C

Initially developed by ICN as an anti-influenza medicine,but not approved.

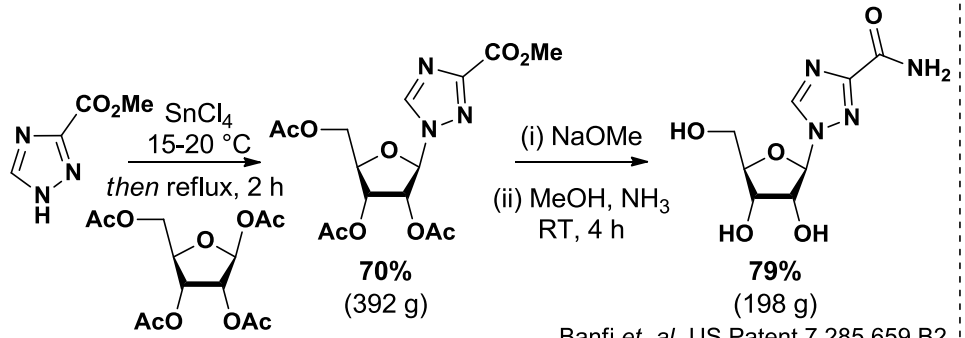
Initial synthesis:



Witkowski. *J. Med. Chem.* **1972**, *15*, 1150-1154.
Robins. *Science* **1972**, *177*, 705-706.

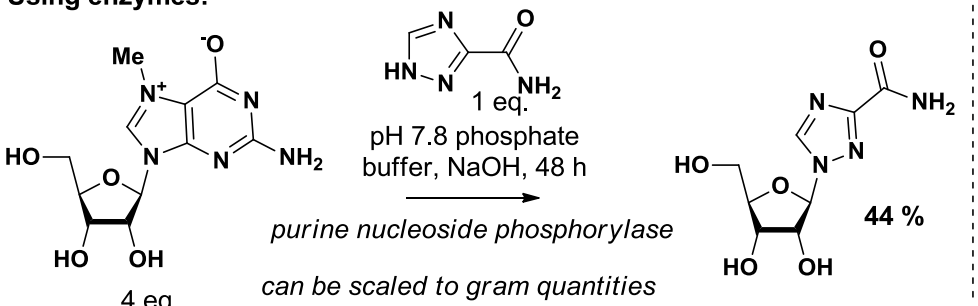
Scaling up:

Original process appears to scale pretty well. Larger scale modifications:



Banfi *et. al.* US Patent 7,285,659 B2

Using enzymes:

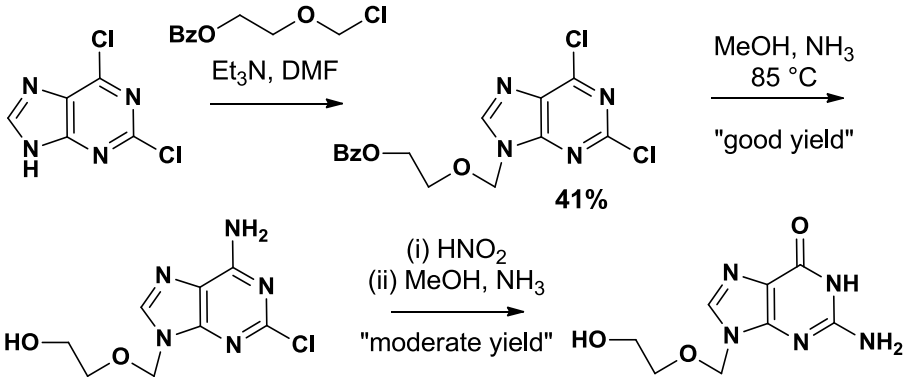


Wong. *J. Org. Chem.* **1989**, *54*, 4692-4695.

Acyclovir (Aciclovir): anti-viral against Herpes Simplex Viruses

studies had shown that intact sugars not necessary to mimic nucleoside binding to enzymes - development of acyclic nucleosides as medicines

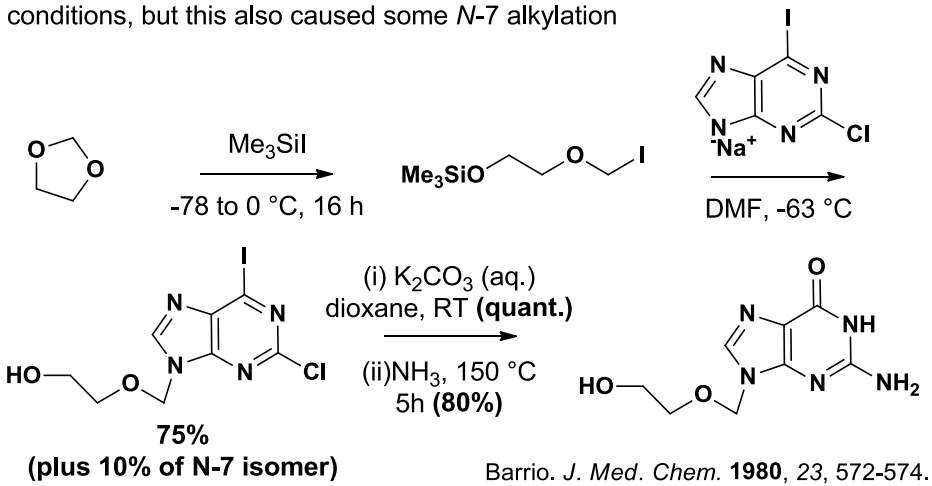
Initial synthesis:



Schaeffer. *Nature* **1978**, *272*, 583-585.

Improved procedure by Barrio:

used 2-chloro-6-iodopurine precursor due to milder nucleophilic displacement conditions, but this also caused some *N*-7 alkylation



Barrio. *J. Med. Chem.* **1980**, *23*, 572-574.
Barrio. *Tet. Lett.* **1979**, *35*, 3263-3265.

Elion and Hitchings work on drugs that exploited differences in nucleic acid metabolism led to the awarding of the 1988 Nobel Prize (along with Black for work on receptor blocking drugs) for discoveries of important principles of drug treatment.