Recent Advances in the Chemical Synthesis and Evaluation of Anticancer Nucleoside Analogues

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Academic Editor: Derek J. McPhee
Received: 2 April 2020; Accepted: 25 April 2020; Published: 28 April 2020

Abstract: Nucleoside analogues have proven to be highly successful chemotherapeutic agents in the treatment of a wide variety of cancers. Several such compounds, including gemcitabine and cytarabine, are the go-to option in first-line treatments. However, these materials do have limitations and the development of next generation compounds remains a topic of significant interest and necessity. Herein, we discuss recent advances in the chemical synthesis and biological evaluation of nucleoside analogues as potential anticancer agents. Focus is paid to 4′-heteroatom substitution of the furanose oxygen, 2′-, 3′-, 4′- and 5′-position ring modifications and the development of new prodrug strategies for these materials.

Keywords: nucleoside analogue; anti-cancer; chemical synthesis; heteroatom replacement; chemotherapeutic; prodrug

1. Introduction

A significant proportion of current chemotherapeutic treatments for cancer involve the use of anti-metabolites, particularly modified nucleoside analogues that possess a capability to mimic native purine or pyrimidine nucleosides which can disrupt metabolic and regulatory pathways [1]. These molecules can be taken up by nucleoside transporters and then phosphorylated to their mono-, di- and triphosphate forms where they are able to interfere with DNA/RNA synthesis and repair; for example, by acting as chain terminators [2] or ribonucleotide reductase inhibitors [3]. Other notable modes of action include epigenetic regulation, through inhibition of DNA regulatory proteins, such as DNA methyltransferase [4]. Selected current examples of anticancer nucleoside analogues approved for chemotherapeutic treatment regimens include capecitabine, gemcitabine 1, clofarabine 2 and cytarabine (Ara-C) 3 (Figure 1).

![Figure 1](image-url)  
*Figure 1. Gemcitabine 1, clofarabine 2 and Ara-C 3. Modifications compared to native n-ribo-configured purine or pyrimidine nucleosides are shown in blue.*
Therapeutic intervention using nucleoside analogues is not without its problems and their use is often limited by poor cellular uptake, low conversion to the active triphosphate metabolite, rapid degradation or clearance and development of resistance profiles in certain cell types [5,6]. Consequently, research continues to develop next generations of nucleoside analogues that overcome some of these limitations and provide new therapeutic options.

This class of antimetabolite also possess a proud history, and current frontline treatment, as antiviral [7–12] and, more recently, antibacterial agents [13]. Indeed, the development of nucleoside analogues has a symbiotic relationship between compound class and final therapeutic treatment. For example, gemcitabine 1 was developed as an antiviral, but was subsequently shown to be very toxic to leukaemia cells.

In this review, we survey developments from 2010 onwards for the chemical synthesis and evaluation of modified nucleoside analogues for anticancer research. Specifically, focusing on alterations to the native furanose ring (Figure 2) and generally retaining native purine or pyrimidine nucleobases. Comprehensive reviews concerning hetero-base modifications and general trends in nucleotide synthesis have been covered elsewhere [14,15]. The review is divided into sections that systematically consider: i) furanose 4'-oxygen atom replacements with N, S, Se and C ii) 2',3',4' or 5'-position furanose ring modifications and iii) new prodrug approaches to deliver nucleoside analogues.

![General scope for nucleoside analogues](image)

**Figure 2.** General scope for nucleoside analogues covered in this review. Base = purine or pyrimidine (i.e., C, U, T, A, G or close derivative thereof). X = heteroatom or carbon and Y and Z = ring functional group or modification of native d-ribo stereochemistry.

## 2. Furanose Oxygen Atom Replacements

### 2.1. Azanucleosides

Azanucleosides were originally defined as nucleoside analogues where the furanosyl oxygen is replaced by nitrogen, however this group of analogues has been extended to include nucleosides where the resultant pyrrolidine core has been replaced by other nitrogen containing rings, including heterocycles, heterotricycles and acyclic nitrogen-containing nucleosides [16,17]. This class of compound have proven successful in the treatment of cancer [18] and have also been established as having antiviral and antibacterial properties [19].

**Development of Forodesine**

Purine nucleoside phosphorylases (PNPs) are responsible for the phosphorolytic metabolism of purine nucleosides to ribose/deoxyribose phosphate and the corresponding nucleobase. Patients with abnormally low levels of PNP possess little T-cell immunity due to a severely reduced degradation of deoxoguanosine, which results in the accumulation of the corresponding triphosphate (dGTP). This then reduces the activity of ribonucleotide reductase and induces apoptosis. As such, human PNP inhibitors are potential treatments for T-cell lymphomas [17]. Immucillin H (forodesine) 4 (Figure 3) is a highly potent PNP inhibitor (IC₅₀ = 0.48–1.57 nM) which is effective against T-cell malignancies and was found to have excellent oral bioavailability in mice (63%) [20].

Forodesine is a gradual onset drug which binds tightly to PNP with a high affinity \(K_i = 0.023\) nM \[21\]. Whilst clinical development of 4 was discontinued in the US and Europe, it was recently approved for use in the treatment of relapsed/refractory peripheral T-cell lymphoma (PCTL) in Japan (April 2017) \[22\].

Forodesine is a guanosine analogue and a transition state inhibitor of PNP with 100–1000-fold higher potency than previously identified inhibitors \[23\]. Due to the combined substitution of the furanosyl oxygen with nitrogen and the C-glycosidic bond, 4 is not incorporated into DNA, acting only as a highly selective PNP inhibitor \[22\]. Also noteworthy is an adenosine mimetic of 4 which is currently under development as a broad-spectrum antiviral \[24\].

In 2000, Tyler et al. described a linear synthesis of 4 in a satisfactory 39% yield over 10 steps (Scheme 1) \[25\]. Starting from 5, synthesised in nine steps by known methods from D-gulonolactone \[26\], the pyrroolidine was treated with N-chlorosuccinimide (NCS), obtaining the 1-chloro anomeric glycoside which subsequently underwent elimination using lithium tetramethyipiperidine (LiTMP) to afford imine 6. The nucleobase was next assembled via addition of lithiated acetonitrile to afford 7, followed by protection of the furanosyl nitrogen giving 8 and treatment with Bredereck’s reagent to afford enamine 9. Acid-catalysed hydrolysis of 9 delivered enol 10 which was reacted with ethyl glycinate to obtain enamine 11. Treatment of 11 with excess benzyl chloroformate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) revealed 12 and subsequent hydrogenolysis of the N-Cbz group provided pyrrole 13. Completion of the carboxyclic nucleobase was achieved via treatment of 13 with formamidine acetate and acidic removal of the silicon, nitrogen and isopropylidene protecting groups, to afford 4.

![Figure 3. Structure of forodesine 4.](image-url)

Scheme 1. Reagents and conditions: (i) NCS, pentane; (ii) LiTMP, −78 °C, 36% over two steps; (iii) n-BuLi, MeCN, THF, −78 °C then tetramethylpiperidine −78 °C, 100%; (iv) (Boc)₂O, CH₂Cl₂; (v) BuOC(O)(NMMe₂)₂, DMF, 70 °C; (vi) THF, AcOH, H₂O, 72% from 7; (vii) H₂NCH₂CO₂Et, HCl, NaOAc, MeOH; (viii) CI₂CO₂Bn, DBU, CH₂Cl₂, reflux, 67% from 10; (ix) H₂, Pd/C, EtOH; (x) H₂NCH=NH AcOH, EtOH, reflux, 91% from 12 and (xi) TFA, 81%.
Forodesine was found to have low oral bioavailability (<11%) in primates, contrary to the case in mice (63% [20]) and was thus originally developed as an intravenous formulation [21]. In 2005, Morris, Jr et al. synthesised BCX-3040, the 2′-deoxy analogue of 4, and comparatively evaluated its oral pharmacokinetic and pharmacodynamic properties in an effort to maintain potency and deliver oral bioavailability [27]. This was hypothesised from 2′-deoxyguanosine exhibiting tight binding to PNP [28] and therefore a possible redundancy for the 2′-OH. Starting from vinyl bromide 14 (Scheme 2), the 9-deazapurine underwent bromine-lithium exchange and addition to imine 6, obtaining nucleoside 15 in 85% yield. Subsequent removal of 5′-O-TBS and 2′,3′-O-isopropylidene groups gave 16, followed by 3′,5′-OTIPDS protection to afford 17. The free 2′-hydroxyl group was then converted to thiocarbonate 18 and deoxygenated via treatment with 1,1′-azobis(cyclohexane-1-carbonitrile) in excellent yield (91%) to give 19. Deprotection was completed in two steps, first cleaving the CH$_2$OCH$_2$Ph (BOM) group, followed by acidic hydrolysis and hydrochloride salt formation to obtain **BCX-3040** in 83% yield (from 19).

![Scheme 2](image)

Scheme 2. Reagents and conditions: (i) 6, BuLi, anisole, ether, −70 °C; (ii) (Boc)$_3$O, CH$_2$Cl$_2$, 85% over two steps; (iii) 1M HCl, MeOH, 30 °C, 96%; (iv) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine, 81%; (v) O-Phenyl chlorothionoformate, MeCN, 90%; (vi) 1,1′-Azobis(cyclohexane-1-carbonitrile), toluene reflux, 91%; (vii) Pd(OH)$_2$, H$_2$, EtOH, conc. NH$_2$OH, 90% and (viii) conc. HCl, MeOH, reflux, 83%.

In vitro biological evaluation of **BCX-3040** confirmed it to be a potent PNP inhibitor, with near identical $IC_{50}$ values to 4 (**BCX-3040** $IC_{50} = 3.1 \pm 0.50$ nM and 4 $IC_{50} = 1.2 \pm 0.21$ nM). Administration of 5.0 mg of 4 had a 12.6-fold greater 2′-deoxyguanosine response than administration of 10.0 mg of **BCX-3040**, indicating a reduced bioavailability for **BCX-3040**. Furthermore, following IV administration of 5 mg/kg of **BCX-3040**, the plasma concentration of **BCX-3040** dropped rapidly to 3.0 ± 0.31 µg/mL. Overall, due to the observed rapid clearance and reduced bioavailability of **BCX-3040**, it was concluded to be a poorer PNP inhibitor candidate in comparison to 4.
2.2. Thionucleosides

4′-Thiofuranoses are of known importance in biological systems, for example as chemical biology or biomedical tools [29], and possess chemotherapeutic activity [30,31]. Furthermore, the thioaminal moiety within 4′-thiofuranosyl nucleosides has been proven to be resistant to metabolic hydrolysis in comparison to native 4′-oxa analogues [32]. In the early 1990s, Secrist, Montgomery and co-workers stimulated interest in this class of molecule with their synthesis and biological evaluation of 2′-deoxy-4′-thiopyrimidine nucleosides [30]. Since then, there has been a resurgence of interest in the synthesis and evaluation of these compounds as potential antiviral and chemotherapeutic agents.

2.2.1. 2′-Modified Thionucleosides

Yoshimura et al. reported the synthesis and biological evaluation of 4′-thia-1-(2-deoxy-2-C-methylene-β-d-erythro-pentofuranosyl)cytosine (4′-thio-DMDC) 28β and 2′-deoxy-2′-fluoro-arabino-4′-thiacytidine 33β as potential antitumour agents [33–35]. Their synthesis of 28 started from 1,2,5,6-diospropylidene-α-glucose 20 and a series of protecting group manipulations delivered 3-O-benzyl α-xylose methyl glycoside 21 (Scheme 3). Mesylation of the 2- and 5-hydroxyl groups enabled reaction with sodium sulfide to afford 2,5-bicyclic intermediate 22 as a mixture of anomers. Following conversion of 22 to 4-thioarabinofuranose 23, the 2-position hydroxyl group was oxidised and the ketone homologated using a Wittig reagent to install the 2-methylene component, with this material then oxidised to sulfoxide 24.

![Scheme 3](image)

Scheme 3. Reagents and conditions: (i) Br, NaH, DMF, THF; (ii) 2M HCl, THF; (iii) NaIO₄, H₂O, MeOH; (iv) NaBH₄, MeOH, 84% from 20; (v) 5% HCl/MeOH, 91%; (vi) MsCl, pyridine; (vii) Na₂S, DMF, α-anomer 78% from 21, β-anomer 73% from 21; (viii) 4M HCl, THF; (ix) NaBH₄, MeOH, 90% from 21; (x) TBPSCI, imidazole, DMF, 87%; (xi) Ac₂O, DMSO; (xii) Ph₃PCH₂Br, NaH, t-amyl alcohol, THF; (xiii) BCl₃, CH₂Cl₂, −78 °C then MeOH, pyridine, 92%; (xiv) m-CPBA, CH₂Cl₂, −78 °C, 74% from 23; (xv) 25, TMSOTf, ClCH₂CH₂Cl, 0 °C, 29%; (xvi) TBAF, THF and (xvii) aqueous NH₃, MeOH then HPLC separation.

The cytidine nucleobase was installed using a Pummerer-type thioglycosylation, via sulphenium ion 26, which afforded 27 as a mixture of anomers. These were fully deprotected to afford 28α and 28β and the desired 28β isolated using HPLC separation.

Additionally, 2′-deoxy-2′-fluoro-arabino-4′-thiacytidine 33 was prepared from intermediate 23 (Scheme 4). Stereospecific DAST fluorination of 23 proceeded through epi-sulphonium intermediate 29 which delivered the 2-deoxy-2′-fluoroarabinose intermediate 30 in 68% yield. m-CPBA oxidation to the
sulfoxide 31 and subsequent Pummerer rearrangement formed anomic acetate 32 in 77% yield from 30. Finally, thioglykosylation was successfully employed to access the corresponding 4'-thionucleoside mixture 33 (54% yield). Comparatively, the group found that using 32 as a donor and employing silyl-Hilbert-Johnson glycosylation conditions yielded 33α/β in higher yield (93%), but still as a mixture of anomers (2.9:1 α/β).

Scheme 4. Reagents and conditions: (i) DAST, CH$_2$Cl$_2$, −78 °C, 77%; (ii) m-CPBA, CH$_2$Cl$_2$, −78 °C; (iii) Ac$_2$O, 100 °C, 77% from 23; (iv) 25, SnCl$_4$, MeCN, 93%; (v) BBr$_3$, MeOH; (vi) NH$_4$F, MeOH, 60 °C and (vii) aqueous NH$_3$, MeOH then HPLC separation, 43% (β-anomer) and 17% (α-anomer) from 32.

Finally, the group synthesised 2'-deoxy-2'-difluoro-1'(4'-thia-α-ribofuranosyl)cytosine 37, a thionucleoside analogue of the potent chemotherapeutic agent gemcitabine 1 (Scheme 5). Intermediate 23 was again utilised and oxidised using Albright-Goldman conditions to obtain ketone 34 which was difluorinated at C2 using DAST. The 3-O-benzyl group was next removed and replaced with benzoate to afford 35 in 79% overall yield from 34. Oxidation to the sulfoxide afforded 36 which subsequently underwent Pummerer rearrangement-glycosylation, before the remaining protecting groups were removed to afford 37α/β (α/β = 2.4:1) in a moderate yield of 51% from 36.

Scheme 5. Reagents and conditions: (i) Ac$_2$O, DMSO; (ii) DAST, benzene, 0 °C-r.t., 48%; (iii) BCl$_3$, CH$_2$Cl$_2$, −78 °C, then MeOH, pyridine; (iv) Bz$_2$O, Et$_3$N, DMAP, MeCN, 79% from 34; (v) m-CPBA, CH$_2$Cl$_2$, −78 °C; (vi) 25, TMSOTf, CICH$_2$CH$_2$Cl, 0 °C, 57% from 35; (vii) TBAF, THF and (viii) aqueous NH$_3$, MeOH, then HPLC separation, 36% (α-anomer) and 15% (β-anomer) from 36.

The antineoplastic activities of 28, 33 and 37 were evaluated and compared to arabinocytidine 3 (Ara-C) and 1-(2-deoxy-2-C-methylene-β-d-erythro-pentofuranosyl)cytosine (DMDC) (Table 1). As expected, all the α-anomer forms were found to be inactive against T-cell leukemia CCRF-HSB-2 cells. However, the β-anomers showed considerable cytotoxic activity against the same cell line. Notably, 28β and 33β were highly potent against both T-cell leukemia CCRF-HSB-2 cells and human solid tumour KB cells, with IC$_{50}$ values of 0.01 µg/mL (CCRF-HSB-2) and 0.05 µg/mL (CCRF-HSB-2) for 28β and 33β, respectively. Indeed, the activity of 28β was significantly higher than that of its native counterpart, DMDC, against both cell lines, with an IC$_{50}$ value 2.4 times lower in CCRF-HSB-2 cells and
3.7 times lower in KB cells. Interestingly, 4′-thiogemcitabine analogue 37β had poorer antineoplastic activity compared to 1, which the authors suggest may be due to a reduction in the phosphorylation efficacy of 37β by deoxycytidine kinase, a key enzyme which converts 2′-deoxycytidine analogues to their corresponding monophosphates.

| Table 1. Antineoplastic activities of 2′-modified-4′-thionucleosides. |
|---------------------------------------------------------------|
| **Compound (Anomer)** | **2′-Substituent** | **CCRF-HSB-2 a** | **KB Cells b** |
|-----------------------|------------------|-----------------|----------------|
| 28α                   | =CH₂             | >10             | ND c           |
| 28β                   | =CH₂             | 0.01            | 0.12           |
| 33α                   | F (arabino)      | >10             | ND             |
| 33β                   | F (arabino)      | 0.05            | 0.02           |
| 37α                   | F₂               | >10             | ND             |
| 37β                   | F₂               | 1.5             | 17             |
| Ara-C 3               |                  | 0.05            | 0.26           |
| DMDC                  |                  | 0.02            | 0.44           |

* a MTT assay [36]; b dye uptake method [36]; c not determined.

2.2.2. 4′-Modified-2′-deoxythionucleosides

Following earlier work by Parker and colleagues, who identified 4′-thia-2′-deoxycytidine (T-dCyd) as being able to inhibit tumour growth [37], Haraguchi and colleagues reported the synthesis and evaluation of a small library of 4′-position modified 4′-thia-2′-deoxycytidine nucleosides 38–41 for their antineoplastic and antiviral activity (Figure 4) [38].

![Figure 4. Structures of 2′-deoxy-4′-thiacytidine nucleosides 38–41.](image)

Towards analogue 38 the group started from thioglycal 42 (Scheme 6), obtained using established procedures from 2-deoxy- α-ribose in 12 steps [39]. This material was transformed into a glycosyl donor through treatment with N-iodosuccinamide (NIS) and pivalic acid, giving one diastereoisomer of 2-iodo derivative 43. Silylated uracil was then glycosylated with 43 using Vorbrüggen-type conditions to give 1β-4′-thiouridine 44 which was 2′-deoxygenated via a Bu3SnH mediated radical reduction to give 45. Subsequent TIPDS deprotection, followed by C3′ and C5′-O-acetylation delivered 46 to enable a four-step procedure to deliver 4′,5′-unsaturated-4′-thiouridine derivative 47. Exo-thioglycal 47 was next converted to silyl-protected 4′-thionucleoside 48, which when treated with Pb(OBz)₄ yielded dibenzoate 49. S₉₂ inversion back to the native 4′-α-ribo configuration and 4′-azide installation was achieved by reaction with MeSiN₃ in the presence of SnCl₄ and afforded the desired 4′-α-azido derivative 50 as the major product. Finally, the nucleoside was converted to the cytidine form via intermediate 51 to give 38 in 7% yield over 18 steps.
In order to access 2'-deoxy-4'-C-fluoromethyl-4'-thiacytidine 39, alcohol 53 was prepared from known aldehyde 52 [40,41]. Treatment of 53 with DAST successfully installed the key 4-α-fluoromethyl group which was then elaborated to 39 (Scheme 7). 4-α-alkynyl and nitrile analogues 40 and 41 were synthesised from aldehyde 54 using a late-stage insertion of the key functional group at the 4'-position in the presence of the nucleobase. Of the four 2'-deoxy-4'-modified thionucleosides 38-41, two analogues, 38 and 39, showed moderate cytotoxicity against human B-cell leukaemia (CCRF-SB; IC50 = 7.14 μM and 3.19 μM for 38 and 39 respectively) and human T-cell leukaemia cell lines (Molt-4; IC50 = 2.72 μM and 2.24 μM for 38 and 39 respectively).
2.3. Selenonucleosides

A first synthesis of pyrimidine 4′-selenonucleosides was reported by Jeong and colleagues in 2008 (Scheme 8), [42] starting from lyxose derivative 55, synthesised from α-gulonic-γ-lactone in four steps using established procedures [43].

![Scheme 8. Reagents and conditions: (i) TBDPSCl, Et3N, DMAP, CH2Cl2, 92%; (ii) NaBH4, MeOH, 98%; (iii) MsCl, Et3N, DMAP, CH2Cl2, 97%; (iv) Se, NaBH4, EtOH, THF, 60 °C, 96%; (v) m-CPBA, CH2Cl2, −78 °C, 85%; (vi) Uracil, Et3N, TMSOTf, toluene, CH2Cl2, 53%; (vii) 50% aq. TFA, 81%; (viii) N3-benzoylcytosine, Et3N, TMSOTf, toluene, CH2Cl2, 35%; (ix) 50% Aq. TFA and (x) NH3, MeOH, 82% over two steps.

Selective protection of the primary alcohol in 55 was achieved by reaction with TBDPSCl, giving hemiacetal 56 which was reduced with NaBH4, furnishing diol 57. Following double mesylation of 57 to give 58, cyclisation to give 4-selenosugar 59 was achieved by treatment with selenium in the presence of NaBH4. Oxidation of 59 to a diastereomeric selenoxide mixture 60 then enabled either a uracil or cytosine nucleobase to be installed via a Pummerer-type glycosylation, furnishing the β-anomers 61 or 63. 4′-selenouridine 62 was obtained after global deprotection of 61 with 50% aqueous TFA in an overall yield of 12% over 11 steps. 4′-selenocytidine 64 was similarly obtained in an overall yield of 9% over 11 steps. The crystal structure of 62 revealed the non-native ring adopted an unusual C2′-endo/C3′-exo twist (Southern confirmation), contrary to uridine, which shows a C2′-exo/C3′-endo twist (Northern conformation). This difference was explained by the introduction of the bulky selenium, whereby stereoelectronic effects observed in 4′-oxanucleosides are outweighed by the size of the heteroatom.

2′-Substituted-4′-selenoribofuranosyl Pyrimidines

Building on their work in this area, Jeong and co-workers reported the synthesis and biological evaluation of a small library of 2′-substituted 4′-selenooribofuranosyl pyrimidine analogues 65–68 (Figure 5) [44,45].

![Figure 5. Structures of 2′-substituted 4′-selenooribofuranosyl pyrimidine analogues 65–68.](image-url)
A 2'-fluoroarabino analogue was obtained via a 2'-position DAST fluorination of intermediate 69, synthesised from 4'-selenouridine 61 using a four-step process [44]. At first attempt, DAST fluorination of 69 led to the formation of the desired 2'-fluoro product 70 as the minor product in 23% yield, along with a major product, 2,2'-O-anhydro nucleoside 71, in 60% yield (Scheme 9). This ratio was subsequently improved by instead fluorinating an N3-benzoyl derivative 72, giving 73 in 45% yield, now as the major product, and 71 in reduced 30% yield.

Following global deprotection and uracil to cytosine conversion, four nucleoside analogues 65–68 were evaluated against several human cancer cell lines (HCT116, A549, SU638, T47D, PC-3 and K562) and compared to the established anticancer nucleosides 1 and 3 (Table 2). From this study, it was found that 2'-fluoro-4'-selenoarabinoctydine 65 was the most potent analogue, showing even greater potency than 3, in the majority of cell lines tested.

Table 2. Anticancer activity of 2'-modified 4'-selenoarabino nucleosides 65–69 compared to 1 and 3 across several human cancer cell lines.

| Compound | HCT116 a | A549 b | SNU638 c | T47D d | PC-3 e | K562 f |
|----------|----------|--------|-----------|--------|--------|--------|
| 65       | 1.1      | 0.47   | 0.14      | 0.79   | 0.58   | 0.63   |
| 66       | 7.13     | 8.83   | 4.72      | ND     | ND     | 86.6   |
| 67       | >100     | >100   | >100      | >100   | >100   | >100   |
| 68       | >100     | >100   | >100      | >100   | >100   | >100   |
| 3        | 5.30     | 1.90   | 0.15      | 2.70   | 55.9   | 0.05   |
| 1        | 0.01     | 0.09   | ND        | ND     | 0.04   | ND     |

Human cancer cell tissue type: a colon; b lung; c stomach; d breast; e prostate; f myelogenous leukemia.

The group have also reported further 2'-substituted-4'-selenoribofuranosides [46], synthesising a series of pyrimidine 2'-substituted analogues and utilising a 2,2'-O-anhydro intermediate to enable regioselective nucleophilic ring opening to the desired ribo-configured products (Figure 6). Only a 2'-fluoro ribose-selenocytosine derivative showed significant activity (uracil, thymine and 5-halo uracil derivatives showed no activity up to 100 μM), but this was less potent than the arabino-configured analogue previously described [45].
2.4. Carbocyclic Nucleosides

In carbocyclic nucleosides the furanosyl oxygen is replaced by CH$_2$, forming a cyclopentane ring. The lack of a hemiaminal linkage between the nucleobase and the sugar leads to an increased chemical stability. Furthermore, due to the lack of a glycosidic bond, these nucleosides show an enhanced resistance towards phosphorylases [47]. Although considered a second generation of nucleoside analogues, there are two naturally occurring carbocyclic nucleosides, aristeromycin 74 and neplanocin A 75 (Figure 7), both of which exhibit substantial biological activity as antitumor agents [47].

Figure 6. 2'-Substituted-4'selenoribofuranosyl nucleosides reported by Jeong.

2.4.1. Fluorinated Derivatives of Neplanocin A

In 1988, Driscoll et al. reported that a cytosine analogue (CPE-C) of 75 showed substantial antitumour and antiviral activity [48]. On the basis of these findings, and the continuing clinical success of fluorine containing nucleoside chemotherapeutics [49,50], Jeong et al. subsequently reported the synthesis and biological evaluation of a small library of fluorocyclopentenyl pyrimidine nucleosides 76–79 (Figure 8) [51].

Figure 7. Structures of aristeromycin, 74, and neplanocin A, 75.

Figure 8. Structures of fluorocyclopentenyl nucleoside analogues 76–79.

Fluorocyclopentenyl cytosine 76 was synthesised from cyclopentenone 80 (Scheme 10), which was obtained from d-ribose in nine steps using established procedures [52,53]. Iodination of 80 was accomplished by treatment with I$_2$ and pyridine in THF, to give 81. Stereo- and regioselective reduction of 76 was achieved using Luche conditions to give 82, followed by TBDPS protection of the resulting alcohol to deliver 83. Following lithium-halogen exchange of 83 using n-BuLi, electrophilic fluorination was achieved by treatment with N-fluorobenzenesulfonylimide (NFSI). Anomeric desilylation with TBAF then gave 84 to which a uracil nucleobase was installed via condensation with N$^3$-benzoyluracil under Mitsunobu conditions. Finally, global deprotection was achieved by sequential treatment with methanolic ammonia and BBr$_3$, providing uracil derivate 85 which was converted to 76 via a standard four-step process.
Of the four nucleoside analogues evaluated (Figure 8), derivative 76 showed significant potency against several human cancer cell lines (Table 3) [51,54,55]. Furthermore, Jeong reported that 76 also showed significant antitumour activity in a nude mouse xenograft model implanted with A549 human lung cancer cells, wherein after 38 days the inhibition of tumour growth was 32% and 58% at 3 and 10 mg/kg doses, respectively [54].

Table 3. Anticancer activity of 76 in human cancer cell lines.

| Cancer Cell Line | IC_{50} (μM) |
|------------------|--------------|
| HTC-116 a         | 0.39         |
| MDA-MB-231 b      | 0.18         |
| PANC-1 c          | 0.62         |
| MCF-7 d           | 0.34         |
| A549 e            | 0.34         |
| MKN45 f           | 0.50         |
| U251 g            | 0.83         |

Human cancer cell tissue type: a colon; b breast; c hormone-dependent breast; d lung; e stomach; f brain.

Carbocyclic nucleoside 76 has now been evaluated in more than 100 different cell lines, as well as several xenograft models, showing high potencies against numerous types of cancer, including gemcitabine resistant cell lines [56,57]. Pharmacokinetics and oral bioavailability for 76 were investigated in phase 0 clinical trials, wherein a small cohort of patients were administered a single oral dose (50 mg or 100 mg) of 76, or a single intravenous dose (20 mg, Table 4) [58]. This study found that the absolute bioavailability for 76 was 56% and 33% for 50 and 100 mg doses, respectively, suggesting it not to be dose-proportional. However, t_{1/2} was found to be 14 h and 21 h for 50 and 100 mg doses, respectively. This may suggest that 76 does exhibit some dose proportionality in some parameters, but not in others and it was noted that this result may be due to the small patient sample size. Analogue 76 is currently in phase II clinical trials for metastatic pancreatic cancer and advanced bladder cancer.

Table 4. Pharmacokinetic data for 76 from phase 0 clinical studies.

| Dose (mg) | Tmax (h) | Cmax (ng/mL) | t_{1/2} (h) | Oral Bioavailability (%) |
|-----------|----------|--------------|-------------|--------------------------|
| 20 *      | 0.3      | 1144         | -           | -                        |
| 50        | 2.2      | 303          | 14          | 56                       |
| 100       | 2.5      | 311          | 21          | 33                       |

* Intravenous.
2.4.2. Norbornane-Derived (C2',C4'-bridged) Carbossyclic Nucleosides

Nencka and colleagues reported the synthesis of norborane, C2',C4'-bridged carbossyclic nucleosides [59], with a hypothesis of locking the nucleoside analogue in the 2'-exo or North conformation which has garnered attention in the field of carbossyclic antiviral nucleoside analogue development [60]. To access these compounds their synthesis started from known hydroxy ester 86 which was stereochemically inverted at the C1 position via an oxidation-reduction sequence to deliver 87 (Scheme 11). This enabled the desired stereochemistry to be attained at C1 when subsequently inserting an azide by nucleophilic displacement of a mesylate to afford 88 which was reduced to give 89. With scaffold 89 in hand, the group then elaborated the amine at C1 to several purine and pyrimidine nucleobase forms (including C6 purine analogues) and evaluated their cytotoxic potential. From this series, derivatives 90 and 91 showed IC50 activities below 100 µM in human T-lymphocyte (CEM) cells (IC50 = 88 µM for 90 and IC50 = 78 µM for 91), but no significant activity was observed in murine leukemia (L1210) or HeLa cell lines.

Scheme 11. Reagents and conditions: (i) PDC, CH2Cl2, 82%; (ii) NaBH4, MeOH, 88%; (iii) MsCl, pyridine, 99%; (iv) NaN3, DMF, 115 °C, 92% and (v) LiAlH4, THF, 59%.

2.4.3. C3',C5'-Bridged Carbossyclic L-Nucleosides

Tănase and colleagues reported the synthesis of an alternative carbossyclic system based on a similar bicyclo[2.2.1]heptane fragment, accessing a small series of 3'-5'-linked L-nucleoside analogues containing C6-amino modifications (Scheme 12) [61]. Their synthesis started from known alcohol 92 and proceeded through azide incorporation (to give 93) and reduction steps in good yields to deliver amine 94. The 6-chloropurine ring was then introduced using standard methods and elaborated at C6 with a series of amines via nucleophilic aromatic substitution. This small library was then screenen in vitro at a single high dose (10^{-5} M) in the full NCI 58 human tumor cell screen panel. Phenethylamine derivative 95 exhibited growth inhibition of 74% on SK-MEL-5 melanoma and UO-31 renal cancer cell lines, but was not deemed sufficiently cytotoxic for studies to proceed further. The group followed up this report with further synthesis of 6-position carbossyclic analogues [62], derived from 94, noting that a 6-(4-methoxy-phenethyl)amino group was active, but again the analogue was not progressed further.
3. 2', 3'- and 5'-Furanose Ring Modifications

3.1. 2'-Furanose Modifications

Clofarabine 2 (Figure 1) is a purine nucleoside analogue which, in its corresponding 5'-O-triphosphate form, inhibits both ribonucleotide reductase and DNA polymerases and prevents effective DNA synthesis. Ultimately, this leads to cell apoptosis, particularly in rapidly proliferating and dormant cancer cells. The nucleoside analogue exhibits excellent cytotoxic activity in vitro, with an \( IC_{50} \) range of 0.028–0.29 \( \mu \)M across a variety of solid tumour and leukaemia cell lines [63], alongside substantial tissue distribution and a half-life of at least 24 h for the active triphosphate metabolite [64]. The use of 2 for treatment of paediatric patients with relapsed or refractory acute lymphoblastic leukaemia was approved by the food and drug administration (FDA) in 2004, the first nucleoside analogue of its kind to be approved in over a decade [65].

In 2010, Sauve and colleagues reported an improved, stereoselective synthesis of 2 [66], in an overall yield of 38% (Scheme 13). This compared favourably to a prior report by ILEX Products Inc., which detailed an overall yield of 14% in six steps, starting from a fully protected ribose derivative. Sauve’s work began from commercially available lactone 96 with 3,5-O protection using TIPS affording 97, which was then diastereoselectively fluorinated at the 2-position, obtaining the arabinino-configured 98 exclusively in 72% yield. Conversion to the anomer chloride (via hemi-acetal 99) delivered 100 which was condensed with 2,6-dichloropurine to afforded 101 in 66% yield over two steps and a \( \beta/\alpha \) ratio of 3.5:1. Access to 2 was gained following diastereomeric separation of 101, 4-position ammonolysis and deprotection.

**Scheme 12.** Reagents and conditions: (i) MsCl, pyridine, \( \text{CH}_2\text{Cl}_2 \), 98%; (ii) \( \text{NaN}_3 \), DMF, 110 °C, 91% and (iii) \( \text{Pd(OH)}_2/C \), MeOH, 87%.

**Scheme 13.** Reagents and conditions: (i) TIPSiCl, imidazole, DMF, 92%; (ii) NFSI, LiHMDS, THF, –78 °C, 72%; (iii) DIBAL-H, toluene, –78 °C, 91%; (iv) MsCl, Et\(_3\)N, \( \text{CH}_2\text{Cl}_2 \), quant.; (v) 2,6-dichloropurine, dichloroethane, reflux; (vi) \( \text{NH}_3 \)/isopropanol, sealed tube, 105 °C, 66% over two steps and (vii) \( \text{Me}_3\text{NF}, \text{AcOH}, \text{DMF}, 90\%\).
3.2. 2'-O,4'-C'-Bridged Nucleosides

In 2011, Nicolaou and colleagues reported the synthesis and evaluation of a small library of 2',4'- and 3',4'-bridged nucleosides, presenting conformationally restrained 3'-endo (North) and 2'-endo (South) systems [67]. These compounds were evaluated for their antiviral, antitumour and antibacterial properties, with 2',4'-bridged purines 105 and 106 showing µM inhibitory activity against CEM (IC_{50} = 0.36 µM for 105 and 7.6 µM for 106) and Raji (IC_{50} = 0.25 µM for 105 and 5.8 µM for 106) cancer cell lines.

The synthesis of 105 and 106 derived from a common 4-disubstituted acetate 102, available using established chemistry from diacetone-α-glucose (Scheme 14) [68,69]. The group used Vorbrüggen glycosylation of either 2,6-diaminopurine or 2,6-dichloro-9H-purine to afford 103 or 104 respectively, with the substitution proceeding in good yields (100% for 103 and 64% for 104), but noting a requirement to control the amount of N,O-bis(trimethylsilyl)acetamide (BSA) used to silylate the purine to 2.5 equivalents. 2',4'-bridged compound 105 was constructed via base mediated cyclisation between C2' and C4', followed by per-benzylation of purine nitrogen and silicon protecting group removal.

![Scheme 14](image)

Scheme 14. Reagents and conditions: (i) 2,6-diaminopurine, BSA, TMSOTf, MeCN, 100%; (ii) 2,6-dichloro-9H-purine, BSA, TMSOTf, 64%; (iii) NaOH, THF, 94%; (iv) BzCl, pyridine, 52%; (v) HF, pyridine, 50%; (vi) Allyl(\(\text{tri-n-butyl} \))tin, PdCl\(_2\)(PPh\(_3\))\(_2\), DMF then NaOH, THF, 42%, 2 steps and (vii) HF, pyridine, 87%.

To access allyl substituted 2',4'-system, Stille couplings were completed at positions 2 and 6 of the purine, followed by a similar base-mediated C2' deacetylation and intramolecular cyclisation to afford, after C5'-protecting group removal, 106. Isomerisation of the allyl group at C6 of the purine ring was observed during the palladium and base-mediated cyclisation steps. The antitumour activities of 105 and 106 were considerably lower than for the known anticancer nucleoside cladribine (CEM IC_{50} = 0.5 nM and Raji IC_{50} = 9.0 nM), as was the activity (IC_{50} >10 µM) of the 2'4'-bridged analogue of cladribine, suggesting that the inclusion of this conformational restraint or the addition of an extra CH\(_2\)O unit was enough to remove antitumour properties.

3.3. 3'-Modified Nucleosides

Cheng and co-workers reported an asymmetric synthesis of 2',3'-dideoxy-3'-boronic acid pyrimidine nucleosides [70]. The group used two highly diastereoselective reactions of chiral boronic esters with (dihalomethyl)lithium reagents to install the ultimate stereochemistry required at C3' and C4' (Scheme 15). Starting from (S,S)-1,2-dicyclohexyl-1,2-ethanediol (DICHED) derived boronate ester 107, homologation was completed with (dichloromethyl)lithium to afford 108, the newly formed stereochemistry of which was inverted to give boronate ester 109. The chiral auxiliary component was next switched from DICHED to a pinanediol (derived from (+)-pinene), delivering 110. A second diastereoselective homologation was completed with (dibromomethyl)lithium, affording bromo boronic ester 111. Nucleophilic displacement and inversion of this bromide with allylmagnesium bromide followed by oxidative cleavage of the alkene afforded aldehyde 112. Hydrogenolysis of the C4-O-Bn group in 112, concomitant cyclisation and anomeric acetylation delivered the final
2-deoxy-ribo configured scaffold 113. This was divergently converted to a series of pyrimidine containing nucleoside analogues containing a unique C3'-boronic acid. Unfortunately, biological evaluation of these compounds demonstrated no significant cytotoxicity towards the HepG2 cell line and all derivatives were observed to undergo gradual hydrolytic cleavage under biological conditions.

Scheme 15. Reagents and conditions: (i) LiCHCl₂, THF then ZnCl₂; (ii) PhCH₂ONa, 97%; (iii) Pinanediol, Et₂O, H₂O, 96%; (iv) CH₂Br₂, LDA, THF then ZnCl₂, 83%; (v) CH₃CHCH₂MgBr, 86%; (vi) NaO₂/K₂OsO₄, 2,6-lutidine, dioxane/H₂O, 63%; (vii) H₂ Pd/C, EtOAc, 81% and (viii) Ac₂O, DMAP, CH₂Cl₂, 98%. Cy = cyclohexyl, T = thymine, 5F-U = 5-fluorouracil, C = cytosine, 5I-U = 5-iodouracil.

More recently, Borbas and colleagues reported a small library of 3'-deoxy-3'-thio substituted xylofuranosyl pyrimidines [71]. Utilising a photoinduced thiol-ene reaction the workers were able to effect addition of several different thiols to an appropriately protected 3'-exomethylene ribopyrimidine system. This afforded p-xylo configured products in high diastereomeric excess which were shown to have cytostatic activity in the low micromolar range.

3.4. C₅'-N-Cyclopropylcarboxamido-C₆'-amino-C₂-alkynylated Analogues

In 2017, Mohan et al. synthesised and evaluated a series of C₅'-N-cyclopropylcarboxamido-C₆'-amino-C₂-alkynylated purine nucleoside analogues (Scheme 16) [72]. Starting from guanosine, the group accessed C₂-aryl iodide derivative 114 which was oxidised at C₅' using KMnO₄, followed by amide coupling to install the C₅'-N-cyclopropylcarboxamido group. Following 2',3'-O-acetonide removal, the C₂-iodide underwent a series of divergent Sonogashira couplings to deliver a library of seven purine analogues. From this library, compounds 115 and 116 showed in vitro cytotoxic effects comparable to doxorubicin against human breast (MDA-MB-2312) and human colon (Caco2) cell lines.
3.5. 5'-β-Hydroxyphosphonate Analogues

In 2014, Peyrottes and co-workers reported their synthesis of a series of β-hydroxyphosphonate nucleosides, targeting 2' and 3' hydroxyl group stereochemistry changes and the introduction of 5-position substituents to the nucleobase (Figure 9) [73]. This report built upon the group’s earlier work developing this class of compound as 5'-nucleotidase inhibitors (specifically the cytosolic cN-II 5'-nucleotidase II) [74]. These enzymes catabolise nucleoside 5'-monophosphates and their expression level is of crucial interest for patients undergoing nucleoside analogue chemotherapy, with a higher expression level often associated with a worsened clinical outcome [75].

The group completed the synthesis of a library of 32 β-hydroxyphosphonate analogues which included β-allo, β-altro, β-manno and β-gluco configurations of the furanose 2'- and 3'-positions along with C2'-C3' ring opened derivatives. They also installed alkynyl, aryl or ethenylaryl groups at C5 of the cytidine or uridine nucleobase using transition-metal cross-couplings of the corresponding C5-iodide. Biological activity data was obtained using recombinant, purified cN-II with inosine monophosphate included.

Figure 9. 5'-β-Hydroxyphosphonate analogues developed by Peyrottes and active compound 117 from this series, Y = NH$_2$ or OH.

3.6. Ferronucleosides

Tucker and colleagues recently described their synthesis of ferronucleosides, an important new development in the field of medicinal bioorganometallic chemistry [76]. Here the furanose ring was
exchanged for the five-membered cyclopentadienyl ring of a ferrocene unit, but retained the nucleobase and hydroxymethyl group as key components appended in a 2,3 relationship to the ferrocene core (compounds 118 and 119, Figure 10). Using thymine or adenine as the nucleobase, the compounds were tested with a control series (where the hydroxymethyl or base component were absent) for their cytostatic activity and compared to established chemotherapeutic agents 5-fluorouracil (5F-U) and cisplatin. In a proliferation activity assay on three tumour cell lines (L1210, HeLa and CEM), both 118 and 119 had activities in the low μM range, with 118 and 119 20 to 50-fold more active than 5F-U in CEM cell cultures (0.9 μM for 118, 0.35 μM for 119 versus 18 μM for 5F-U). The compounds also performed promisingly in cell growth inhibition (oesophageal cancer cell line) and cellular viability (MTT assay) studies, with the data indicating that both functional groups appended to the ferrocene component were required for optimum cytostatic activity.

![Figure 10. Ferronucleosides developed by Tucker.](image)

4. Nucleoside Analogue Prodrugs

4.1. Phosphorodiamidate Prodrugs

In 2018, Slusarczyk’s team reported the synthesis of phosphorodiamidates for a series of established anticancer nucleoside analogues [77]. This was envisioned following the successful ProTide technology developed by McGuigan [78], to deliver nucleoside monophosphate analogues into cells and recent reports of phosphorothioamidate systems [79]. A lack of chirality at phosphorus in a phosphorodiamidate was envisioned to confer advantages in not having to resolve diastereomeric mixtures, often a requirement in earlier generations of ProTides, as S and R diastereoisomers exhibited markedly different biological activities [80]. Accordingly, seven different anticancer nucleosides (1, FUdR, 8-chloroadenosine, fludarabine, AraG, thioinosine and thioguanosine) were converted to their phosphorodiamidate form using a one-pot, two-stage strategy (Scheme 17). First the 5’-OH was converted to a phosphorodichloridate intermediate 120, followed by double phosphoramidation using an appropriate amino acid ester salt. The panel of analogues were then evaluated in vitro against a wide range of solid tumour and haematological cell lines with the potential for this approach confirmed for FUdR and 8-chloroadenosine, where similar or improved inhibitory activities compared to the parent nucleosides were observed. FUdR and its phosphorodiamidate prodrug showed activity in the sub-micromolar range with IC_{50} values of 0.0046–0.073 μM for FUdR and 0.01–0.40 μM for the FUdR phosphorodiamidates against the wild-type cell lines. Also of note was the inactivity of arabino configured analogues, suggesting conversion to the monophosphate might be prevented through their being poor substrates for phosphoramidase activity [81]. Enzymatic studies were undertaken to investigate the bioactivation pathway of this class of nucleoside prodrug with a carboxypeptidase Y assay and ^31^P-NMR confirming the hydrolysis of both esters followed by loss of one phosphoramidate group.
4.2. Vitamin E Phosphate Prodrugs

Nucleoside analogue therapy can suffer from inductive and constitutive resistance, which limits the efficacy of the treatment. Isoforms of vitamin E, in particular δ-tocopherol and tocotrienols have displayed anticancer activity. As such, vitamin E phosphate nucleoside analogue prodrugs were envisaged to combat two mechanisms of resistance: i) downregulation of metabolic kinases (deoxycytidine kinase, dCK, in the case of 1) and ii) nucleoside transport [82,83]. Accordingly, Daifuku and co-workers synthesised and evaluated four isoforms of vitamin E conjugated to 1 (Figure 11, compounds 121–124) [84].

The in vitro GI_{50} of 1, vitamin E phosphate (VEP) isoforms and compounds 121–124 showed these VEP-gemcitabine prodrugs to exhibit anticancer activity, consistent with their catabolism to vitamin E and gemcitabine monophosphate. Conjugate 122 displayed the best activity with GI_{50} values <5 μM in breast MDA (MB-231), non-small cell lung (NCI-H460) and colon (HCT-116) cell lines. The authors suggested this was due to steric hindrance, from the methyl groups proximal to the vitamin E-phosphate bond, reducing the rate of enzymatic cleavage to the monophosphate counterpart.

Prodrugs 122 and 123 were then evaluated in the presence of an inhibitor of nucleoside transport, dipyridamole (DP, Table 5) with the data indicating that both were largely unaffected by the presence of
DP in comparison to 1. This suggested that these prodrugs bypass nucleoside membrane transporters and may thus be beneficial in the treatment of patients with gemcitabine resistant cells.

Table 5. GI50 values of 1, 122 and 123 in the presence or absence of dipyridamole.

| Cancer Cell Line | Breast MDA-MB-231 (µM) | Non-Small Cell Lung NCI-H460 (µM) | Colon HCT-116 (µM) |
|------------------|------------------------|----------------------------------|-------------------|
| Compound         | DP (−) | DP (20 µM) | DP (−) | DP (20 µM) | DP (−) | DP (20 µM) |
| 1                | 3.08   | 56.8       | 0.02   | 0.82       | 0.03   | 2.39       |
| 122              | 30.3   | 27.8       | 7.16   | 16.0       | 5.55   | 12.6       |
| 123              | 17.2   | 23.3       | 2.14   | 1.47       | 3.07   | 6.74       |

In particular, as conjugate 123 displayed significant potency against the three DP(−) cell lines and moderate potency against the DP-dosed cell lines (Table 5), it was selected to further compare activity against 1 in in vitro wild-type leukemic CEM cells and CEM cells deficient in dCK. In dCK(−) cells 1 is not phosphorylated, with GI50 values increasing from 0.002 µM in wild-type to 124.5 µM in dCK(−). For 123 the GI50 increase was significantly lower than that of 1 (from 0.59 µM to 19.2 µM). Furthermore, the half-life of 123 in mice was shown to be 4 h, a 13.9-fold increase to that of 1 (0.3 h [85]). Overall, this study showed an interesting proof of concept for VEP-1 prodrugs against resistant cancer cell lines. However, further optimisation will be required to obtain promising candidate compounds with increased potencies.

5. Conclusions

Nucleoside analogues are an historically accomplished class of drugs with highly diversifiable scaffolds and proven potential to treat a wide range of cancer cell types. Recent synthetic trends have focused on furanose oxygen substitution with heteroatoms or carbon and fluorocyclopentenyl cytosine is proving a promising new clinical candidate in this regard. Alongside this, examples of templating such modifications onto established nucleoside analogue scaffolds, such as Ara-C and forodesine, are emerging. As further pharmacokinetic and pharmacodynamic parameters are evaluated for these architectures, their pharmaceutical utility will be established. Finally, the continued advancement of prodrug strategies to deliver these compounds more effectively and provide options to treat drug-resistant cancer cell types sets an exciting future for nucleoside analogue chemotherapeutics and the underpinning requirement of chemical synthesis in realising this.

Author Contributions: G.J.M., M.G. and C.B. conceptualised and wrote the manuscript. M.G., C.B., M.S., G.J.M. reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: Riboscience LLC and Keele University are thanked for PhD scholarship funding to M.G and C.B.

Conflicts of Interest: M.S. is a Founder and owns equity in Riboscience LLC.

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