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 iodouridine, 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 antiprotoszoal 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 Galt, M. Nucleic Acids in Chemistry and Biology.
Vaghefi, M. Nucleoside Triphosphates and their Analogs: Chemistry, Biotechnology, and Biological Applications.
Common disconnections towards nucleosides:

A. Formation of the glycosidic bond

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

useful for unusual sugars and carbocyclic derivatives:

Tsuiji-Trost allylic alkylation

Major methods for the construction of nucleosides:

useful for purines and analogues:

construct base around a C-1′-substituted sugar

construct purine around a C-1′-imidazole sugar

typically transfers sugars from pyrimidines to more basic purines

transglycosylation: either chemically or enzymatically
A. Formation of the glycosidic bond

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

**Fischer-Helfrich procedure:** first syntheses of adenosine and guanosine

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

Nucleoside Chemistry


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


(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:

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


*Fischer and Helfrich, Ber., 1914, 47, 210.*
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

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


(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

...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:


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


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

(i) HMDS, TMSCl MeCN, 80 °C [concentrate]
(ii) 1.1 eq. TMSOTf MeCN, 80 °C
(iii) MeOH, NH_3
(iv) MeOH, 80 °C

doesn't work for 2'-deoxyribose

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

\[
\begin{align*}
\text{TrO-} & \quad \text{Boc} \\
\text{TMS-juracil} & \quad \text{MeCN, -23 °C} \\
(i) \quad \text{PhSeBr} & \quad \text{MeOH} \\
\text{ZnBr}_2 & \quad \text{MeOH} \\
\text{H}_2\text{O}_2 & \quad \text{dioxane} \\
\text{NaHCO}_3 & \quad 87\% \\
\text{(10:1 β:α)} & \quad \text{aza-D4U} \\
\text{Pd/C, H}_2 & \quad \text{86% aza-DDU}
\end{align*}
\]


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

\[
\begin{align*}
\text{HO} & \quad \text{Pd(OAc)}_2 \\
\text{AsPh}_3 & \quad \text{5-iodouracil} \\
\text{TBDPSO} & \quad \text{TBDPSO} \\
\text{i} & \quad \text{TBAF} \\
\text{naHB(OAc)}_3 & \quad \text{HO} \quad 63%
\end{align*}
\]


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

\[
\begin{align*}
\text{HO} & \quad \text{EtO}_2\text{C} \\
\text{N} & \quad \text{CN} \\
\text{Bu} & \quad \text{OBz} \\
\text{NH}_2 & \quad \text{MeC(OEt)}_3 \\
\text{OEt} & \quad \text{reflux} \\
\text{AcOH, H}_2\text{O} & \quad 25% \text{ from azide}
\end{align*}
\]


C. Construct purine around a C-1' imidazole sugar
in this case used to get regioselectivity for an unusual N-7 purine nucleoside

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{DMSO (84%)} & \quad \text{HO} \quad \text{by fusion} \\
\text{i} & \quad \text{KCN, KI} \\
\text{Pd/C, H}_2 (87%) & \quad \text{72% brsm} \\
\text{(i) MeC(OEt)}_3 & \quad \text{reflux} \\
\text{(ii) NH}_3 & \quad \text{65%}
\end{align*}
\]


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 ribavirin)

\[
\begin{align*}
\text{HO} & \quad \text{N} \\
\text{purine nucleoside} & \quad \text{purine (1.1-2 eq.)} \\
\text{phosphorylase} & \quad \text{buffer, 35 °C, 72 h} \\
\text{thymidine phosphorylase} & \quad \text{11-69%}
\end{align*}
\]


Useful tricks for glycosidic bond formation on heterocyclic sugar analogues:

\[
\begin{align*}
\text{OR} & \quad \text{Nu'} \\
(i) \quad \text{NCS} & \quad \text{OR} \\
(ii) \quad \text{base} & \quad \text{Nu'} \\
\text{Pummerer-type with Ac}_2\text{O or TMS-base TMSOTf and Et}_3\text{N}
\end{align*}
\]

C-Nucleosides: nucleosides with a C-1'-Base C-C bond can be naturally occurring (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

(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

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
Synthesis of Lagociclovir Valactate: anomic ratio is a big problem

Synthesis of Clolarabine: extensive screening to improve anomic ratio DNA polymerase inhibitor, treat resistant leukaemias:

Initial route:

Selectivity an issue?
"Production of a series of nucleosides"

DCE, 4 Å MS
100 °C, 16 h

Improvements on larger scale (particularly focusing on anomic ratio):

Adjusted anomic 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


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

A brief look at some relevant sugar chemistry...

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

\[
\text{HO} - \text{O} - \text{HO} \quad \xrightarrow{\text{Ph}_3\text{CCl, pyridine, 37 °C, then 70 °C, 30 min}} \quad \text{HO} - \text{O} - \text{Ac} - \text{O} - \text{OAc} \\
\quad \xrightarrow{\text{Ac}_2\text{O, pyridine, 5 °C to RT}} \quad 75\%
\]

(i) \text{PtO}_2, \text{H}_2, \text{AcOH, 35 °C}

(ii) \text{Ac}_2\text{O, pyridine}

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

70%

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


A more practical approach for scale up...

\[
\text{HO} - \text{O} - \text{OH} \\
\quad \xrightarrow{\text{MeOH, H}^+} \quad \text{HO} - \text{O} - \text{Me} \\
\quad \xrightarrow{\text{Ac}_2\text{O, base}} \quad \text{HO} - \text{O} - \text{Ac} - \text{O} - \text{OAc}
\]

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

Guthrie. Chem. Ind. 1968, 547.

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

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

\[
\text{HO} - \text{O} - \text{OH} \quad \xrightarrow{\text{MeOH, H}_2\text{SO}_4, \text{RT, 3 h}} \quad \text{HO} - \text{O} - \text{Ac} - \text{O} - \text{OAc} \\
\quad \xrightarrow{\text{then Li}_2\text{CO}_3 and remove MeOH}} \quad \text{HO} - \text{O} - \text{Ac} - \text{O} - \text{OAc}
\]

\[
\xrightarrow{\text{Ac}_2\text{O, AcOH, then workup, after distilling at 90-120 °C, 30 mbar}} \quad \text{AcO} - \text{O} - \text{Ac}
\]

\[
\text{H}_2\text{SO}_4, 25 °C, 30 min \quad \text{Ac}_2\text{O, 2.5 h then workup}
\]

74%

251 g


A scalable example of making a halosugar:

\[
\text{BzO} - \text{O} - \text{F} - \text{HBr (33% in AcOH, CH}_2\text{Cl}_2, \text{RT, 18.5 h}} \quad \text{BzO} - \text{O} - \text{F} - \text{Br}
\]

88% (36 g)

Nucleoside Chemistry

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

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


(i) [(i-Pr)₂SiCl]₂O pyridine, RT, 2 h
(ii) PhOCSCI DMAP, MeCN RT, 6 h
(iii) Bu₃SnH, AiBN toluene, 75 °C, 3 h
(iv) TBAF

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...
Pd/C, H₂

88 % (3 steps)

Other ways of making 2',3'-didehydro-2',3'-dideoxynucleosides:
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 X= NRaza-nucleosides (2' or 3') thionucleosides (L- or Levovirin) acyclic nucleosides (see Acyclovir)

Anhydronucleosides are also very useful for:
displacing with nucleophiles Nu = halide, thiol hydride, azide, NH₃... eliminating - access to DD and D₄ nucleosides e.g. DDC (early HIV drug)


if you have a purine, then epoxides can allow similar manipulations:
Carbocyclic nucleosides: all carbon cyclopentane "sugar" ring stable to hydrolysis, enhanced biostability. Natural products include aristomycin 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

Abacavir: anti-viral medicine (HIV and AIDS)
Synthesis of Abacavir from Vince Lactam: example of building base up from an "amino sugar"

Vince Lactam (commercial)

Abacavir

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

**Nucleoside Chemistry**

**Lamivudine (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

![Chemical Structures](image)


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

- SO₂Cl, DMF
- CH₂Cl₂
- Et₃N, toluene, pyrimidine
- (iii) n-hexane, Et₃N, H₂O


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


**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

![Chemical Structures](image)


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

- one step from cordycepin
- 4 steps from adenosine

Ribavirin (Virazole): anti-viral for severe RSV and hepatitis C
Initially developed by ICN as an anti-influenza medicine, but not approved.

Initial synthesis:

\[
\begin{align*}
&\text{CO}_2\text{Me} \quad \text{(i) NH(C(SiMe_3)_2)}_2 \\
&\text{reflux} \\
&\text{(ii) MeCN, RT, 72 h} \\
&\text{Br} \\
&\text{AcO} \\
&\text{AcO} \\
&\text{51:46 N'-N}^2
\end{align*}
\]

70% (392 g)

Scaling up:

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

\[
\begin{align*}
&\text{CO}_2\text{Me} \quad \text{SnCl}_4 \\
&15-20 \degree \text{C} \\
&\text{then reflux, 2 h} \\
&\text{AcO} \\
&\text{AcO} \\
&\text{70%} \quad \text{(392 g)}
\end{align*}
\]

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:

\[
\begin{align*}
&\text{Cl} \\
&\text{BzO} \\
&\text{Cl} \\
&\text{Et}_3\text{N, DMF} \\
&\text{MeOH, NH}_3 \\
&85 \degree \text{C} \\
&\text{"good yield"}
\end{align*}
\]

Improved procedure by Barrio:


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

Using enzymes:


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.