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The evolution of antiviral nucleoside analogues: A review for chemists and non-chemists. Part II: Complex modifications to the nucleoside scaffold

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ABSTRACT

This is the second of two invited articles reviewing the development of nucleoside analogue antiviral drugs, written for a target audience of virologists and other non-chemists, as well as chemists who may not be familiar with the field. As with the first paper, rather than providing a chronological account, we have chosen to examine particular examples of structural modifications made to nucleoside analogues that have proven fruitful as various antiviral, anticancer, and other therapeutics. The first review covered the more common, and in most cases, single modifications to the sugar and base moieties of the nucleoside scaffold. This paper focuses on more recent developments, especially nucleoside analogues that contain more than one modification to the nucleoside scaffold. We hope that these two articles will provide an informative historical perspective of some of the successfully designed analogues, as well as many candidate compounds that encountered obstacles.

1. Introduction

This is the second of two invited articles reviewing the development of nucleoside analogue antiviral drugs, written for a target audience of virologists and other non-chemists, as well as chemists who may not be familiar with the field. As with the first paper, rather than providing a chronological account, we have chosen to examine particular examples of structural modifications made to nucleoside analogues that have proven fruitful as various antiviral, anticancer, and other therapeutics. The first review covered the more common, and in most cases, single modifications to the base and sugar moieties of the nucleosides scaffold. This second paper focuses on more recent developments in the field, especially nucleoside analogues that contain more than one modification to the nucleoside scaffold.

The term “nucleoside” was first used by [Levene and Jacobs \(1909\)](#). A nucleoside is composed of a sugar moiety and nucleobase, whereas a nucleotide is composed of a sugar, nucleobase, and at least one phosphate (or phosphate-like) group ([Fig. 1](#)). Both nucleosides and nucleotides play important roles in the replication and transcription of genetic information, and, as such, have been utilized for decades for chemotherapy, antiparasitic, antibacterial or antiviral therapeutics ([De Clercq, 2004](#); [Perigaud et al., 1992](#); [De Clercq and Li, 2016](#); [De Clercq, 2012](#); [Field and De Clercq, 2004](#)). Ideally, a nucleoside/tide analogue would mimic the structure of a natural nucleoside enough to be recognized by cellular or viral enzymes and be incorporated into the DNA

or RNA replication cycle, however, these analogues would possess one or more modifications that would then lead to the disruption and/or termination of replication ([De Clercq and Neyts, 2009](#); [De Clercq, 2005a, 2005b](#)). Over the years, numerous modifications to the nucleoside scaffold have been made, including alterations to the sugar, nucleobase, glycosidic bond, and phosphate group ([Fig. 1](#)). As described in the first paper, these modifications range from adding a substituent or group to the heterocyclic base or sugar, replacing an atom in either moiety, by moving an atom to a different position, or a combination of these approaches ([Perigaud et al., 1992](#); [Jordheim et al., 2013](#)). More recently, researchers have employed the latter, utilizing a combination of many different types of modifications, which has led to the development of a wide array of potent nucleoside therapeutics, with complex structures. For convenience, this review is organized based on modifications to the various positions on the sugar moiety, however many of the nucleoside analogues discussed also contain modifications to the nucleobase moiety as well.

2. 1'-Sugar modifications

The sugar modifications explored in the first review featured nucleosides with 2'-OH, 3'-OH, a combination of 2' and 3'-OH modifications, carbocyclic nucleosides, alternative ring sizes, acyclic nucleosides, and acyclic nucleoside phosphonates. More recently, modifications to the 1'-carbon of the sugar, some in combination with

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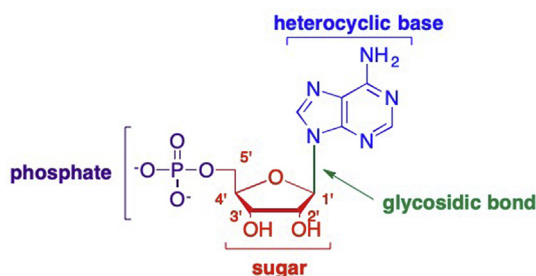


Fig. 1. Sites for potential modifications to nucleos(t)ide analogues.

prodrug strategies, have been pursued. This section details these analogues and later, their prodrugs.

2.1. Early 1'-Modifications

Structure-activity relationship (SAR) studies are common in drug design and typically involve “walking” around the nucleoside's scaffold making a particular change and observing its subsequent effect on biological activity. One such study by Siddiqi et al. found that moving a methyl substituent to the different positions on the sugar of an adenosine nucleoside yielded profound differences in activity (Siddiqi et al., 1995). For example, replacement of the 4'-hydrogen with a methyl group in adenosine analogues led to a large decrease in activity against adenosine receptors, whereas replacing the 3'-hydrogen with a methyl group increased activity (Siddiqi et al., 1995). Further analysis by Cappellacci et al. found that the replacement of the 1'-hydrogen with a methyl group had a different effect on different adenosine receptors, most notably, A₁ and A_{2A}, however, overall the 1'-methyl analogues demonstrated little or no activity (Fig. 2) (Cappellacci et al., 2002).

Other early 1'-modified nucleoside analogues included the 1'-fluoromethyladenosine analogues originally synthesized by Damont et al. (Fig. 2). (Damont et al., 2007) These analogues utilized electrophilic fluorination of an exocyclic double bond at the C-1 carbon in order to install the fluoromethyl group at the 1' position (Damont et al., 2007). Unfortunately, like the 1'-methyl analogues, the 1'-fluoromethyl analogues also did not demonstrate any antiviral activity against bovine viral diarrhea virus (BVDV) or against hepatitis C virus (HCV) in a subgenomic replicon assay, however notably, the analogues were not toxic (Damont et al., 2007). Due to the lack of any antiviral activity, they were not pursued further.

2.2. 1'-Cyano analogues

Further research led scientists to believe that the lack of activity demonstrated by the early 1'-analogues was due to instability of the glycosidic bond with the addition of the 1'-methyl group (Cappellacci et al., 2002; Cho et al., 2012). In general, the glycosidic bond is stable under physiological conditions, however, cleavage of this bond can occur and is dependent on various factors including pH, type of nucleobase, and 1'-substituents (Cho et al., 2012; Temburnikar and Seley-Radtke, 2018; Rios et al., 2015; Lindahl and Karlström, 1973; Levy and

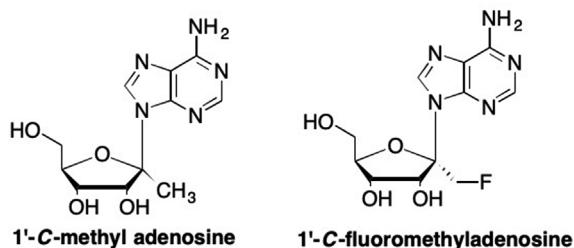


Fig. 2. Examples of early 1'-modified nucleoside analogues.

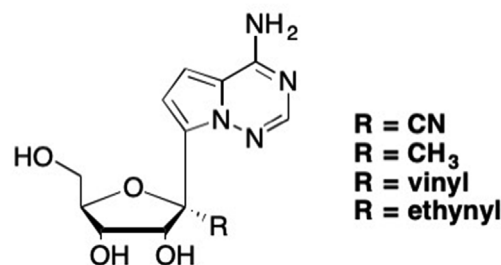


Fig. 3. Gilead's first generation 1'-substituted 4-aza-7,9-dideazaadenosine C-nucleosides.

Miller, 1998). Since the glycosidic bond cleavage occurs either by nucleophilic attack on the 1' carbon of the sugar or by stabilization of the leaving group, changing the substituent from a hydrogen to any other group at the 1' position could have a profound effect on glycosidic bond cleavage, either through steric or electronic effects (Temburnikar and Seley-Radtke, 2018; Berti and McCann, 2006; Lenz et al., 2016). Scientists reasoned however, that if they replaced the hemiaminal (O–C–N) glycosidic bond with the O–C–C bond found in C-nucleosides, then they would be able to add 1' substituents without compromising the integrity of the glycosidic bond (Temburnikar and Seley-Radtke, 2018; De Clercq, 2016; Stambaský et al., 2009; Siegel et al., 2017). This subsequently led to the development of a number of 1'-substituted C-nucleoside analogues.

In that regard, some of the most promising 1'-substituted C-nucleosides pursued recently were the 1'-substituted 4-aza-7,9-dideazaadenosine C-nucleosides developed by Gilead (Fig. 3). (Cho et al., 2012, 2014; Siegel et al., 2017; Lo et al., 2017; Sheahan et al., 2017; Warren et al., 2016; Metobo et al., 2012) An SAR study focused on various 1'-substituted analogues found that the 1'-cyano analogue displayed a broader spectrum of antiviral activity against viruses such as HCV, yellow fever virus (YFV), dengue-2 virus (DENV-2), influenza A, parainfluenza 3, Ebola virus (EBOV) and severe acute respiratory syndrome coronavirus (SARS-CoV), with the best antiviral activity against EBOV in HMVEC cells (EC₅₀ = 0.78 μM) (Cho et al., 2012; Siegel et al., 2017). These findings were especially interesting since both the 1'-methyl and 1'-vinyl analogues showed reduced potency, a much narrower spectrum of antiviral activity, and in some instances, higher toxicity compared to the 1'-cyano analogue (Cho et al., 2012; Siegel et al., 2017). In contrast, the 1'-ethynyl analogue demonstrated no antiviral activity (Cho et al., 2012).

Due to the surprising broad-spectrum antiviral activity found with the 1'-cyano analogue, researchers at Gilead performed a computational docking study with the triphosphate analogue of the 1'-cyano analogue and various RNA virus polymerases. It was found that the 1'-cyano group occupies a unique pocket present in only the viral polymerase binding site, which leads to the increased selectivity of the 1'-cyano analogues for viral polymerases over human polymerases (Siegel et al., 2017; Warren et al., 2016). Moreover, in order to increase the delivery of this analogue, they also employed the McGuigan ProTide (PROdrug nucleoTIDE) approach (Pertusati et al., 2012; McGuigan et al., 1996, 2006; Mehellou et al., 2009, 2018; Mehellou, 2016).

The ProTide approach has proven extremely valuable for delivery of nucleotide analogues, as well as to overcome the rate-limiting first phosphorylation step. During DNA/RNA replication, nucleosides (and nucleoside analogues) are phosphorylated by various host cell or viral kinases into their triphosphate form, which are then recognized by DNA polymerases, RNA polymerases, or reverse transcriptase and incorporated into the growing chain (De Clercq and Neyts, 2009; Deval, 2009). Since the triphosphate cannot be administered directly due to the highly charged nature of the phosphate groups, the prodrug helps deliver the nucleotide into the cell. A second limitation associated with nucleoside drugs is that the first phosphorylation step is often highly

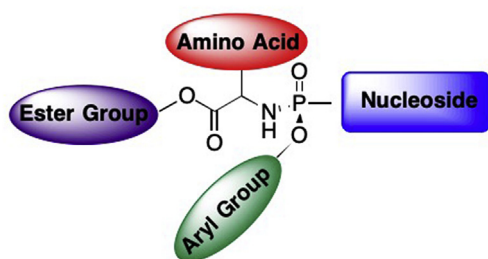


Fig. 4. General structure of a McGuigan ProTide.

specific and rate-limiting, thus the nucleoside analogue is often not recognized and appears inactive (McGuigan et al., 1996, 2006; Mehellou et al., 2018; Herdewijn et al., 1987; Balzarini et al., 1987, 1989). To overcome this obstacle, McGuigan et al. created ProTides that would efficiently deliver the monophosphate nucleoside analogue into the target cell, bypassing the rate-limiting first phosphorylation step (Siegel et al., 2017; McGuigan et al., 1996, 1999, 2006; Mehellou et al., 2009, 2018; Mehellou, 2016). These ProTides utilize a unique structure, with three “tunable” positions - the aryl, the amino acid, and the ester groups (Fig. 4). (Siegel et al., 2017; McGuigan et al., 1996, 2006; Mehellou et al., 2018) The aryl group and the amino acid ester mask the negative charges on the monophosphate, allowing the ProTide to efficiently cross the cell membrane (Siegel et al., 2017; McGuigan et al., 2006; Mehellou et al., 2009, 2018; Mehellou, 2016). Following metabolism by various host enzymes, the monophosphate nucleotide analogue is successfully delivered and is subsequently phosphorylated into the active triphosphate (Siegel et al., 2017; Mehellou et al., 2009, 2018; Mehellou, 2016).

Using the McGuigan ProTide approach with the 1'-cyano compound produced GS-5734 (Remdesivir), which increased the overall anti-EBOV activity ($EC_{50} = 0.06 \mu\text{M}$ compared to $0.78 \mu\text{M}$ for the parent). In addition, this also increased the spectrum of the antiviral activity to include viruses that the parent nucleoside was not active against, including West Nile virus (WNV), Lassa fever virus, and Middle East respiratory syndrome coronavirus (MERS-CoV) (Fig. 5). (Lo et al., 2017; Sheahan et al., 2017; Warren et al., 2016) Further studies found that GS-5734 was an effective post-exposure therapeutic in EBOV-infected rhesus monkeys at 10 mg/kg (Siegel et al., 2017; Warren et al., 2016). Studies with healthy human volunteers are currently underway in order to evaluate pharmacokinetics and clinical safety, specifically in male Ebola survivors with EBOV persistence in semen (NCT02818582).

3. 2'-Modifications

The first review focused on 2'-OH modifications, including the arabinose or “Ara” analogues in which the configuration of the 2'-OH is inverted, as well as the mono- and di-fluoro substituted 2'-modified nucleoside analogues. This was due to their role in the development of some of the first medically relevant nucleoside analogues, which greatly impacted the field of drug design. In this second article, we focus on more recent 2'-modifications, as well as 2'-modified analogues that

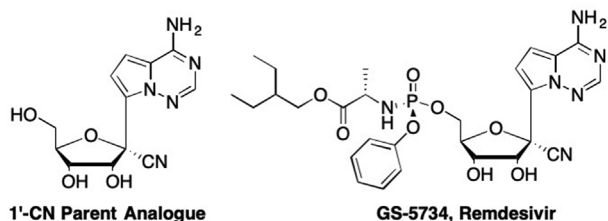


Fig. 5. Structure of the 1'-CN parent analogue and the McGuigan ProTide GS-5734.

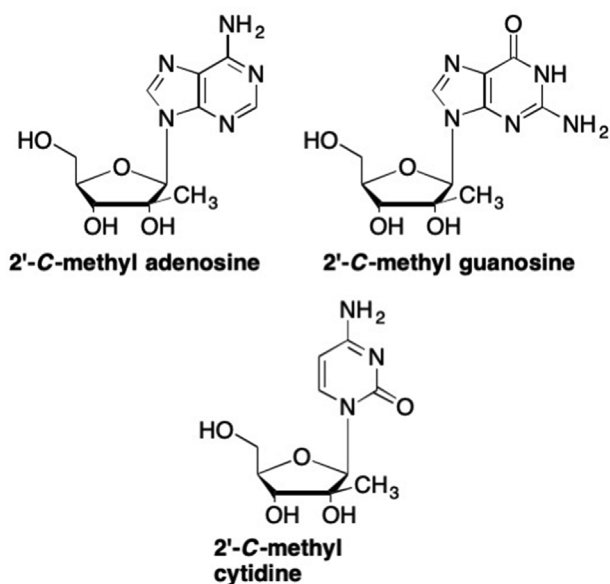


Fig. 6. First generation 2'-methyl nucleoside analogues for HCV therapy.

contain additional modifications, especially to the nucleobase scaffold.

3.1. 2'-Methyl Modifications

Of the numerous 2' modified nucleoside analogues that have been developed, some of the most promising have been the 2'-methyl analogues, particularly those that have exhibited activity against HCV (Zhang et al., 2011; Carroll et al., 2003). The presence of this 2'-methyl group alters the configuration of the sugar which prevents the binding of subsequent nucleotides to the enzyme active site, thus these analogues are considered non-obligate chain terminators (Migliaccio et al., 2003). Of these, 2'-methyl adenosine, 2'-methyl guanosine, and 2'-methyl cytidine (NM107) were initially pursued and all displayed micromolar levels of activity against HCV in whole-cell replicon assays (Fig. 6). (Zhang et al., 2011; Carroll et al., 2003; Eldrup et al., 2004a, 2004b; Pierra et al., 2006) The adenosine analogue proved to be the most potent, with an EC_{50} of $0.26 \mu\text{M}$ compared to $3.5 \mu\text{M}$ for the guanosine analogue and $1.23 \mu\text{M}$ for the cytidine analogue (Eldrup et al., 2004a, 2004b; Pierra et al., 2006). Interestingly, none of these compounds demonstrated any cytotoxicity *in vitro* (Carroll et al., 2003; Eldrup et al., 2004a, 2004b; Pierra et al., 2006). Unfortunately, they displayed low bioavailability as well as a high rate of deamination of the nucleobase in the 2'-methyl adenosine analogues and increased glycosidic bond cleavage by purine nucleoside pyrophosphorylase (Eldrup et al., 2004a, 2004b). The guanosine analogue also exhibited low bioavailability due to insufficient phosphorylation and decreased cellular uptake (Eldrup et al., 2004a, 2004b; McGuigan et al., 2009, 2010a).

While these initial studies were disappointing, they provided researchers with a starting point for developing the next generation of HCV nucleoside therapeutics. Through the initial studies with 2'-methyl adenosine and 2'-methyl guanosine, researchers determined that the bioavailability of these analogues could potentially be increased by removing the N7 of the purine ring system to yield a “deaza” analogue. Deazapurine nucleosides are naturally found in secondary metabolites produced by *Streptomyces* bacteria, and, due to their strong resemblance to natural purine nucleosides, the deazapurines have been shown to disrupt various biological functions, thereby leading to potent therapeutics (McCarty and Bandarian, 2008). Of the initial deaza-methyl purines synthesized, the most promising was 7-deaza-2'-methyl adenosine (MK-0608) (Fig. 7), which demonstrated broad-spectrum activity against numerous flaviviruses, including HCV, DENV, Zika virus

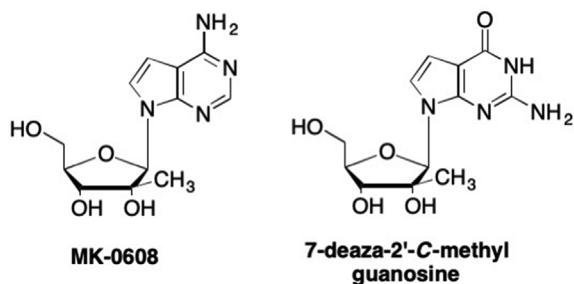


Fig. 7. Modified 7-deaza-2'-methyl analogues.

(ZIKV), tick-borne encephalitis (TBEV), and YFV with EC_{50} values ranging from 5 to 15 μM (Zmurko et al., 2016; Olsen et al., 2004; Schul et al., 2007; Eyer et al., 2015, 2016, 2017; Di Francesco et al., 2012a). Further analysis of MK-0608 found that this analogue was not associated with cellular toxicity at 100 μM after 24 or 72 h in Huh7 cells, and HCV RNA replication was inhibited at 0.3 μM in a subgenomic replicon assay (Olsen et al., 2004; Di Francesco et al., 2012b; Carroll et al., 2006). In comparison to the parent analogue 2'-methyl adenosine, MK-0608 demonstrated a dramatic increase in half-life and oral bioavailability, since MK-0608 was no longer susceptible to deamination or cleavage by adenosine-metabolizing enzymes (Olsen et al., 2004; Bloch et al., 1967).

Unfortunately, this approach proved ineffective with the corresponding guanosine analogues once viral strain mutations were introduced, thus only the 7-deaza-2'-methyl adenosine analogues were originally pursued (Olsen et al., 2004; Di Francesco et al., 2012b). Subsequently, however, researchers found that the addition of prodrug moieties, such as the aforementioned McGuigan ProTides, greatly enhanced the antiviral activity of 2'-methyl guanosine (McGuigan et al., 2009; Sizun et al., 2015). Of these, one of the most successful analogues was IDX184 (Fig. 8), a 2'-methylguanosine prodrug originally developed by Idenix, which utilized an *S*-pivaloyl-2-thioethyl (tBuSATE) moiety functionalized with an *N*-benzylphosphoramidate group (*O*-(HO)tBuSATE *N*-benzylphosphoramidate) to impart a significant increase in anti-HCV activity compared to the parent compound (Sizun et al., 2015; Cretton-Scott et al., 2008). Through an SAR study, Sizun et al. found that the (*O*-(HO)tBuSATE *N*-benzylphosphoramidate) derivative IDX184 was the most potent analogue, with an EC_{50} of

0.2 \pm 0.03 μM and no associated cytotoxicity in an HCV subgenomic luciferase replicon system (Sizun et al., 2015; Cretton-Scott et al., 2008). Further analysis revealed that IDX184 was well tolerated in a chimpanzee model, thus researchers turned to testing IDX184 in human patients (Standring et al., 2008). In phase II clinical trials, it was determined that IDX184 was effective in patients with chronic HCV infections, thus supporting further study for clinical application (Sizun et al., 2015; Zhou et al., 2011; Lalezari et al., 2012).

In related studies by McGuigan et al., it was found that the addition of a ProTide moiety to 2'-methylguanosine resulted in a 10-fold increase in activity against HCV in Huh 5-2 cells, as well as a lack of cytotoxicity at 50 μM (McGuigan et al., 2009). Further investigation by McGuigan led to the development of INX-189 (BMS-986094), which utilized a phosphoramidate ProTide moiety with a naphthyl group as the aromatic component, an *L*-alanine as the amino acid component, and a *t*-BuCH₂ group as the aliphatic component (Fig. 8). (McGuigan et al., 2010a, 2010b) This analogue was considered a double prodrug, since the carbonyl group at the C6 position of the guanine base was replaced with a methoxy group, which, following hydrolysis, reverts to the carbonyl (McGuigan et al., 2010b). This modification proved to be essential, in that it consistently improved the activity against the HCV replicon across all ProTide derivatives, compared to the parent 2'-methylguanosine (McGuigan et al., 2010b). Further analysis revealed INX-189 to be the most potent analogue with an EC_{50} value of 0.01 μM and a CC_{50} value of 7 μM (Mehellou, 2016; McGuigan et al., 2010b). Most importantly, this approach delivered substantially higher levels of the 5'-triphosphate of 2'-methylguanosine compared to the parent analogue, and increased the half-life to over 24 h (McGuigan et al., 2010b; Vernachio et al., 2011). Similarly, studies found that combination therapy with INX-189 and ribavirin resulted in significant synergistic anti-DENV activity *in vitro*, with a synergy score of 2.2 ± 0.048 (Yeo et al., 2015). These promising results prompted further clinical analysis of INX-189, including Phase III trials of INX-189 and another anti-HCV experimental drug daclatasvir (Vernachio et al., 2011). Unfortunately, severe cardiotoxicity complications were soon discovered, thus INX-189 was withdrawn from further clinical study (Baumgart et al., 2016; Feng et al., 2017; Gentile et al., 2015). For similar toxicity reasons, and due to the fact that both IDX184 and INX-189 share the same parent analogue, IDX184 was also pulled from clinical trials (Gentile et al., 2015; Luo et al., 2016).

3.2. 2'-Methyl-fluoro modification

As mentioned in the first review, researchers have capitalized on the unique properties that fluorine imparts to nucleoside analogues for decades (Liu et al., 2008; Pankiewicz and Watanabe, 1993; Pankiewicz, 2000). Fluorine is often used as an isosteric replacement since it is similar in size to hydrogen, but is also similar in electronegativity to the hydroxyl groups found in nucleosides (Liu et al., 2008). The presence of fluorine on the sugar ring greatly influences the conformation of the "sugar pucker" in the ring system, which in turn has an effect on the overall conformation of the entire nucleoside, as well as recognition by different enzymes (Saenger and Cantor, 1984; Ikeda et al., 1998; Wojtowicz-Rajchel, 2012). Furthermore, studies have demonstrated that the presence of a fluorine at the 2'-position of the sugar greatly decreases the nucleoside's susceptibility to enzymatic cleavage of the glycosidic bond (Liu et al., 2008; Wojtowicz-Rajchel, 2012; Bohm et al., 2004; Kirk, 2006; Park et al., 2001; Gudmundsson et al., 2000). One early successful 2'-fluorine analogue was 2'-deoxy-2'-fluorocytidine, which demonstrated potent HCV inhibition with an EC_{90} of 5 μM and no cytotoxicity up to 100 μM (Stuyver et al., 2004). Unfortunately, while 2'-deoxy-2'-fluorocytidine targeted the HCV non-structural NS5B polymerase, it was also shown to target cellular polymerases, thus was not pursued further (Stuyver et al., 2004; Brox et al., 1974; Richardson et al., 2000).

Due to the success of the 2'-methyl analogues, as well as the known

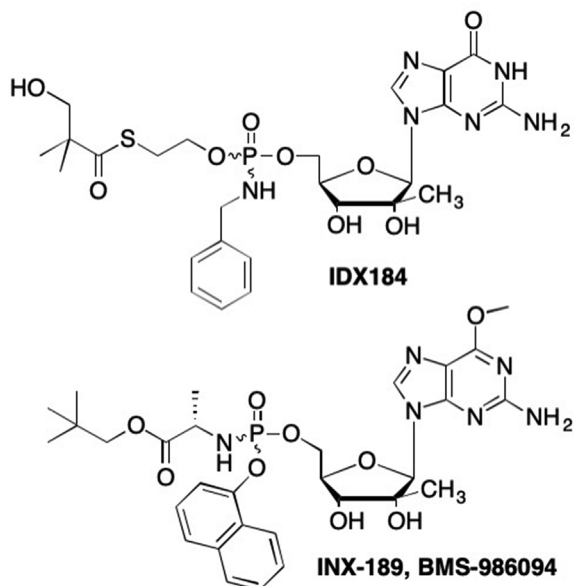


Fig. 8. Second generation 2'-methylguanosine analogues.

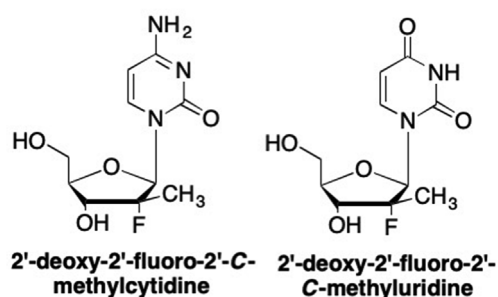


Fig. 9. First generation 2'-deoxy-2'-fluoro-2'-methyl analogues.

impact of using fluorine in nucleoside drug design, Clark et al. sought to combine these two modifications to yield 2'-deoxy-2'-fluoro-2'-methyl analogues (Fig. 9). (Clark et al., 2005) The hope was that by combining both 2' substituents these novel analogues would demonstrate potent antiviral activity, and that the addition of the 2'-methyl group would target these analogues to the viral polymerases rather than the human polymerases, thus resulting in lower cytotoxicity compared to the 2'-deoxy-2'-fluoro analogues. In an HCV replicon assay, the cytidine analogue demonstrated an EC_{90} of $5.40 \pm 2.6 \mu\text{M}$ with no associated cytotoxicity up to $100 \mu\text{M}$ while the uridine analogue was inactive, although also not cytotoxic (Clark et al., 2005).

As mentioned previously, one of the limitations of nucleoside analogues is the first phosphorylation step by viral or cellular kinases. Interestingly, while the 2'-deoxy-2'-fluoro-2'-methyluridine analogue did not demonstrate any antiviral activity in the HCV replicon assays, the triphosphate of this analogue demonstrated potent inhibitory activity against the HCV NS5B, with an IC_{50} of $1.19 \mu\text{M}$ (Ma et al., 2007; Murakami et al., 2008). This suggested that the 2'-deoxy-2'-fluoro-2'-methyluridine analogue was not efficiently phosphorylated to the monophosphate, thus the use of the phosphoramidate ProTide method could potentially increase antiviral activity. Through an extensive SAR study, Sofia et al. found that a phosphoramidate structure with an isopropyl alkyl chain, L-alanine, and phenyl aromatic substituent (Fig. 10) greatly increased antiviral activity of the uridine analogue, PS-7851, with an EC_{90} of $0.52 \mu\text{M}$ (Sofia et al., 2010; Lam et al., 2010). Further analysis to determine safety found that this analogue demonstrated no cytotoxicity up to $100 \mu\text{M}$ against numerous cell lines including the human hepatocyte cell lines Huh7 and HepG2, the human pancreatic cell line BxBC3, and the human T-lymphoblast cell line CEM (Sofia et al., 2010). As PS-7851 is a mixture of diastereomers at the phosphorus center, it was important to determine which isomer demonstrated a greater antiviral activity, was potentially more toxic, or whether the two isomers worked synergistically. Sofia et al. found that the Sp isomer (PS-7977, sofosbuvir) was indeed more active, with an EC_{90} of $0.42 \mu\text{M}$ while the Rp isomer (PS-7976) had an EC_{90} of $7.5 \mu\text{M}$ (Sofia et al., 2010). Neither analogue demonstrated cytotoxicity at concentrations up to $100 \mu\text{M}$ (Sofia et al., 2010). Most importantly, sofosbuvir consistently produced high levels of triphosphate in liver cells across all species tested (Sofia et al., 2010; Lam et al., 2010; Murakami et al., 2010). These promising results led to the development of sofosbuvir in clinical trials, and ultimately sofosbuvir was approved

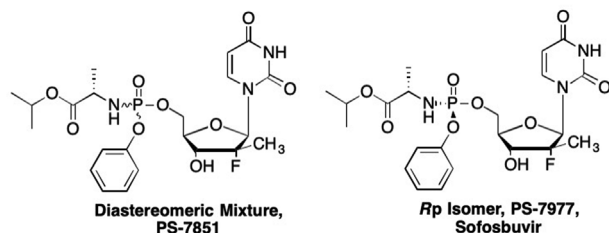


Fig. 10. Phosphoramidate prodrug of 2'-deoxy-2'-fluoro-2'-methyluridine.

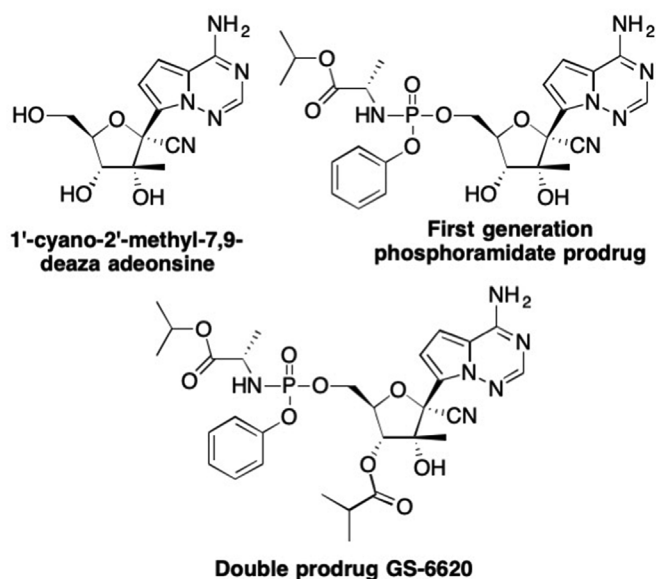


Fig. 11. Structure of the parent analogue, first generation phosphoramidate prodrug, and the double prodrug GS-6620.

by the FDA (under the name[®] Sovaldi). Moreover, sofosbuvir was also approved combination with other drugs such as ribavirin and ledipasvir for use in prophylaxis after liver transplantation, as a treatment for recurring HCV infection, as well as against numerous HCV genotypes (Charlton et al., 2015; Curry et al., 2015; Desnoyer et al., 2016; Foster et al., 2015; Gane et al., 2013, 2014; Jacobson et al., 2013; Lawitz et al., 2013).

3.3. Other 2'-Combination approaches

The field of nucleoside analogue drug design has greatly benefitted from combination approaches, whereby researchers merged different structural modifications that proved essential for antiviral activity into one analogue. This was highlighted with GS-5734, which combines a 1'-sugar modification, a deaza purine nucleobase, substitution of the N9 with a carbon to create a C-nucleoside, and the McGuigan ProTide technology (Siegel et al., 2017; Lo et al., 2017; Sheahan et al., 2017). Another analogue to use this strategy is GS-6620 (Fig. 11), a C-nucleoside adenosine analogue originally developed by researchers at Gilead Sciences for HCV treatment (Cho et al., 2014). As mentioned previously, the 1'-cyano group occupies a pocket in the viral polymerase binding site, which leads to the increased selectivity of the 1'-CN analogues for viral over human polymerases (Siegel et al., 2017; Warren et al., 2016). Like GS-5734, employing a C-nucleoside replaces the hemiaminal (O–C–N) glycosidic bond with an O–C–C bond, thus decreasing the nucleoside analogue's susceptibility to enzymatic glycosidic bond cleavage (Temburnikar and Seley-Radtke, 2018; De Clercq, 2016; Stambaský et al., 2009; Siegel et al., 2017). This was also observed in studies with 7-deaza-2'-methyladenosine analogues, which exhibited an increase in antiviral activity, half-life, and oral bioavailability, since the 7-deaza analogues were no longer susceptible to deamination or cleavage by adenosine-metabolizing enzymes (Zmurko et al., 2016; Olsen et al., 2004; Di Francesco et al., 2012b; Bloch et al., 1967).

As previously mentioned, addition of a 2'-methyl group has also proved fruitful, since once incorporated into the growing strand, analogues with this modification prevent incoming nucleoside triphosphates from binding to the active site of HCV NS5B, thus acting as non-obligate chain terminators (Cho et al., 2014; Migliaccio et al., 2003). With these modifications in mind, Cho et al. synthesized a novel 1'-cyano-2'-methyl-7,9-deaza adenosine analogue (Fig. 11) and screened it

for activity against HCV (Cho et al., 2014). Unfortunately, this analogue failed to display antiviral activity up to 89 μM in whole-cell replicon assays. This was subsequently shown to be due to the presence of the 1'-cyano group, which limited the first phosphorylation step, likely due to the changes in the sugar pucker, which can affect recognition by kinases (and other enzymes) (Cho et al., 2012, 2014). Surprisingly however, the corresponding triphosphate displayed an IC_{50} value of 0.29 μM , thus the ProTide approach was employed to overcome the rate-limiting phosphorylation step, as well as to increase the amount of triphosphate delivered to the target cell (Cho et al., 2014). This proved successful, and it was found that the phosphoramidate prodrug (Fig. 11) demonstrated moderate HCV activity in the replicon assay ($\text{EC}_{50} = 1.05 \mu\text{M}$) and was efficiently converted to the active triphosphate (Cho et al., 2014). Subsequent pharmacokinetic studies to determine the triphosphate levels in hamster liver revealed that the phosphoramidate analogue did not yield adequate amounts of triphosphate following oral absorption (Cho et al., 2014). In order to overcome this limitation, Cho et al. then utilized a double prodrug approach, in which the 3'-OH was protected using an isobutyrate group (Fig. 11). (Cho et al., 2014) This modification not only increased lipophilicity and oral absorption, it also resulted in much higher levels of triphosphate in primary human hepatocytes (Cho et al., 2014). Similar to sofosbuvir, the double-prodrug analogue was a diastereomeric mixture, thus further analysis found that the Sp isomer was 6-fold more potent in HCV replicon assays and delivered 3-fold higher levels of triphosphate to primary human hepatocytes as compared to the Rp isomer (Cho et al., 2014). Due to the promising antiviral profile of the Sp isomer (later known as GS-6620), this analogue was chosen for further clinical evaluation (Cho et al., 2014; Feng et al., 2014; Murakami et al., 2014).

3.4. 2'-Cyano modification

Not just confined to the 1'-position, the cyano group has also found use at other positions on the nucleoside sugar moiety. As mentioned previously, some of the first nucleosides discovered to have medicinal properties were the arabinose or "Ara" analogues, such as Ara-C (Fig. 12), which demonstrated potent activity against numerous cancers including non-Hodgkin's lymphoma, myeloid leukemia, acute myeloblastic leukemia, and many others (Reese and Schiller, 2013; Schilsky et al., 1987; Bodey et al., 1969; Hiddemann, 1991; Livingston and

Carter, 1968). Unfortunately, there are numerous drawbacks to using Ara-C in anticancer treatment including a short half-life in plasma, inactivation by deamination to the inactive uracil metabolite by cytidine deaminase, development of resistance, and ineffectiveness against solid tumors (Chabner et al., 1979; Prince et al., 1969; Ho, 1973; Plunkett and Gandhi, 1993). It was later shown that adding various groups to the 2'- β position of 2'-deoxycytidine could have profound effects on the stability of this analogue by decreasing its susceptibility to cytidine deaminase. Studies found that the introduction of an electron-withdrawing substituent at the 2'- β position increased the acidity of the 2'- α proton, thus β -elimination could occur, resulting in DNA strand breaks (Azuma et al., 1993, 1995; Matsuda et al., 1991a, 1991b; Matsuda and Azuma, 1995). This was particularly interesting, due to the hypothesis that radiation therapy causes DNA strand breaks, leading to tumor cell death (Baskar et al., 2012). Thus, the addition of a 2'- β -cyano group was employed to yield 2'-C-cyano-2'-deoxy-1- β -D-arabinofuranosylcytosine (CNDAC, Fig. 12) which demonstrated potent *in vitro* activity against numerous human tumor cells such as sarcomas, osteosarcomas, fibroblastomas, and carcinomas (Azuma et al., 1993, 1995, 1997, 2001; Matsuda et al., 1991a; Matsuda and Azuma, 1995; Tanaka et al., 1992). In comparison to the parent analogue Ara-C, CNDAC demonstrated potent cytotoxicity in 14 tumor cell lines, with IC_{50} values from 0.04 to 6.8 $\mu\text{g}/\text{mL}$, whereas Ara-C was only active against 6 of these cell lines, with IC_{50} values of 0.09–4.5 $\mu\text{g}/\text{mL}$ (Matsuda et al., 1991a). Furthermore, CNDAC is very effective against solid tumors, whereas Ara-C and the widely used 5-fluorouracil and 5'-deoxy-5-fluorouridine were not active against these solid tumors (Azuma et al., 1995; Matsuda et al., 1991a; Tanaka et al., 1992). This led to a series of clinical trials, including one in which CNDAC is currently in Phase I/II trials against acute myeloid leukemia and acute lymphatic leukemia (NCT01702155) (Kantarjian et al., 2015a, 2015b, 2016), and another in which a prodrug of CNDAC, sapacitabine (Fig. 12), is in Phase III trials for acute myeloid leukemia and myelodysplastic syndrome (NCT01303796) (Kantarjian et al., 2010, 2012).

3.5. 2'-Ethynyl modification

From the studies with 2'-cyano groups, researchers found that acetylene-derived analogues were an important sugar modification associated with potent antiviral effects. Furthermore, modification at the N7 position of 2'-acetylene adenosine analogues, such as removal of the nitrogen to yield a 7-deaza analogue or addition of a carbamoyl moiety, led to profound antiviral activity against DENV (Chen et al., 2010a, 2010b; Deng et al., 2016). While the analogue, NITD449, demonstrated moderate anti-DENV activity ($\text{EC}_{50} = 2.0 \mu\text{M}$), it was also associated with cytotoxicity (Chen et al., 2010b). Interestingly, the 7-deaza analogue NITD008 (Fig. 13) demonstrated similar potency without the associated cytotoxicity (Yin et al., 2009; Shang et al., 2014; Lo et al., 2016). Studies found that this analogue was not cytotoxic up to 50 μM across numerous cell lines and inhibited DENV-2 with an EC_{50} of 0.64 μM (Yin et al., 2009).

More importantly, NITD008 was also effective against the other three serotypes (Yin et al., 2009). This is critical, because a patient is

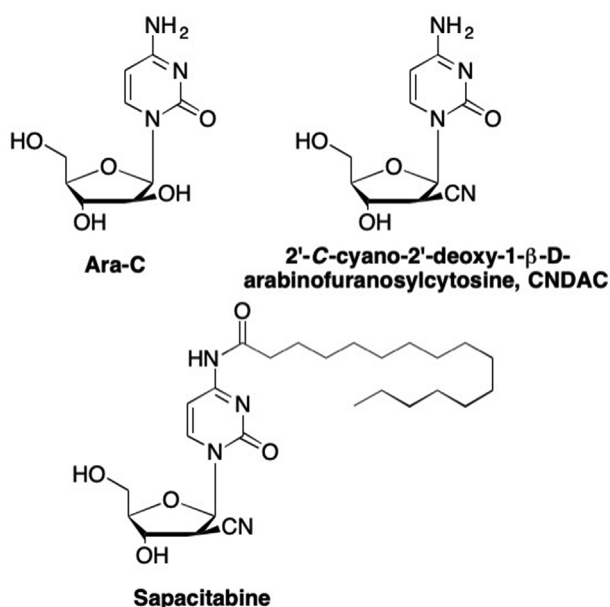


Fig. 12. Structure of 2'- β modified 2'-deoxycytosine analogues Ara-C, CNDAC, and the prodrug Sapacitabine.

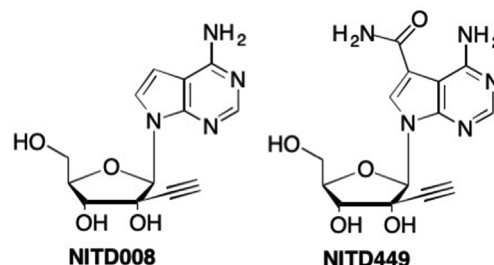


Fig. 13. Structure of the adenosine based analogues NITD008 and NITD449.

more likely to develop severe dengue hemorrhagic fever or dengue shock syndrome when infected a second time, by a different serotype, thus an analogue with activity against all serotypes is highly sought after (Katzelnick et al., 2017; Low et al., 2017). Furthermore, studies found that NITD008 was also effective against other flaviviruses including WNV, YFV, HCV, ZIKV, and TBEV, as well as against enterovirus 71 (Deng et al., 2014, 2016; Yin et al., 2009; Shang et al., 2014; Lo et al., 2016; Qing et al., 2016). Elucidation of the mechanism of action determined that the triphosphate of NITD008 interacted with the flavivirus RdRp with an IC_{50} of 0.31 μ M and served as a chain terminator in a similar fashion as the 2'-methyl analogues, thereby halting viral RNA elongation (Deng et al., 2016; Yin et al., 2009). Other *in vivo* studies determined NITD008 could suppress peak viremia in infected mice and completely protected them from death (Yin et al., 2009). Further studies with NITD008 and other flaviviruses are currently under way to determine if NITD008 could prove to be a broad-spectrum therapeutic against a variety of flaviviruses (Deng et al., 2016; Lo et al., 2016; Qing et al., 2016).

4. 3' Modifications

Early examples of modifications at the 3' position of nucleoside sugars led to the development of novel analogues such as the 3'-methyl nucleosides (Mikhailov et al., 1983; Franchetti et al., 2005; Cappellacci et al., 2006), however, the 2', 3'-dideoxy nucleosides (Veal et al., 1995; Horwitz et al., 1967; Mitsuya and Broder, 1986) and 2'-deoxy-3'-modified nucleosides (Smith et al., 2008; Hostetler et al., 1994) ultimately proved to be more promising. The development of these analogues was covered extensively in the previous review and is not discussed here (Seley-Radtke and Yates, 2018).

5. 4' Modifications

Until the discovery of naturally occurring 4'-modified nucleoside analogues (Thomas et al., 1956), modifications to the 4' position of the furanose ring was rather uncommon in drug design, mainly due to the synthetic challenges. As more facile synthetic routes were developed, more researchers began to pursue these interesting analogues. Researchers soon noted that modifications to the 4' position of the furanose ring changed the sugar pucker from a C2'-exo/C3'-endo "north" conformation, as is common in natural RNA nucleosides (Koole et al., 1992), to a C2'-endo/C3'-exo "south" conformation (Waga et al., 1993). As mentioned previously, this affects recognition by different enzymes, thus can have a significant impact on their biological activity (Saenger and Cantor, 1984; Ikeda et al., 1998).

5.1. 4'-Fluoro modification

As mentioned previously, fluorine substitution has been utilized at a number of positions on both the nucleobase and the sugar moiety, however, one of the lesser explored positions has been substitution at the 4'-carbon of nucleoside sugars. One of the first 4'-modified nucleoside analogues was isolated from *Streptomyces calvus* in 1957 (Thomas et al., 1956), but it wasn't until 1969 that the correct structure of this analogue, later named nucleocidin, was elucidated (Fig. 14). (Jenkins et al., 1971, 1976; Morton et al., 1969; Owen et al., 1976) While this analogue was one of the first examples of a 4'-modified furanose sugar, it also possessed a novel 5'-sulfamoyl group (Jenkins et al., 1971, 1976; Morton et al., 1969; Owen et al., 1976). This unique structure endowed nucleocidin with a broad antiparasitic spectrum, particularly against trypanosomes, however, the practical use of this analogue as a therapeutic was severely limited due to its toxicity (Thomas et al., 1956; Hewitt et al., 1956).

The initial discovery of nucleocidin prompted researchers to pursue other 4'-fluoro modified nucleoside analogues in an effort to decrease the overall toxicity. One such example was developed by Guillem et al.

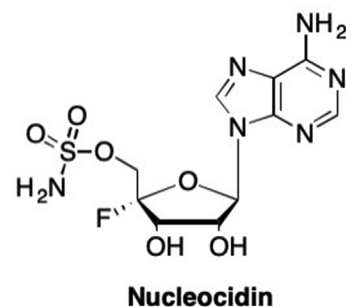


Fig. 14. Structure of the first 4'-modified furanose nucleoside Nucleocidin.

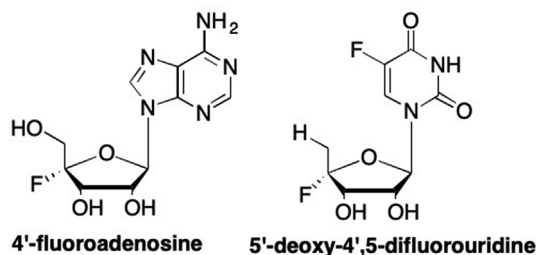


Fig. 15. Second generation 4'-fluoro analogues.

as a potential *S*-adenosyl-L-homocysteine hydrolase (SAHase) inhibitor (Fig. 15). (Guillem et al., 1995) Based on the mechanism of action of SAHase, it was hypothesized that the lack of a 4'-proton on 4'-fluoro-adenosine would completely inhibit further catalysis, however, when tested against SAHase it was determined that 4'-fluoro-adenosine had a 100-fold lower affinity for SAHase compared to natural adenosine, thus this modification proved unsuccessful (Guillem et al., 1995).

Another 4'-fluoro analogue related to nucleocidin was 5'-deoxy-4',5'-difluorouridine (Fig. 15), a 5-fluorouracil analogue that demonstrated similar inhibition of growth of L1210 mouse leukemia cells as the parent 5-fluorouracil, but 10-fold greater activity than previously reported prodrugs (Ajmera et al., 1988a). Since 5'-deoxy-4',5'-difluorouridine does not have a 5'-hydroxyl group, this analogue cannot be converted into the nucleotide and be incorporated by polymerases. Instead, the presence of the 4'-fluoro group makes the glycosidic bond unusually more acid-labile and increases glycosidic bond cleavage by uridine phosphorylase, thus delivering increased amounts of 5-fluorouracil to tumor sites (Ajmera et al., 1988a, 1988b). In comparison to another 5-fluorouracil prodrug, 5'-deoxy-5-fluorouridine (discussed below), 5'-deoxy-4',5'-difluorouridine demonstrated an IC_{50} of 0.3 μ M and a 500-fold increase in glycosidic bond hydrolysis, whereas the IC_{50} of 5'-deoxy-5-fluorouridine was 3.0 μ M, and it failed to undergo significant hydrolysis (Ajmera et al., 1988b). While this analogue appeared promising as a potential anticancer agent, further studies have yet to be reported.

5.2. 4'-Methyl modification

Another common isosteric modification in nucleoside drug design is the substitution of a methyl group for a hydrogen. While this modification has been explored at the 1' (Cappellacci et al., 2002), 2' (Zhang et al., 2011; Carroll et al., 2003; Migliaccio et al., 2003; Eldrup et al., 2004a, 2004b; Pierra et al., 2006), and 3' (Mikhailov et al., 1983; Franchetti et al., 2005; Cappellacci et al., 2006; Orlov et al., 2017) positions on the furanose ring, little research had been reported on the presence of a methyl group at the 4' position. As mentioned previously, it was found that the addition of a 4'-modification altered the reactivities of the 3' and 5'-hydroxyl groups compared to natural nucleosides due to steric effects (Waga et al., 1993), thus 4'-methyl modifications

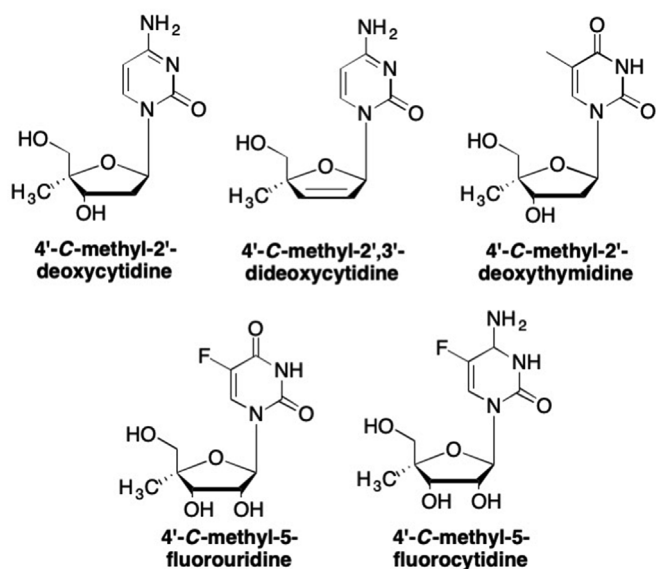


Fig. 16. Examples of 4'-methyl nucleoside analogues.

were considered an interesting avenue to pursue.

Synthesis of 4'-methyl analogues was pioneered by Waga et al. in the early 90s for use as potential anticancer and/or antiviral therapeutics (Waga et al., 1993, 1996). While the change in sugar pucker due to the presence of the 4'-methyl substituent imparted these analogues with interesting characteristics, Waga et al. also hypothesized that the 4'-methyl modification could act synergistically with other sugar modifications (Fig. 16). (Waga et al., 1996) Combining the 4'-methyl modification with dideoxy sugars, 2'-deoxy sugars, and saturated dideoxy sugars with various nucleobases, the resulting analogues were screened against HIV-1 in MT-4 cells (Waga et al., 1996). Most of the analogues failed to display any notable antiviral activity, with IC_{50} values ranging from 21 μ M to over 500 μ M, however, the 4'-methyl-thymidine analogue and the 4'-methyl-2'-deoxycytidine analogue displayed potent activity with IC_{50} values of 7.2 μ M and 0.072 μ M respectively (Waga et al., 1996; Nomura et al., 1999). While the thymidine analogue did not display cytotoxicity up to 100 μ M, the cytidine analogue was quite toxic with a CC_{50} value of 0.13 μ M, thus these analogues were not pursued further (Waga et al., 1996; Nomura et al., 1999).

Years later, the 4'-methyl modification was revisited when Gosselin et al. synthesized a ribose series that focused on nucleobase modifications instead of the sugar modifications seen in the study by Waga et al. (Fig. 16). (Griffon et al., 2003) Like the analogues synthesized by Waga, the 4'-methyl ribose analogues were evaluated for their inhibitory effects against HIV-1 replication in MT-4 cells, however, none demonstrated any meaningful antiviral activity (Griffon et al., 2003). These analogues were also tested for activity against other viruses including HBV in HBV DNA-transfected Hep-G2 cells (2.2.15 cells) and against YFV in BHK cells. Again, no antiviral activity or cytotoxicity was observed with any of the analogues against either virus (Griffon et al., 2003), so pursuit of the 4'-methyl modification approach was abandoned.

5.3. 4'-Azido modifications

With the early success of 2'-deoxy-3'-azidothymidine (AZT, zidovudine) (Smith et al., 2008; Hostetler et al., 1994; Shirasaka et al., 1995; Horwitz et al., 1964), which bears a 3'-azido modification, researchers sought to utilize this modification at different positions on the sugar ring to either increase antiviral activity, or, perhaps most importantly, decrease cellular toxicity. One example that garnered early

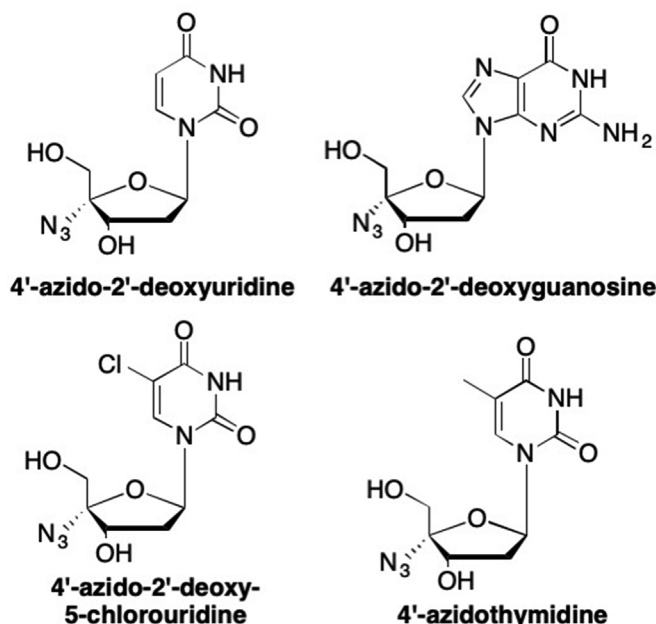


Fig. 17. Novel 4'-azido nucleoside analogues with potent anti-HIV activity.

attention was the addition of the azide group at the 4'-position of the sugar, due to the result the electron-withdrawing effects of the azide group on the sugar pucker (Maag et al., 1992). Thus, a series of 2'-deoxy-4'-azido nucleosides was synthesized and demonstrated potent anti-HIV activity. These were the first nucleoside analogues with potent anti-HIV activity that had an azido group in any position other than the 3' position (Fig. 17). (Maag et al., 1992; Chen et al., 1992) Interestingly, the presence of the 4'-azido group in these analogues affected the 3' and 5'-hydroxyl groups in a way that brought these two groups into closer proximity (Maag et al., 1992; Chen et al., 1992). Due to its electro-negativity, the 4'-azido group prefers a pseudoaxial orientation, which then forces both the 3' and 5'-hydroxyl groups into pseudo-equatorial positions, causing the sugar to adopt a C3'-endo/C2'-exo RNA-type conformation (Maag et al., 1992). Thus, while these analogues retain the 3'-hydroxyl moiety in contrast to AZT and other HIV chain terminators, the change in sugar pucker allows the 4'-azido analogues to still act as DNA chain terminators, however designated as pseudo-obligate chain terminators (Maag et al., 1992; Chen et al., 1992; Yamada et al., 2015).

While none of the analogues was more active than AZT against HIV-1 isolates, they did demonstrate potent activity (Maag et al., 1992; Chen et al., 1992). The IC_{50} for the 4'-azido-2'-deoxyuridine analogue was 0.8 μ M with no associated cytotoxicity up to 200 μ M, whereas the 4'-azido-2'-deoxy-5-chlorouridine analogue possessed an IC_{50} value of 0.056 μ M, also with no cytotoxicity (Maag et al., 1992). Furthermore, the 4'-azidothymidine analogue demonstrated equipotent activity against HIV-1 with an IC_{50} value of 0.01 μ M compared to AZT in A3.01 cells (Chen et al., 1992). Even more significant was the observation that 4'-azidothymidine was effective against strains of HIV-1 that were AZT-resistant (Maag et al., 1992; Chen et al., 1992). Unfortunately, this analogue proved to have increased cytotoxicity compared to AZT, and never progressed to the clinic.

In contrast, one of the most successful 4'-azido analogues was 4'-azidocytidine, later termed R1479, which demonstrated potent anti-HCV activity (Fig. 18). (Smith et al., 2007; Klumpp et al., 2006) This analogue was originally discovered during a 4'-modification SAR study by Roche, where it was found that 4'-azidocytidine showed an IC_{50} of 1.28 μ M in an HCV replicon proliferation assay, and the triphosphate of 4'-azidocytidine inhibited the HCV NS5B with an IC_{50} of 0.30 μ M (Smith et al., 2007). Further analysis found that R1479 was also active

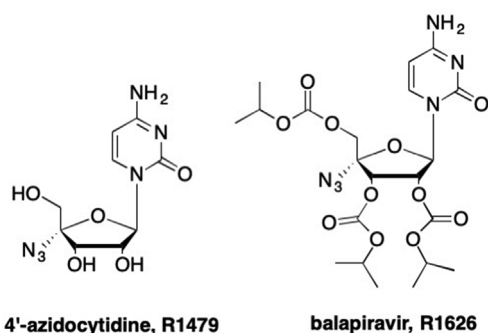


Fig. 18. Structure of 4'-azidocytidine and its tri-isobutyl ester prodrug balapiravir.

against mutant strains of HCV (Klumpp et al., 2006). These promising results, along with low cytotoxicity, led researchers to further investigate 4'-azidocytidine against other viruses. It was found that R1479 was effective against HCV and against DENV serotypes 1, 2, and 4 (EC₅₀ range 1.3–3.2 μM) in primary human macrophages (Nguyen et al., 2013), as well as against respiratory syncytial virus (RSV), for which R1479-TP had an IC₅₀ of 0.24 μM (Hotard et al., 2017; Wang et al., 2015). More recent studies found micromolar activity against the henipaviruses, Nipah and Hendra (Hotard et al., 2017). Unfortunately, like many nucleoside analogues, R1479 suffered from low bioavailability, thus a tri-isobutyl ester prodrug moiety was introduced to give balapiravir (R1626, Fig. 18). (Klumpp and Smith, 2011; Roberts et al., 2008; Toniutto et al., 2008; Brandl et al., 2008) Although balapiravir demonstrated increased antiviral activity compared to the parent analogue R1479 and was efficacious in clinical trials against HCV (Klumpp and Smith, 2011; Roberts et al., 2006, 2008; Robson et al., 2007; Pockros et al., 2008a), the development of more potent nucleoside analogues, such as sofosbuvir, as well as adverse toxicity (Pockros et al., 2008b), halted further advancement of this analogue as an HCV therapeutic. Later, balapiravir was analyzed in a clinical study against DENV (Nguyen et al., 2013; Chen et al., 2014), however, treatment was not well tolerated due to adverse effects, nor did it decrease viral load or fever clearance time, thus clinical trials were terminated.

Other 4'-azido analogues of early interest include 4'-azido-aracytidine (RO-9187) and 4'-azido-2'-methyl nucleosides (Fig. 19) for use as anti-HCV analogues (Eyer et al., 2016; Klumpp et al., 2008; Smith et al., 2009; Rondla et al., 2009; Nilsson et al., 2012). RO-9187 features a 4'-azido group and the 2' ara, or “up” hydroxyl group and was discovered through an SAR study in which the authors were attempting to develop more potent 4'-azido ribonucleosides against HCV (Smith et al., 2007; Klumpp et al., 2008). Interestingly, RO-9187 proved to be the most potent analogue tested, with an IC₅₀ of 0.171 μM, and no associated cytotoxicity up to 1000 μM (Smith et al., 2007; Klumpp et al., 2008). Furthermore, studies found that not only was it a potent anti-HCV analogue, but it also was effective against TBEV with an EC₅₀ of 0.3 ± 0.01 μM and no associated cytotoxicity up to 50 μM (Eyer et al.,

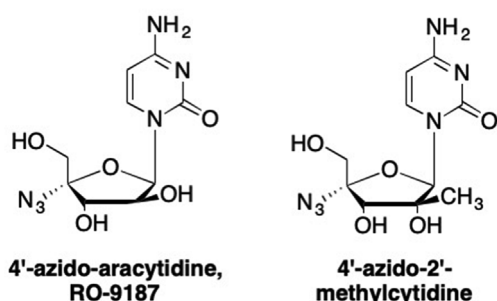


Fig. 19. Novel structure of 4'-azido-aracytidine and 4'-azido-2'-methylcytidine.

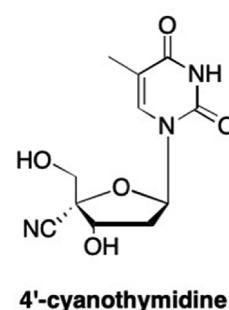


Fig. 20. Potent HIV inhibitor 4'-cyanothymidine.

2016). Similarly, researchers analyzed the change in antiviral activity when the ara hydroxyl group was substituted with a methyl group, yielding 2'-methyl analogues such as 4'-azido-2'-methylcytidine (Fig. 19). (Rondla et al., 2009; Nilsson et al., 2012) Unfortunately, it was determined that this analogue did not demonstrate potent anti-HCV activity, however, the addition of a phosphoramidate prodrug moiety greatly increased antiviral activity, with EC₅₀ values ranging from 3.0 to 4.9 μM (Rondla et al., 2009; Nilsson et al., 2012). This increase in activity has led researchers to pursue other phosphoramidate modifications in order to increase the anti-HCV activity even further, and these studies are currently under way.

5.4. 4'-Cyano modifications

Due to the initial successes of the various 4'-azido analogues, scientists sought more potent candidates, utilizing other moieties with terminal nitrogen atoms such as a 4'-cyano group. This led to the early development of 4'-cyanothymidine (Fig. 20), which demonstrated activity against HIV, with an EC₅₀ of 0.002 μM (O-Yang et al., 1992). Unfortunately, when studied in a mouse model, 4'-cyanothymidine demonstrated toxicity at 0.3 mg/kg dose per day (O-Yang et al., 1992), thus studies were discontinued.

After these initial findings of toxicity, researchers sought additional 4'-cyano analogues in an effort to retain their biological activity and decrease their cytotoxicity. In an SAR study focusing on different 4'-modifications, Nomura et al. determined that the 4'-cyano-2'-deoxycytidine analogue (Fig. 21) demonstrated activity against L1210 tumor cells, herpes simplex virus (HSV) 1, HSV-2, and very potent activity against HIV-1 (EC₅₀ = 0.0012 μM) with no accompanying cytotoxicity (Nomura et al., 1999). Interestingly, the corresponding ribose derivative, 4'-cyanocytidine, demonstrated similar antiviral activity but

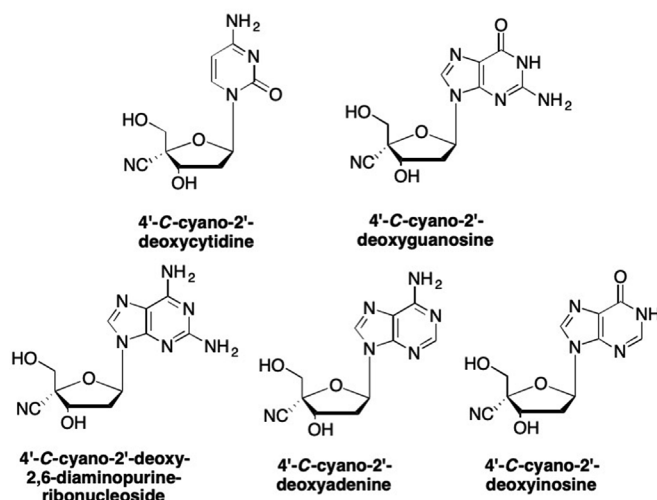


Fig. 21. Second generation 4'-cyano nucleoside analogues.

exhibited much greater levels of cytotoxicity (Smith et al., 2007), thus the deoxyribose analogues were selected for further studies.

Synthesis of these analogues initially was challenging, since 4'-modified sugars exhibit low reactivity in glycosylation reactions, thus making it difficult to add the necessary heterocyclic bases, but researchers subsequently developed novel synthetic approaches by modifying naturally occurring deoxyribonucleosides as starting materials, which allowed for more readily available 4'-C-modified analogues (Nomura et al., 1999; Kohgo et al., 2004). Subsequent studies showed that 4'-cyano-2'-deoxyguanosine (CdG) and 4'-cyano-2'-deoxy-2',6-diaminopurine-ribonucleoside (CAaA) analogues (Fig. 21) exhibited sub-nanomolar levels of activity against HIV-1 ($EC_{50} = 0.19$ nM and $EC_{50} = 0.79$ nM respectively), but were associated with significant toxicity (Kohgo et al., 2004). By comparison, the 4'-cyano-2'-deoxyadenosine (CdA) and 4'-cyano-2'-deoxyinosine analogues (CdI) (Fig. 21) exhibited sub-micromolar activity ($EC_{50} = 0.05$ μ M for both) with little to no cytotoxicity (Kohgo et al., 2004). Both CAaA and CdG were analyzed further by Takamatsu et al., who found that both analogues demonstrated potent activity against HIV-1, but also had potent activity against hepatitis B virus (HBV) ($EC_{50} = 0.4$ nM for both analogues) with less cytotoxicity than previously reported (Kohgo et al., 2004; Takamatsu et al., 2015).

5.5. 4'-Combination approach

Before the discovery of the anti-HIV activity of 4'-modified nucleoside analogues, many scientists believed that only HIV therapeutics lacking a 3'-hydroxyl group could act as chain terminators. While the absence of a 3'-hydroxyl group did impart potent anti-HIV effects, there were also several disadvantages including poor phosphorylation and reduced recognition by the polymerases (Kirby et al., 2013; Gallois-Montbrun et al., 2002; Hattori et al., 2009; Nakata et al., 2007). Researchers therefore attempted to design novel analogues that would retain antiviral potency, as well as the recognition required for activation and incorporation.

One such analogue was 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA, Fig. 22). Notably, EFdA demonstrated potent anti-HIV activity with an EC_{50} of 0.05 nM, but also significant activity against nucleoside reverse transcriptase inhibitor (NRTI)-resistant strains (Kirby et al., 2013; Hattori et al., 2009; Ohruai, 2006; Ohruai et al., 2007). The design of EFdA's scaffold was a result of combining several strategic structural modifications. For example, the fluorine moiety at the 2-position of the adenosine nucleobase was chosen due to the observation that a fluorine or another halogen at the 2-position of 4'-modified nucleoside analogues significantly enhances antiviral activity (Kirby et al., 2013; Kawamoto et al., 2008). This increase in activity was due to the decreased susceptibility of EFdA to adenosine deaminase, as a result of the highly electronegative fluorine at the 2-position (Kirby et al., 2013; Kawamoto et al., 2008). The addition of the 4'-ethynyl moiety also plays a role in decreasing deamination of EFdA by adenosine

deaminase, thus the two modifications work synergistically (Kirby et al., 2013).

The second modification was the retention of the 3'-OH to ensure recognition by the kinases, as this was also known to be important. Related, to this, addition of the 4'-ethynyl group leads EFdA to strongly favor the "north" (C2'-exo/C3'-endo) conformation. A number of studies have determined that HIV-1 reverse transcriptase (RT) prefers NRTIs and/or incoming nucleotides with a north confirmation, thus EFdA binding with HIV-1 RT is optimized (Kirby et al., 2011, 2013; Boyer et al., 2005; Marquez et al., 1998; Michailidis et al., 2009). Furthermore, while EFdA is readily recognized and incorporated by HIV-1 RT, it does not inhibit human DNA polymerases α or β , or mitochondrial DNA polymerase γ , thus EFdA displays a superior toxicity profile compared to other HIV-1 NRTIs (Ohruai, 2006; Ohruai et al., 2007; Michailidis et al., 2009; Sohl et al., 2012).

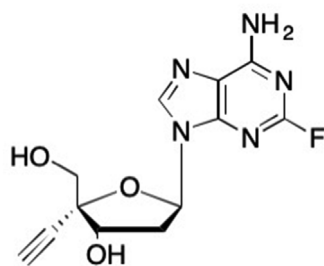
While the addition of the 4'-ethynyl group had a profound effect on the sugar pucker, and thus the increased binding affinity with HIV-1 RT, the 4'-ethynyl moiety also endowed EFdA with two unique mechanisms of action. Instead of obligate, non-obligate, delayed or pseudo-obligate chain termination typical of other nucleoside analogues, EFdA acts as a translocation-defective reverse transcriptase inhibitor (TDRTI) (Michailidis et al., 2009; Muftuoglu et al., 2014; Salie et al., 2016). Once EFdA-TP is incorporated by RT at the 3'-primer terminus, the unique structure of EFdA blocks translocation of the primer strand on the viral polymerase, thus further nucleotides cannot be incorporated (Michailidis et al., 2009; Muftuoglu et al., 2014; Salie et al., 2016). Interestingly, EFdA can also act as a traditional non-obligate chain terminator since it retains the 3'-OH (Michailidis et al., 2009; Muftuoglu et al., 2014; Salie et al., 2016). Due to its low toxicity profile and highly potent anti-HIV activity, EFdA has progressed to clinical trials under the name MK-8591, sponsored by Merck (NCT03272347, NCT02217904).

6. 5' Modifications

While modifications to various positions of the furanose ring are very common, the importance of the 5'-hydroxyl group in nucleotide incorporation for both DNA and RNA synthesis initially caused researchers to avoid modifications at the 5'-position. In some instances however, as seen with the 5'-deoxy, 5'-nor, and truncated carbocyclic nucleosides related to aristeromycin and neplanocin developed by Schneller, Seley, Borchardt, and others, removal or replacement of the 5'-methylene group and/or 5'-hydroxyl group proved beneficial since these analogues could no longer be phosphorylated, thus the toxicity observed with the parent analogues aristeromycin and neplanocin did not occur with the truncated analogues (Seley-Radtke and Yates, 2018; Das and Schneller, 2014; Barnard et al., 2001; Hegde et al., 1999, 2000; Seley et al., 1997a, 1997b, 1997c, 1997d, 1997e, 1997f; Hasobe et al., 1987). Other researchers have also utilized these approaches to their advantage in order to decrease overall toxicity of various nucleoside analogues.

6.1. 5'-Deoxy-5-fluorouridine

One early nucleoside analogue that incorporated the 5' substitution was 5'-deoxy-5-fluorouridine (5'-dFUrd, doxifluridine, Fig. 23), in which instead of a 5'-hydroxymethylene group, 5'-dFUrd has a methyl at that position (Armstrong and Diasio, 1980, 1981; Bollag and Hartmann, 1980; Cook et al., 1979). Like other 5'-modified analogues, 5'-dFUrd cannot be phosphorylated and converted into the corresponding triphosphate. Instead, 5'-dFUrd exerts its effect by acting as a prodrug and releases 5-fluorouracil as a result of glycosidic bond cleavage by human phosphorylases (Kono et al., 1983; Sommadossi et al., 1983). This analogue has demonstrated a broad range activity against numerous cancers including colorectal, leukemia, and melanomas (Armstrong and Diasio, 1980, 1981; Cook et al., 1979;



4'-ethynyl-2-fluoro-2'-deoxyadenosine, EFdA

Fig. 22. Unique structure of 4'-ethynyl-2-fluoro-2'-deoxyadenosine.

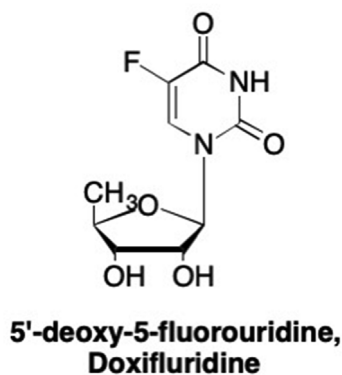


Fig. 23. Structure of one of the first 5'-truncated nucleoside analogues, 5'-deoxy-5-fluorouridine.

Sommadossi et al., 1983; Ishitsuka et al., 1980; Kramer et al., 1979; Tevæarai et al., 1992). Furthermore, compared to other fluorinated pyrimidines, 5'-dFurd exhibits a higher therapeutic index and is less immunosuppressive (Armstrong and Diasio, 1980, 1981; Kramer et al., 1979; Ohta et al., 1980).

Since its initial discovery in the mid 1970's, 5'-FdUrd has progressed from Phase I to numerous Phase II clinical trials for treatment of squamous cell carcinomas, advanced breast cancer, advanced colorectal adenocarcinoma and others (Alberto et al., 1988; Abele et al., 1983, 1984; van Oosterom et al., 1991). Despite these successes, the FDA has yet to approve 5'-dFurd as an anticancer treatment in the United States, thus more research is needed in order to better determine potential side effects that could be associated with this analogue.

7. Additional modifications

This final section is dedicated to describing several types of nucleoside analogues that utilize novel and unique structural modifications, as well as explaining how some are used in unconventional ways.

7.1. Tricyclic analogues

In the first article, various expanded purine nucleobases were described, including Nelson Leonard's benzyl-expanded nucleosides (Leonard et al., 1975, 1976, 1978; Leonard, 1982), and Seley-Radtke's thieno-expanded nucleosides (Seley-Radtke et al., 2008; Wauchope et al., 2012a; Chen et al., 2015a, 2015b). Other examples of interesting tricyclic analogues are the dual-faced bases or the Janus nucleosides, named after Janus, the Roman god of gates and doors (Fig. 24). (Zhou et al., 2013; Chung et al., 1980) These unique nucleosides can present Watson-Crick donor/acceptor base pairing from two different faces of the nucleobase through rotation about the glycosidic bond, thus forming stable base pairs with more than one complementary

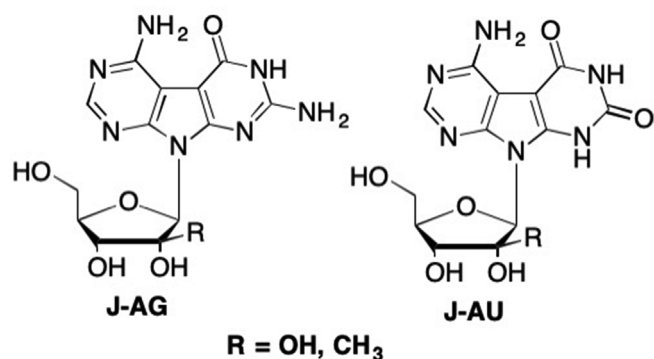


Fig. 24. Janus-type nucleosides that feature two pyrimidine faces.

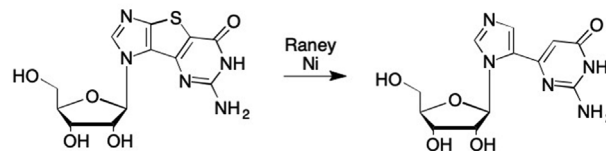


Fig. 25. Origins of fleximer analogues from treatment of thienophene expanded nucleosides with Raney Nickel.

nucleoside (Zhou et al., 2013; Chung et al., 1980). Synthesis of both ribose and 2'-methyl Janus-type nucleosides met with challenges, however, the ribose J-AU and J-AG analogues demonstrated moderate activity against HCV, with EC₅₀ values of 5.7 μM and 3 μM respectively (Zhou et al., 2013). Unfortunately, they all demonstrated significant toxicity in numerous cell lines (Zhou et al., 2013), thus were not extensively pursued.

7.2. Fleximer analogues

A serendipitous outcome of some of the early studies with Seley-Radtke's thiophene-expanded tricyclic analogues led to a new class of novel nucleoside analogues. By treating the tricyclic nucleosides with Raney nickel, the sulfur in the middle ring was removed leaving the two outer rings intact, thereby resulting in a flexible nucleoside. These unique analogues were termed "fleximers" (Fig. 25). (Seley et al., 2001, 2002, 2005a, 2005b) They feature a purine ring that is "split" into the imidazole and pyrimidine moieties, which remain connected by a single carbon-carbon bond from the C4 of the imidazole to the C5 of the pyrimidine in proximal fleximers, or from the C5 of the imidazole to the C6 of the pyrimidine in distal fleximers (Fig. 26). (Seley et al., 2001, 2002, 2005a, 2005b; Wauchope et al., 2012b) This design retains the hydrogen bonding pattern necessary for nucleoside-recognizing enzymes while creating an increase in flexibility that allows for alternative interactions in the enzyme binding site, resulting in a number of highly beneficial properties (Seley et al., 2001, 2002, 2003, 2005a, 2005b).

The inherent flexibility of these analogues allows for free rotation about the carbon-carbon bond, increasing the rotational degrees of freedom and allowing the fleximer to interact with alternative amino acids in the binding pocket, that were previously unattainable by the parent nucleoside (Seley et al., 2003, 2005a, 2005b; Polak et al., 2004; Quirk and Seley, 2005a, 2005b). Further studies found that the introduction of the flexible nucleoside scaffold corresponds to an increase in binding affinity compared to corresponding rigid inhibitors, as well as the ability to circumvent point mutations in the binding site, thus overcoming the development of drug resistance (Seley et al., 2003, 2005a, 2005b; Polak et al., 2004; Quirk and Seley, 2005a, 2005b).

To date a number of different types of fleximer and thiophene analogues of FDA-approved nucleoside analogues have been synthesized by Seley-Radtke et al. (Chen et al., 2015a, 2015b; Seley et al., 2005a, 2005b; Zimmermann et al., 2013, 2014), however, the most

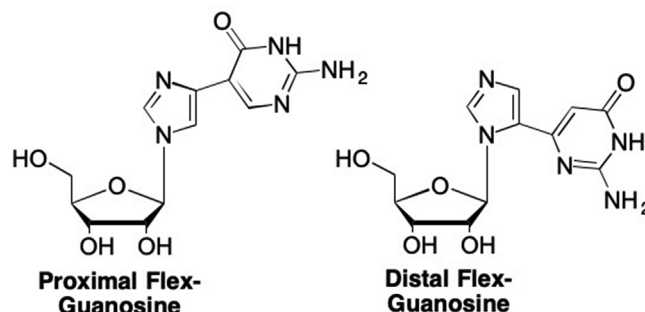


Fig. 26. Structures of proximal and distal fleximers.

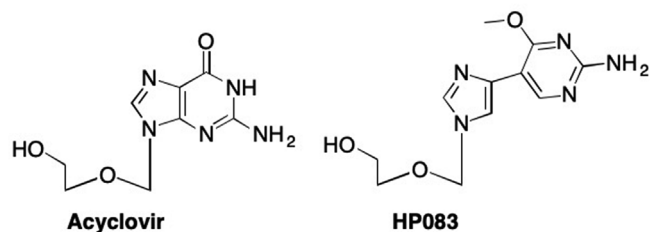


Fig. 27. Structure of Acyclovir compared to the potent antiviral acyclic fleximer analogue HP083.

promising analogues are the more recent acyclic fleximers based on the FDA-approved drug acyclovir. These doubly flexible nucleosides have demonstrated potent micromolar activity against numerous RNA viruses (Fig. 27). (Yates et al., 2017; Peters et al., 2015) The most potent analogue, a methoxy-prodrug of Flex-Acyclovir (HP083), has an EC_{50} of $8.8 \pm 1.5 \mu\text{M}$ against MERS-CoV, and also has potent activity against EBOV at $2.2 \mu\text{M}$ (Yates et al., 2017; Peters et al., 2015). More recent studies with this analogue have found sub-micromolar activity against DENV ($EC_{50} = 0.057 \mu\text{M}$) and YFV ($EC_{50} = 0.37 \mu\text{M}$), as well as potent inhibitory activity of the corresponding triphosphate against both DENV and ZIKV ($IC_{50} = 8.4 \mu\text{M}$ and $1.7 \mu\text{M}$ respectively) (Yates et al., 2018; Seley-Radtke, 2018a, 2018b). Most importantly, these analogues have demonstrated little to no cytotoxicity (Yates et al., 2017, 2018; Peters et al., 2015). Due to their broad-spectrum antiviral activity, they are currently undergoing further analysis to determine their mechanism of action.

8. Concluding remarks

As detailed in this and the previous review, nucleoside analogues remain the cornerstone of antiviral and anticancer therapeutics, particularly in combination therapies. As additional structural and biological information becomes available, new and more complex modifications will continue to be pursued. We hope that these two articles have increased our readers' understanding of the historical modifications to the nucleoside scaffold, the justification for these modifications, and their significance in modern-day therapeutics.

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References

Abele, R., Alberto, P., Kaplan, S., Siegenthaler, P., Hofmann, V., Rysse, H.J., Hartmann, D., Holdener, E.E., Cavalli, F., 1983. Phase II study of doxifluridine in advanced colorectal adenocarcinoma. *J. Clin. Oncol.* 1, 750–754.

Abele, R., Kaplan, E., Grossenbacher, R., Schmid, H.J., Cavalli, F., 1984. Phase II study of doxifluridine in advanced squamous cell carcinoma of the head and neck. *Eur. J. Cancer Clin. Oncol.* 20, 333–336.

Ajmera, S., Bapat, A.R., Danenberg, P.V., Stephanian, E., 1988a. Synthesis and interaction with uridine phosphorylase of 5'-deoxy-4,5-difluorouridine, a new prodrug of 5-fluorouracil. *J. Med. Chem.* 31, 1094–1098.

Ajmera, S., Bapat, A.R., Danenberg, P.V., Stephanian, E., 1988b. Synthesis and interaction with uridine phosphorylase of 5'-deoxy-4,5-difluorouridine, a new prodrug of 5-fluorouracil. *J. Med. Chem.* 31, 1094–1098.

Alberto, P., Jungi, W.F., Siegenthaler, P., Mermillod, B., Obrecht, J.P., Decoster, G., Cavalli, F., 1988. A phase II study of doxifluridine in patients with advanced breast cancer. *Eur. J. Cancer* 24, 565–566.

Armstrong, R.D., Diasio, R.B., 1980. Metabolism and biological activity of 5'-deoxy-5-fluorouridine, a novel fluoropyrimidine. *Cancer Res.* 40, 3333–3338.

Armstrong, R.D., Diasio, R.B., 1981. Selective activation of 5'-deoxy-5-fluorouridine by tumor cells as a basis for an improved therapeutic index. *Cancer Res.* 41, 4891–4894.

Azuma, A., Nakajima, Y., Nishizono, N., Minakawa, N., Suzuki, M., Hanaoka, K., Kobayashi, T., Tanaka, M., Sasaki, T., Matsuda, A., 1993. Nucleosides and nucleotides. 122. 2'-C-cyano-2'-deoxy-1-beta-D-arabinofuranosylcytosine and its derivatives. A new class of nucleoside with a broad antitumor spectrum. *J. Med. Chem.* 36, 4183–4189.

Azuma, A., Hanaoka, K., Kurihara, A., Kobayashi, T., Miyauchi, S., Kamo, N., Tanaka, M., Sasaki, T., Matsuda, A., 1995. Nucleosides and nucleotides. 141. Chemical stability of a new antitumor nucleoside, 2'-C-cyano-2'-deoxy-1-beta-D-arabino-pentofuranosylcytosine in alkaline medium: formation of 2'-C-cyano-2'-deoxy-1-beta-D-ribo-pentofuranosylcytosine and its antitumor activity. *J. Med. Chem.* 38, 3391–3397.

Azuma, A., Huang, P., Matsuda, A., Plunkett, W., 1997. Intracellular metabolism and action of an antitumor nucleoside, 2'-C-Cyano-2'-Deoxy-1-beta-D-arabinofuranosylcytosine (CNDAC). *Nucleosides Nucleotides* 16, 1037–1039.

Azuma, A., Huang, P., Matsuda, A., Plunkett, W., 2001. Cellular pharmacokinetics and pharmacodynamics of the deoxycytidine analog 2'-C-cyano-2'-deoxy-1-beta-D-arabino-pentofuranosylcytosine (CNDAC). *Biochem. Pharmacol.* 61, 1497–1507.

Balzarini, J., Kang, G.J., Dalal, M., Herdewijn, P., De Clercq, E., Broder, S., Johns, D.G., 1987. The anti-HTLV-III (anti-HIV) and cytotoxic activity of 2',3'-dideohydro-2',3'-dideoxyribonucleosides: a comparison with their parental 2',3'-dideoxyribonucleosides. *Mol. Pharmacol.* 32, 162–167.

Balzarini, J., Herdewijn, P., De Clercq, E., 1989. Differential patterns of intracellular metabolism of 2',3'-dideohydro-2',3'-dideoxythymidine and 3'-azido-2',3'-dideoxythymidine, two potent anti-human immunodeficiency virus compounds. *J. Biol. Chem.* 264, 6127–6133.

Barnard, D.L., Stowell, V.D., Seley, K.L., Hegde, V.R., Das, S.R., Rajappan, V.P., Schneller, S.W., Smeed, D.F., Sidwell, R.W., 2001. Inhibition of measles virus replication by 5'-nor carbocyclic adenosine analogues. *Antivir. Chem. Chemother.* 12, 241–250.

Baskar, R., Lee, K.A., Yeo, R., Yeoh, K.W., 2012. Cancer and radiation therapy: current advances and future directions. *Int. J. Med. Sci.* 9, 193–199.

Baumgart, B.R., Wang, F., Kwagh, J., Storck, C., Euler, C., Fuller, M., Simic, D., Sharma, S., Arnold, J.J., Cameron, C.E., Van Vleet, T.R., Flint, O., Bunch, R.T., Davies, M.H., Graziano, M.J., Sanderson, T.P., 2016. Effects of BMS-986094, a guanosine nucleotide analogue, on mitochondrial DNA synthesis and function. *Toxicol. Sci.* 153, 396–408.

Berti, P.J., McCann, J.A., 2006. Toward a detailed understanding of base excision repair enzymes: transition state and mechanistic analyses of N-glycosidic hydrolysis and N-glycosidic transfer. *Chem. Rev.* 106, 506–555.

Bloch, A., Leonard, R.J., Nichol, C.A., 1967. On the mode of action of 7-deaza-adenosine (tubercidin). *Biochim. Biophys. Acta* 138, 10–25.

Bodey, G.P., Freireich, E.J., Monto, R.W., Hewlett, J.S., 1969. Cytosine arabinoside (NSC-63878) therapy for acute leukemia in adults. *Cancer Chemother. Rep.* 53, 59–66.

Bohm, H., Banner, D., Bendels, S., Kansy, M., Kuhn, B., Muller, K., Obst-Sander, U., Stahl, M., 2004. Fluorine in medicinal chemistry. *ChemBiochem* 5, 637–643.

Bollag, W., Hartmann, H.R., 1980. Tumor inhibitory effects of a new fluorouracil derivative: 5'-deoxy-5-fluorouridine. *Eur. J. Cancer* 16, 427–432.

Boyer, P.L., Julias, J.G., Marquez, V.E., Hughes, S.H., 2005. Fixed conformation nucleoside analogs effectively inhibit excision-proficient HIV-1 reverse transcriptases. *J. Mol. Biol.* 345, 441–450.

Brandl, M., Wu, X., Holper, M., Hong, L., Jia, Z., Birudaraj, R., Reddy, M., Alfredson, T., Tran, T., Larrabee, S., Hadig, X., Sarma, K., Washington, C., Hill, G., Smith, D.B., 2008. Physicochemical properties of the nucleoside prodrug R1626 leading to high oral bioavailability. *Drug Dev. Ind. Pharm.* 34, 683–691.

Brox, L.W., LePage, G.A., Hendler, S.S., Shannahoff, D.H., 1974. Studies on the growth inhibition and metabolism of 2'-deoxy-2'-fluorocytidine in cultured human lymphoblasts. *Cancer Res.* 34, 1838–1842.

Cappellacci, L., Barboni, G., Palmieri, M., Pasqualini, M., Grifantini, M., Costa, B., Martini, C., Franchetti, P., 2002. Ribose-modified nucleosides as ligands for adenosine receptors: synthesis, conformational analysis, and biological evaluation of 1'-C-methyl adenosine analogues. *J. Med. Chem.* 45, 1196–1202.

Cappellacci, L., Franchetti, P., Petrelli, R., Riccioni, S., Vita, P.N., Jayaram, H., Grifantini, M., 2006. Purine and pyrimidine nucleoside analogs of 3'-C-methyladenosine as antitumor agents. *Collect. Czech Chem. Commun.* 71.

Carroll, S.S., Tomassini, J.E., Bosserman, M., Getty, K., Stahlhut, M.W., Eldrup, A.B., Bhat, B., Hall, D., Simcoe, A.L., LaFemina, R., Rutkowski, C.A., Wolanski, B., Yang, Z., Migliaccio, G., De Francesco, R., Kuo, L.C., MacCoss, M., Olsen, D.B., 2003. Inhibition of hepatitis C virus RNA replication by 2'-modified nucleoside analogs. *J. Biol. Chem.* 278, 11979–11984.

Carroll, S.S., Davies, M.E., Handt, L., Koeplinger, K., Zhang, R., Ludmerer, S.W., MacCoss, M., Hazuda, D.J., Olsen, D.B., 2006. Robust suppression of viral replication by a nucleoside polymerase inhibitor in chimpanzees infected with hepatitis C virus. *Hepatology* 44.

Chabner, B.A., Hande, K.R., Drake, J.C., 1979. Ara-C metabolism: implications for drug resistance and drug interactions. *Bull. Cancer* 66, 89–92.

Charlton, M., Gane, E., Manns, M.P., Brown, R.S., Curry, M.P., Kwo, P.Y., Fontana, R.J., Gilroy, R., Teperman, L., Muir, A.J., McHutchison, J.G., Symonds, W.T., Brainard, D., Kirby, B., Dvory-Sobol, H., Denning, J., Arterburn, S., Samuel, D., Fornis, X., Terrault, N.A., 2015. Sofosbuvir and ribavirin for treatment of compensated recurrent hepatitis C virus infection after liver transplantation. *Gastroenterology* 148, 1088–1117.

Chen, M.S., Suttman, R.T., Wu, J.C., Priske, E.J., 1992. Metabolism of 4'-azidothymidine. A compound with potent and selective activity against the human immunodeficiency virus. *J. Biol. Chem.* 267, 257–260.

Chen, Y.L., Yin, Z., Lakshminarayana, S.B., Qing, M., Schul, W., Duraiswamy, J., Kondreddi, R.R., Goh, A., Xu, H.Y., Yip, A., Liu, B., Weaver, M., Dartois, V., Keller, T.H., Shi, P.Y., 2010a. Inhibition of dengue virus by an ester prodrug of an adenosine analog. *Antimicrob. Agents Chemother.* 54, 3255–3261.

Chen, Y.L., Yin, Z., Duraiswamy, J., Schul, W., Lim, C.C., Liu, B., Xu, H.Y., Qing, M., Yip, A., Wang, G., Chan, W.L., Tan, H.P., Lo, M., Liang, S., Kondreddi, R.R., Rao, R., Gu, H., He, H., Keller, T.H., Shi, P.Y., 2010b. Inhibition of dengue virus RNA synthesis by an adenosine nucleoside. *Antimicrob. Agents Chemother.* 54, 2932–2939.

Chen, Y.L., Abdul Ghafar, N., Karuna, R., Fu, Y., Lim, S.P., Schul, W., Gu, F., Herve, M., Yokohama, F., Wang, G., Cerny, D., Fink, K., Blasco, F., Shi, P.Y., 2014. Activation of

- peripheral blood mononuclear cells by dengue virus infection depositions bala-piravir. *J. Virol.* 88, 1740–1747.
- Chen, Z., Jochmans, D., Ku, T., Paeshuyse, J., Neyts, J., Seley-Radtke, K.L., 2015a. Bicyclic and tricyclic “expanded” nucleobase analogues of sofosbuvir: new scaffolds for hepatitis C therapies. *ACS Infect. Dis.* 1, 357–366.
- Chen, Z., Ku, T.C., Seley-Radtke, K.L., 2015b. Thiophene-expanded guanosine analogues of Gemcitabine. *Bioorg. Med. Chem. Lett.* 25, 4274–4276.
- Cho, A., Saunders, O.L., Butler, T., Zhang, L., Xu, J., Vela, J.E., Feng, J.Y., Ray, A.S., Kim, C.U., 2012. Synthesis and antiviral activity of a series of 1'-substituted 4-aza-7,9-dideazaadenosine C-nucleosides. *Bioorg. Med. Chem. Lett.* 22, 2705–2707.
- Cho, A., Zhang, L., Xu, J., Lee, R., Butler, T., Metobo, S., Aktoudianakis, V., Lew, W., Ye, H., Clarke, M., Doerfler, E., Byun, D., Wang, T., Babusis, D., Carey, A.C., German, P., Sauer, D., Zhong, W., Rossi, S., Fenaux, M., McHutchison, J.G., Perry, J., Feng, J., Ray, A.S., Kim, C.U., 2014. Discovery of the first C-nucleoside HCV polymerase inhibitor (GS-6620) with demonstrated antiviral response in HCV infected patients. *J. Med. Chem.* 57, 1812–1825.
- Chung, F.L., Schram, K.H., Panzica, R.P., Earl, R.A., Wotrung, L.L., Townsend, L.B., 1980. Synthesis of certain [6:5:6] linear tricyclic nucleosides as potential antitumor agents. *J. Med. Chem.* 23, 1158–1166.
- Clark, J.L., Hollecker, L., Mason, J.C., Stuyver, L.J., Tharnish, P.M., Lostia, S., McBrayer, T.R., Schinazi, R.F., Watanabe, K.A., Otto, M.J., Furman, P.A., Stec, W.J., Patterson, S.E., Pankiewicz, K.W., 2005. Design, synthesis, and antiviral activity of 2'-deoxy-2'-fluoro-2'-C-methylcytidine, a potent inhibitor of hepatitis C virus replication. *J. Med. Chem.* 48, 5504–5508.
- Cook, A.F., Holman, M.J., Kramer, M.J., Trown, P.W., 1979. Fluorinated pyrimidine nucleosides. 3. Synthesis and antitumor activity of a series of 5'-deoxy-5-fluoropyrimidine nucleosides. *J. Med. Chem.* 22, 1330–1335.
- Cretton-Scott, E., Perigaud, C., Peyrottes, S., Licklider, L., Camire, M., Larsson, M., La Colla, M., Hildebrand, E., Lallo, L., Billello, J., McCarville, J., Seifer, M., Liuzzi, M., Pierra, C., Badaroux, B., Gosselin, G., Surleraux, D., Standring, D.N., 2008. In vitro antiviral activity and pharmacology of IDX184, a novel and potent inhibitor of HCV replication. *J. Hepatol.* 48.
- Curry, M.P., Forns, X., Chung, R.T., Terrault, N.A., Brown, R., Fenkel, J.M., Gordon, F., O'Leary, J., Kuo, A., Schiano, T., Everson, G., Schiff, E., Befeler, A., Gane, E., Saab, S., McHutchison, J.G., Subramanian, G.M., Symonds, W.T., Denning, J., McNair, L., Arterburn, S., Svarovskaia, E., Moonka, D., Afdhal, N., 2015. Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology* 148, 100–107 e1.
- Damont, A., Dukhan, D., Gosselin, G., Peyronnet, J., Storer, R., 2007. Synthesis of 1'-C-fluoromethyladenosine. *Nucleos Nucleot. Nucleic Acids* 26, 1431–1434.
- Das, S.R., Schnell, S.W., 2014. The 5'-nor aristeromycin analogues of 5'-deoxy-5'-methylthioadenosine and 5'-deoxy-5'-thiophenyladenosine. *Nucleos Nucleot. Nucleic Acids* 33, 668–677.
- De Clercq, E., 2004. Antivirals and antiviral strategies. *Nat. Rev. Microbiol.* 2, 704–720.
- De Clercq, E., 2005a. Antiviral drug discovery and development: where chemistry meets with biomedicine. *Antivir. Res.* 67, 56–75.
- De Clercq, E., 2005b. Recent highlights in the development of new antiviral drugs. *Curr. Opin. Microbiol.* 8, 552–560.
- De Clercq, E., 2012. Milestones in the discovery of antiviral agents: nucleosides and nucleotides. *Acta Pharm. Sin.* B 2, 535–548.
- De Clercq, E., 2016. C-nucleosides to be revisited. *J. Med. Chem.* 59, 2301–2311.
- De Clercq, E., Li, G., 2016. Approved antiviral drugs over the past 50 years. *Clin. Microbiol. Rev.* 29, 695–747.
- De Clercq, E., Neyts, J., 2009. Antiviral agents acting as DNA or RNA chain terminators. *Handb. Exp. Pharmacol.* 53–84.
- Deng, C.L., Yeo, H., Ye, H.Q., Liu, S.Q., Shang, B.D., Gong, P., Alonso, S., Shi, P.Y., Zhang, B., 2014. Inhibition of enterovirus 71 by adenosine analog NITD008. *J. Virol.* 88, 11915–11923.
- Deng, Y.Q., Zhang, N.N., Li, C.F., Tian, M., Hao, J.N., Xie, X.P., Shi, P.Y., Qin, C.F., 2016. Adenosine analog NITD008 is a potent inhibitor of Zika virus. *Open Forum Infect. Dis.* 3 ofw175.
- Desnoyer, A., Pospai, D., Lê, M.P., Gervais, A., Heurgué-Berlot, A., Laradi, A., Harent, S., Pinto, A., Salmon, D., Hillaire, S., Fontaine, H., Zucman, D., Simonpoli, A.M., Muret, P., Larrouy, L., Bernard Chabert, B., Descamps, D., Yazdanpanah, Y., Peytavin, G., 2016. Pharmacokinetics, safety and efficacy of a full dose sofosbuvir-based regimen given daily in hemodialysis patients with chronic hepatitis C. *J. Hepatol.* 65, 40–47.
- Deval, J., 2009. Antimicrobial strategies: inhibition of viral polymerases by 3'-hydroxyl nucleosides. *Drugs* 69, 151–166.
- Di Francesco, M.E., Avolio, S., Dessole, G., Koch, U., Pompei, M., Pucci, V., Rowley, M., Summa, V., 2012a. Synthesis and antiviral properties of novel tetracyclic nucleoside inhibitors of hepatitis C NS5B polymerase. *Nucleos Nucleot. Nucleic Acids* 31, 592–607.
- Di Francesco, M.E., Avolio, S., Pompei, M., Pesci, S., Monteagudo, E., Pucci, V., Giuliano, C., Fiore, F., Rowley, M., Summa, V., 2012b. Synthesis and antiviral properties of novel 7-heterocyclic substituted 7-deaza-adenine nucleoside inhibitors of Hepatitis C NS5B polymerase. *Bioorg. Med. Chem.* 20, 4801–4811.
- Eldrup, A.B., Prhac, M., Brooks, J., Bhat, B., Prakash, T.P., Song, Q., Bera, S., Bhat, N., Dande, P., Cook, P.D., Bennett, C.F., Carroll, S.S., Ball, R.G., Bosserman, M., Burlein, C., Colwell, L.F., Fay, J.F., Flores, O.A., Getty, K., LaFemina, R.L., Leone, J., MacCoss, M., McMasters, D.R., Tomassini, J.E., Von Langen, D., Wolanski, B., Olsen, D.B., 2004a. Structure-activity relationship of heterobase-modified 2'-C-methyl ribonucleosides as inhibitors of hepatitis C virus RNA replication. *J. Med. Chem.* 47, 5284–5297.
- Eldrup, A.B., Allerson, C.R., Bennett, C.F., Bera, S., Bhat, N., Bosserman, M.R., Brooks, J., Burlein, C., Carroll, S.S., Cook, P.D., Getty, K.L., MacCoss, M., McMasters, D.R., Olsen, D.B., Prakash, T.P., Prhac, M., Song, Q., Tomassini, J.E., Xia, J., 2004b. Structure-activity relationship of purine ribonucleosides for inhibition of hepatitis C virus RNA-dependent RNA polymerase. *J. Med. Chem.* 47, 2283–2295.
- Eyer, L., Valdés, J.J., Gil, V.A., Nencka, R., Hřebabecský, H., Šála, M., Salát, J., Černý, J., Palus, M., De Clercq, E., Růžek, D., 2015. Nucleoside inhibitors of tick-borne encephalitis virus. *Antimicrob. Agents Chemother.* 59, 5483–5493.
- Eyer, L., Šmídová, M., Nencka, R., Neča, J., Kastl, T., Palus, M., De Clercq, E., Růžek, D., 2016. Structure-activity relationships of nucleoside analogues for inhibition of tick-borne encephalitis virus. *Antivir. Res.* 133, 119–129.
- Eyer, L., Kondo, H., Zouharova, D., Hirano, M., Valdés, J.J., Muto, M., Kastl, T., Kobayashi, S., Haviernik, J., Igarashi, M., Kariwa, H., Vaculovicova, M., Cerny, J., Kizek, R., Kröger, A., Lienenklaus, S., Dejmeke, M., Nencka, R., Palus, M., Salát, J., De Clercq, E., Yoshii, K., Ruzek, D., 2017. Escape of tick-borne flavivirus from 2'-C-methylated nucleoside antivirals is mediated by a single conservative mutation in NS5 that has a dramatic effect on viral fitness. *J. Virol.* 91.
- Feng, J.Y., Cheng, G., Perry, J., Barauskas, O., Xu, Y., Fenaux, M., Eng, S., Tirunagari, N., Peng, B., Yu, M., Tian, Y., Lee, Y.J., Stepan, G., Laggapan, L.L., Jin, D., Hung, M., Ku, K.S., Han, B., Kitirinos, K., Perron, M., Birkus, G., Wong, K.A., Zhong, W., Kim, C.U., Carey, A., Cho, A., Ray, A.S., 2014. Inhibition of hepatitis C virus replication by GS-6620, a potent C-nucleoside monophosphate prodrug. *Antimicrob. Agents Chemother.* 58, 1930–1942.
- Feng, J.Y., Tay, C.H., Ray, A.S., 2017. Role of mitochondrial toxicity in BMS-986094-induced toxicity. *Toxicol. Sci.* 155, 2.
- Field, H.J., De Clercq, E., 2004. Antiviral drugs - a short history of their discovery and development. *Microbiol. Today* 31, 58–61.
- Foster, G.R., Pianko, S., Brown, A., Forton, D., Nahass, R.G., George, J., Barnes, E., Brainard, D.M., Massetto, B., Lin, M., Han, B., McHutchison, J.G., Subramanian, G.M., Cooper, C., Agarwal, K., Group, B.S., 2015. Efficacy of sofosbuvir plus ribavirin with or without peginterferon-alfa in patients with hepatitis C virus genotype 3 infection and treatment-experienced patients with cirrhosis and hepatitis C virus genotype 2 infection. *Gastroenterology* 149, 1462–1470.
- Franchetti, P., Cappellacci, L., Pasqualini, M., Petrelli, R., Vita, P., Jayaram, H.N., Horvath, Z., Szekeres, T., Grifantini, M., 2005. Antitumor activity of C-methyl-beta-D-ribofuranosyladenine nucleoside ribonucleotide reductase inhibitors. *J. Med. Chem.* 48, 4983–4989.
- Gallois-Montbrun, S., Schneider, B., Chen, Y., Giacomoni-Fernandes, V., Mulard, L., Morera, S., Janin, J., Deville-Bonne, D., Veron, M., 2002. Improving nucleoside diphosphate kinase for antiviral nucleotide analogs activation. *J. Biol. Chem.* 277, 39953–39959.
- Gane, E.J., Stedman, C.A., Hyland, R.H., Ding, X., Svarovskaia, E., Symonds, W.T., Hinds, R.G., Berrey, M.M., 2013. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N. Engl. J. Med.* 368, 34–44.
- Gane, E.J., Stedman, C.A., Hyland, R.H., Ding, X., Svarovskaia, E., Subramanian, G.M., Symonds, W.T., McHutchison, J.G., Pang, P.S., 2014. Efficacy of nucleotide polymerase inhibitor sofosbuvir plus the NS5A inhibitor ledipasvir or the NS5B non-nucleoside inhibitor GS-9669 against HCV genotype 1 infection. *Gastroenterology* 146, 736–743 e1.
- Gentile, I., Buonomo, A.R., Zappulo, E., Borgia, G., 2015. Discontinued drugs in 2012 - 2013: hepatitis C virus infection. *Expert Opin. Investig. Drugs* 24, 239–251.
- Griffon, J.F., Dukhan, D., Pierra, C., Benzaria, S., Loi, A.G., La Colla, P., Sommadossi, J.P., Gosselin, G., 2003. 4'-C-methyl-beta-D-ribofuranosyl purine and pyrimidine nucleosides revisited. *Nucleos Nucleot. Nucleic Acids* 22, 707–709.
- Gudmundsson, K.S., Freeman, G.A., Drach, J.C., Townsend, L.B., 2000. Synthesis of fluorosugar analogues of 2,5,6-trichloro-1-(beta-D-ribofuranosyl)benzimidazole as antivirals with potentially increased glycosidic bond stability. *J. Med. Chem.* 43, 2473–2478.
- Guillerm, D., Muzard, M., Allart, B., Guillerm, G., 1995. Synthesis of 4'-fluoroadenosine as an inhibitor of S-adenosyl-L-homocysteine hydrolase. *Bioorg. Med. Chem. Lett.* 5, 1455–1460.
- Hasobe, M., McKee, J.G., Borcharding, D.R., Borchardt, R.T., 1987. 9-(trans-2',trans-3'-Dihydroxycyclopent-4'-enyl)-adenine and -3-deazaadenine: analogs of neplanocin A which retain potent antiviral activity but exhibit reduced cytotoxicity. *Antimicrob. Agents Chemother.* 31, 1849–1851.
- Hattori, S., Ide, K., Nakata, H., Harada, H., Suzu, S., Ashida, N., Kohgo, S., Hayakawa, H., Mitsuya, H., Okada, S., 2009. Potent activity of a nucleoside reverse transcriptase inhibitor, 4'-ethynyl-2'-fluoro-2'-deoxyadenosine, against human immunodeficiency virus type 1 infection in a model using human peripheral blood mononuclear cell-transplanted NOD/SCID Janus kinase 3 knockout mice. *Antimicrob. Agents Chemother.* 53, 3887–3893.
- Hegde, V.R., Seley, K.L., Chen, X., Schnell, S.W., 1999. The synthesis of carbocyclic 5'-nor thymidine and an isomer as oligonucleotide monomers. *Nucleosides Nucleotides* 18, 1905–1910.
- Hegde, V.R., Seley, K.L., Schnell, S.W., 2000. Carbocyclic 5'-noruridine. *Nucleos Nucleot. Nucleic Acids* 19, 269–273.
- Herdewijn, P., Balzarini, J., De Clercq, E., Pauwels, R., Baba, M., Broder, S., Vanderhaeghe, H., 1987. 3'-substituted 2',3'-dideoxynucleoside analogues as potential anti-HIV (HTLV-III/LAV) agents. *J. Med. Chem.* 30, 1270–1278.
- Hewitt, R.I., Gumble, A.R., Taylor, L.H., Wallace, W.S., 1956. The activity of a new antibiotic, nucleocidin, in experimental infections with Trypanosoma equiperdum. *Antibiot. Annu.* 722–729.
- Hiddemann, W., 1991. Cytosine arabinoside in the treatment of acute myeloid leukemia: the role and place of high-dose regimens. *Ann. Hematol.* 62, 119–128.
- Ho, D.H., 1973. Distribution of kinase and deaminase of 1-beta-D-arabino-furanosylcytosine in tissues of man and mouse. *Cancer Res.* 33, 2816–2820.
- Horwitz, J.P., Chua, J., Noel, M., Nucleosides, V., 1964. The monomesylates of 1-(2'-Deoxy-beta-D-lyxofuranosyl)thymine. *J. Org. Chem.* 29, 2076–2078.
- Horwitz, J.P., Chua, J., Noel, M., Donatti, J.T., 1967. Nucleosides. XI. 2',3'-

- dideoxycytidine. *J. Org. Chem.* 32, 817–818.
- Hosteler, K.Y., Richman, D.D., Sridhar, C.N., Felgner, P.L., Felgner, J., Ricci, J., Gardner, M.F., Selleseth, D.W., Ellis, M.N., 1994. Phosphatidylazidothymidine and phosphatidyl-dC: assessment of uptake in mouse lymphoid tissues and antiviral activities in human immunodeficiency virus-infected cells and in Rauscher leukemia virus-infected mice. *Antimicrob. Agents Chemother.* 38, 2792–2797.
- Hotard, A.L., He, B., Nichol, S.T., Spiropoulou, C.F., Lo, M.K., 2017. 4'-Azidocytidine (R1479) inhibits henipaviruses and other paramyxoviruses with high potency. *Antivir. Res.* 144, 147–152.
- Ikeda, H., Fernandez, R., Wilk, A., Barchi, J.J., Huang, X., Marquez, V.E., 1998. The effect of two antipodal fluorine-induced sugar puckers on the conformation and stability of the Dickerson-Drew dodecamer duplex [d(CGCGAATTCGCG)]₂. *Nucleic Acids Res.* 26, 2237–2244.
- Ishitsuka, H., Miwa, M., Takemoto, K., Fukuoaka, K., Itoga, A., Maruyama, H.B., 1980. Role of uridine phosphorylase for antitumor activity of 5'-deoxy-5-fluorouridine. *Gann* 71.
- Jacobson, I.M., Gordon, S.C., Kowdley, K.V., Yoshida, E.M., Rodriguez-Torres, M., Sulkowski, M.S., Shiffman, M.L., Lawitz, E., Everson, G., Bennett, M., Schiff, E., Al-Assi, M.T., Subramanian, G.M., An, D., Lin, M., McNally, J., Brainard, D., Symonds, W.T., McHutchison, J.G., Patel, K., Feld, J., Pianko, S., Nelson, D.R., Study, P., Study, F., 2013. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N. Engl. J. Med.* 368, 1867–1877.
- Jenkins, I.D., Verheyden, J.P., Moffatt, J.G., 1971. Synthesis of the nucleoside antibiotic nucleocidin. *J. Am. Chem. Soc.* 93, 4323–4324.
- Jenkins, I.D., Verheyden, J.P., Moffatt, J.G., 1976. 4'-Substituted nucleosides. 2. synthesis of the nucleoside antibiotic nucleocidin. *J. Am. Chem. Soc.* 98, 3346–3357.
- Jordheim, L.P., Durantel, D., Zoulim, F., Dumontet, C., 2013. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug Discov.* 12, 447–464.
- Kantarjian, H., Garcia-Manero, G., O'Brien, S., Faderl, S., Ravandi, F., Westwood, R., Green, S.R., Chiao, J.H., Boone, P.A., Cortes, J., Plunkett, W., 2010. Phase I clinical and pharmacokinetic study of oral sapacitabine in patients with acute leukemia and myelodysplastic syndrome. *J. Clin. Oncol.* 28, 285–291.
- Kantarjian, H., Faderl, S., Garcia-Manero, G., Luger, S., Venugopal, P., Maness, L., Wetzler, M., Coutre, S., Stock, W., Claxton, D., Goldberg, S.L., Arellano, M., Strickland, S.A., Seiter, K., Schiller, G., Jabbour, E., Chiao, J., Plunkett, W., 2012. Oral sapacitabine for the treatment of acute myeloid leukaemia in elderly patients: a randomised phase 2 study. *Lancet Oncol.* 13, 1096–1104.
- Kantarjian, H., Jabbour, E., Garcia-Manero, G., Kadia, T., DiNardo, C., Daver, N., Borthakur, G., Jain, N., Waukau, J., Kwari, M., Anderson, B.D., Iizuka, K., Jin, C., Plunkett, W., 2015a. Results of a phase I/II study of DFP-10917, a nucleoside analog, given by continuous infusion (CI) in patients (pts) with relapsed or refractory acute leukemia. *Blood* 126 3804-3804.
- Kantarjian, H.M., Jabbour, E., Garcia-Manero, G., Kadia, T.M., Dinardo, C.D., Daver, N.G., Borthakur, G., Jain, N., Waukau, J., Kwari, M., Anderson, B.D., Iizuka, K., Jin, C., Plunkett, W., 2015b. First report of a phase I/II study of DFP-10917, a nucleoside analog, given by continuous infusion (CI) in patients (pts) with relapsed or refractory acute leukemia. *J. Clin. Oncol.* 33 7077-7077.
- Kantarjian, H.M., Jabbour, E.J., Garcia-Manero, G., Kadia, T.M., DiNardo, C.D., Daver, N.G., Borthakur, G., Jain, N., Waukau, J., Kwari, M., Anderson, B.D., Iizuka, K., Jin, C., Zhang, C., Ravandi, F., Plunkett, W., 2016. Phase I/II study of DFP-10917 in relapsed/refractory AML demonstrates efficacy and safety profile suitable for phase III study. *Blood* 128.
- Katzelnick, L.C., Coloma, J., Harris, E., 2017. Dengue: knowledge gaps, unmet needs, and research priorities. *Lancet Infect. Dis.* 17, e88–e100.
- Kawamoto, A., Kodama, E., Sarafianos, S.G., Sakagami, Y., Kohgo, S., Kitano, K., Ashida, N., Iwai, Y., Hayakawa, H., Nakata, H., Mitsuya, H., Arnold, E., Matsuoka, M., 2008. 2'-deoxy-4'-C-ethynyl-2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants. *Int. J. Biochem. Cell Biol.* 40, 2410–2420.
- Kirby, K.A., Singh, K., Michailidis, E., Marchand, B., Kodama, E.N., Ashida, N., Mitsuya, H., Parniak, M.A., Sarafianos, S.G., 2011. The sugar ring conformation of 4'-ethynyl-2-fluoro-2'-deoxyadenosine and its recognition by the polymerase active site of HIV reverse transcriptase. *Cell. Mol. Biol. (Noisy-Le-Grand)* 57, 40–46.
- Kirby, K.A., Michailidis, E., Fetterly, T.L., Steinbach, M.A., Singh, K., Marchand, B., Leslie, M.D., Hagedorn, A.N., Kodama, E.N., Marquez, V.E., Hughes, S.H., Mitsuya, H., Parniak, M.A., Sarafianos, S.G., 2013. Effects of substitutions at the 4' and 2' positions on the bioactivity of 4'-ethynyl-2-fluoro-2'-deoxyadenosine. *Antimicrob. Agents Chemother.* 57, 6254–6264.
- Kirk, K., 2006. Fluorine in medicinal chemistry: recent therapeutic applications of fluorinated small molecules. *J. Fluorine Chem.* 127, 1013–1029.
- Klump, K., Smith, D.B., 2011. Discovery and Clinical Evaluation of the Nucleoside Analog Balapiravir (R1626) for the Treatment of HCV Infection. pp. 287–304.
- Klump, K., Lévêque, V., Le Pogam, S., Ma, H., Jiang, W.R., Kang, H., Grancycome, C., Singer, M., Laxton, C., Hang, J.Q., Sarma, K., Smith, D.B., Heindl, D., Hobbs, C.J., Merrett, J.H., Symons, J., Gammack, N., Martin, J.A., Devos, R., Najera, I., 2006. The novel nucleoside analog R1479 (4'-azidocytidine) is a potent inhibitor of NS5B-dependent RNA synthesis and hepatitis C virus replication in cell culture. *J. Biol. Chem.* 281, 3793–3799.
- Klump, K., Kalayanov, G., Ma, H., Le Pogam, S., Leveque, V., Jiang, W.R., Inocencio, N., De Witte, A., Rajyaguru, S., Tai, E., Chanda, S., Irwin, M.R., Sund, C., Winquist, A., Maltseva, T., Eriksson, S., Usova, E., Smith, M., Alker, A., Najera, I., Gammack, N., Martin, J.A., Johansson, N.G., Smith, D.B., 2008. 2'-deoxy-4'-azido nucleoside analogs are highly potent inhibitors of hepatitis C virus replication despite the lack of 2'-alpha-hydroxyl groups. *J. Biol. Chem.* 283, 2167–2175.
- Kohgo, S., Yamada, K., Kitano, K., Iwai, Y., Sakata, S., Ashida, N., Hayakawa, H., Nameki, D., Kodama, E., Matsuoka, M., Mitsuya, H., Ohru, H., 2004. Design, efficient synthesis, and anti-HIV activity of 4'-C-cyano- and 4'-C-ethynyl-2'-deoxy purine nucleosides. *Nucleos Nucleot. Nucleic Acids* 23, 671–690.
- Kono, A., Hara, Y., Sugata, S., Karube, Y., Matsushima, Y., Ishitsuka, H., 1983. Activation of 5'-deoxy-5-fluorouridine by thymidine phosphorylase in human tumors. *Chem. Pharm. Bull. (Tokyo)* 31, 175–178.
- Koole, L.H., Plavec, J., Liu, H., Vincent, B.R., Dyson, M.R., Coe, P.L., Walker, R.T., Hardy, G.W., Rahim, S.G., Chattopadhyaya, J., 1992. Conformation of two 4'-thio-2'-deoxynucleoside analogs studied by 5000-MHz proton NMR spectroscopy and x-ray crystallography. *J. Am. Chem. Soc.* 114, 9936–9943.
- Kramer, M.J., Trown, P.W., Cleeland, R., Cook, A.F., Grunberg, E., 1979. 5'-deoxy-5-fluorouridine—a New orally active antitumor agent. comparative activity with 5-fluorouracil, 2'-deoxy-5-fluorouridine and florafur against transplant-able tumors in mice and rats. *Subj. Strain Bibliogr.* 1519.
- Lalezari, J., Asmuth, D., Casiró, A., Vargas, H., Lawrence, S., Dubuc-Patrick, G., Chen, J., McCarville, J., Pietropaolo, K., Zhou, X.J., Sullivan-Bolyai, J., Mayers, D., 2012. Short-term monotherapy with IDX184, a liver-targeted nucleotide polymerase inhibitor, in patients with chronic hepatitis C virus infection. *Antimicrob. Agents Chemother.* 56, 6372–6378.
- Lam, A.M., Murakami, E., Espiritu, C., Steuer, H.M., Niu, C., Keilman, M., Bao, H., Zennou, V., Bourne, N., Julander, J.G., Morrey, J.D., Smees, D.F., Frick, D.N., Heck, J.A., Wang, P., Nagarithnam, D., Ross, B.S., Sofia, M.J., Otto, M.J., Furman, P.A., 2010. PSI-7851, a pronucleotide of beta-D-2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate, is a potent and pan-genotype inhibitor of hepatitis C virus replication. *Antimicrob. Agents Chemother.* 54, 3187–3196.
- Lawitz, E., Mangia, A., Wyles, D., Rodriguez-Torres, M., Hassanein, T., Gordon, S.C., Schultz, M., Davis, M.N., Kayali, Z., Reddy, K.R., Jacobson, I.M., Kowdley, K.V., Nyberg, L., Subramanian, G.M., Hyland, R.H., Arterburn, S., Jiang, D., McNally, J., Brainard, D., Symonds, W.T., McHutchison, J.G., Sheikh, A.M., Younossi, Z., Gane, E.J., 2013. Sofosbuvir for previously untreated chronic hepatitis C infection. *N. Engl. J. Med.* 368, 1878–1887.
- Lenz, S.A., Kohout, J.D., Wetmore, S.D., 2016. Hydrolytic glycosidic bond cleavage in RNA nucleosides: effects of the 2'-hydroxy group and acid-base catalysis. *J. Phys. Chem. B* 120, 12795–12806.
- Leonard, N.J., 1982. Dimensional probes of enzyme-coenzyme binding-sites. *Acc. Chem. Res.* 15, 128–135.
- Leonard, N.L., Morrice, A.G., Sprecker, M.A., 1975. Linear benzoadenine. A stretched-out analog of adenine. *J. Org. Chem.* 40, 356–363.
- Leonard, N.J., Sprecker, M.A., Morrice, A.G., 1976. Defined dimensional changes in enzyme substrates and cofactors. Synthesis of lin-benzoadenosine and enzymatic evaluation of derivatives of the benzopurines. *J. Am. Chem. Soc.* 98, 3987–3994.
- Leonard, N.J., Scopes, D.I., VanDerLijn, P., Barrio, J.R., 1978. Dimensional probes of the enzyme binding sites of adenine nucleotides. Biological effects of widening the adenine ring by 2.4 Å. *Biochemistry* 17, 3677–3685.
- Levene, P.A., Jacobs, W.A., 1909. Über Inosinsäure. *Berichte der deutschen chemischen Gesellschaft.* 42, 1198–1203.
- Levy, M., Miller, S.L., 1998. The stability of the RNA bases: implications for the origin of life. *Proc. Natl. Acad. Sci. U. S. A.* 95, 7933–7938.
- Lindahl, T., Karlström, O., 1973. Heat-induced depyrimidination of deoxyribonucleic acid in neutral solution. *Biochemistry* 12, 5151–5154.
- Liu, P., Sharon, A., Chu, C.K., 2008. Fluorinated nucleosides: synthesis and biological implication. *J. Fluorine Chem.* 129, 743–766.
- Livingston, R.B., Carter, S.K., 1968. Cytosine arabinoside (NSC-63878)—clinical brochure. *Cancer Chemother. Rep.* 3 (1), 179–205.
- Lo, M.K., Shi, P.Y., Chen, Y.L., Flint, M., Spiropoulou, C.F., 2016. In vitro antiviral activity of adenosine analog NITD008 against tick-borne flaviviruses. *Antivir. Res.* 130, 46–49.
- Lo, M.K., Jordan, R., Arvey, A., Sudhamsu, J., Shrivastava-Ranjan, P., Hotard, A.L., Flint, M., McMullan, L.K., Siegel, D., Clarke, M.O., Mackman, R.L., Hui, H.C., Perron, M., Ray, A.S., Cihlar, T., Nichol, S.T., Spiropoulou, C.F., 2017. GS-5734 and its parent nucleoside analog inhibit fluo-, pneumo-, and paramyxoviruses. *Sci. Rep.* 7, 43395.
- Low, J.G., Ooi, E.E., Vasudevan, S.G., 2017. Current status of dengue therapeutics research and development. *J. Infect. Dis.* 215, S96–S102.
- Luo, S., Rush, R., Standing, D., 2016. Single- and repeat-dose toxicity of IDX14184, a nucleotide prodrug with antiviral activity for hepatitis C viral infection, in mice, rats, and monkeys. *Hum. Exp. Toxicol.* 35, 472–490.
- Ma, H., Jiang, W.R., Robledo, N., Leveque, V., Ali, S., Lara-Jaime, T., Masjedizadeh, M., Smith, D.B., Gammack, N., Klump, K., Symons, J., 2007. Characterization of the metabolic activation of hepatitis C virus nucleoside inhibitor beta-D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) and identification of a novel active 5'-triphosphate species. *J. Biol. Chem.* 282, 29812–29820.
- Maag, H., Ryzdzewski, R.M., McRoberts, M.J., Crawford-Ruth, D., Verheyden, J.P., Prisbe, E.J., 1992. Synthesis and anti-HIV activity of 4'-azido- and 4'-methoxynucleosides. *J. Med. Chem.* 35, 1440–1451.
- Marquez, V.E., Ezzitouni, A., Russ, P., Siddiqui, M.A., Ford Jr., H., Feldman, R.J., Mitsuya, H., George, C., Barchi, J., J. J., 1998. Lessons from the pseudorotational cycle: conformationally rigid AZT carbocyclic nucleosides and their interaction with reverse transcriptase. *Nucleosides Nucleotides* 17, 1881–1884.
- Matsuda, A., Azuma, A., 1995. 2'-C-Cyano-2'-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC): a mechanism-based DNA-strand-breaking antitumor nucleoside. *Nucleosides Nucleotides* 14, 461–471.
- Matsuda, A., Nakajima, Y., Azuma, A., Tanaka, M., Sasaki, T., 1991a. Nucleosides and nucleotides. 100. 2'-C-cyano-2'-deoxy-1-beta-D-arabinofuranosyl-cytosine (CNDAC): design of a potential mechanism-based DNA-strand-breaking antineoplastic nucleoside. *J. Med. Chem.* 34, 2917–2919.
- Matsuda, A., Takenuki, K., Tanaka, M., Sasaki, T., Ueda, T., 1991b. Nucleosides and nucleotides. 97. Synthesis of new broad spectrum antineoplastic nucleosides, 2'-deoxy-2'-methylidencytidine (DMDC) and its derivatives. *J. Med. Chem.* 34,

- 812–819.
- McCarthy, R.M., Bandarian, V., 2008. Deciphering deazapurine biosynthesis: pathway for pyrrolopyrimidine nucleosides toyocamycin and sangivamycin. *Chem. Biol.* 15, 790–798.
- McGuigan, C., Cahard, D., Sheeka, H.M., De Clercq, E., Balzarini, J., 1996. Aryl phosphoramidate derivatives of d4T have improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. *J. Med. Chem.* 39, 1748–1753.
- McGuigan, C., Yarnold, C.J., Jones, G., Velázquez, S., Barucki, H., Brancale, A., Andrei, G., Snoeck, R., De Clercq, E., Balzarini, J., 1999. Potent and selective inhibition of varicella-zoster virus (VZV) by nucleoside analogues with an unusual bicyclic base. *J. Med. Chem.* 42, 4479–4484.
- McGuigan, C., Hassan-Abdallah, A., Srinivasan, S., Wang, Y., Siddiqui, A., Daluge, S.M., Gudmundsson, K.S., Zhou, H., McLean, E.W., Peckham, J.P., 2006. Application of phosphoramidate ProTide technology significantly improves antiviral potency of carbocyclic adenosine derivatives. *J. Med. Chem.* 49, 7215–7226.
- McGuigan, C., Perrone, P., Madela, K., Neyts, J., 2009. The phosphoramidate ProTide approach greatly enhances the activity of beta-2'-C-methylguanosine against hepatitis C virus. *Bioorg. Med. Chem. Lett.* 19, 4316–4320.
- McGuigan, C., Gilles, A., Madela, K., Aljarah, M., Holl, S., Jones, S., Vernachio, J., Hutchins, J., Ames, B., Bryant, K.D., Gorovits, E., Ganguly, B., Hunley, D., Hall, A., Kolykhalov, A., Liu, Y., Muhammad, J., Raja, N., Walters, R., Wang, J., Chamberlain, S., Henson, G., 2010a. Phosphoramidate ProTides of 2'-C-methylguanosine as highly potent inhibitors of hepatitis C virus. Study of their in vitro and in vivo properties. *J. Med. Chem.* 53, 4949–4957.
- McGuigan, C., Madela, K., Aljarah, M., Gilles, A., Brancale, A., Zonta, N., Chamberlain, S., Vernachio, J., Hutchins, J., Hall, A., Ames, B., Gorovits, E., Ganguly, B., Kolykhalov, A., Wang, J., Muhammad, J., Patti, J.M., Henson, G., 2010b. Design, synthesis and evaluation of a novel double pro-drug: INX-08189. A new clinical candidate for hepatitis C virus. *Bioorg. Med. Chem. Lett.* 20, 4850–4854.
- Mehellou, Y., 2016. The ProTides boom. *ChemMedChem* 11, 1114–1116.
- Mehellou, Y., Balzarini, J., McGuigan, C., 2009. Aryloxy phosphoramidate triesters: a technology for delivering monophosphorylated nucleosides and sugars into cells. *ChemMedChem* 4, 1779–1791.
- Mehellou, Y., Rattan, H.S., Balzarini, J., 2018. The ProTide prodrug technology: from the concept to the clinic. *J. Med. Chem.* 61, 2211–2226.
- Metobo, S., Xu, J., Saunders, O.L., Butler, T., Aktoudianakis, E., Cho, A., Kim, C.U., 2012. Practical synthesis of 1'-substituted Tubercidin C-nucleoside analogs. 53, 484–486.
- Michailidis, E., Marchand, B., Kodama, E.N., Singh, K., Matsuoka, M., Kirby, K.A., Ryan, E.M., Sawani, A.M., Nagy, E., Ashida, N., Mitsuya, H., Parniak, M.A., Sarafianos, S.G., 2009. Mechanism of inhibition of HIV-1 reverse transcriptase by 4'-Ethinyl-2-fluoro-2'-deoxyadenosine triphosphate, a translocation-defective reverse transcriptase inhibitor. *J. Biol. Chem.* 284, 35681–35691.
- Migliaccio, G., Tomassini, J.E., Carroll, S.S., Tomei, L., Altamura, S., Bhat, B., Bartholomew, L., Bosserman, M.R., Ceccacci, A., Colwell, L.F., Cortese, R., De Francesco, R., Eldrup, A.B., Getty, K.L., Hou, X.S., LaFemina, R.L., Ludmerer, S.W., MacCoss, M., McMasters, D.R., Stahlhut, M.W., Olsen, D.B., Hazuda, D.J., Flores, O.A., 2003. Characterization of resistance to non-obligate chain-terminating ribonucleoside analogs that inhibit hepatitis C virus replication in vitro. *J. Biol. Chem.* 278, 49164–49170.
- Mikhailov, S.N., Beigelman, L.N., Gurskaya, G.V., Padyukova, N.S., Yakovlev, G.I., Karpeisky, M.Y., 1983. Synthesis and properties of 3'-C-methylnucleosides and their phosphoric esters. *Carbohydr. Res.* 124, 75–96.
- Mitsuya, H., Broder, S., 1986. Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides. *Proc. Natl. Acad. Sci. U. S. A.* 83, 1911–1915.
- Morton, G.O., Lancaster, J.E., Van Lear, G.E., Fulmor, W., Meyer, W.E., 1969. The structure of nucleocidin. 3. (A new structure). *J. Am. Chem. Soc.* 91, 1535–1537.
- Muftuoglu, Y., Sohl, C.D., Mislak, A.C., Mitsuya, H., Sarafianos, S.G., Anderson, K.S., 2014. Probing the molecular mechanism of action of the HIV-1 reverse transcriptase inhibitor 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) using pre-steady-state kinetics. *Antivir. Res.* 106, 1–4.
- Murakami, E., Niu, C., Bao, H., Micolochick Steuer, H.M., Whitaker, T., Nachman, T., Sofia, M.A., Wang, P., Otto, M.J., Furman, P.A., 2008. The mechanism of action of beta-D-2'-deoxy-2'-fluoro-2'-C-methylcytidine involves a second metabolic pathway leading to beta-D-2'-deoxy-2'-fluoro-2'-C-methyluridine 5'-triphosphate, a potent inhibitor of the hepatitis C virus RNA-dependent RNA polymerase. *Antimicrob. Agents Chemother.* 52, 458–464.
- Murakami, E., Tolstykh, T., Bao, H., Niu, C., Steuer, H.M., Bao, D., Chang, W., Espiritu, C., Bansal, S., Lam, A.M., Otto, M.J., Sofia, M.J., Furman, P.A., 2010. Mechanism of activation of PSI-7851 and its diastereoisomer PSI-7977. *J. Biol. Chem.* 285, 34337–34347.
- Murakami, E., Wang, T., Babusis, D., Lepist, E.I., Sauer, D., Park, Y., Vela, J.E., Shih, R., Birkus, G., Stefanidis, D., Kim, C.U., Cho, A., Ray, A.S., 2014. Metabolism and pharmacokinetics of the anti-hepatitis C virus nucleotide prodrug GS-6620. *Antimicrob. Agents Chemother.* 58, 1943–1951.
- Nakata, H., Amano, M., Koh, Y., Kodama, E., Yang, G., Bailey, C.M., Kohgo, S., Hayakawa, H., Matsuoka, M., Anderson, K.S., Cheng, Y.C., Mitsuya, H., 2007. Activity against human immunodeficiency virus type 1, intracellular metabolism, and effects on human DNA polymerases of 4'-ethynyl-2-fluoro-2'-deoxyadenosine. *Antimicrob. Agents Chemother.* 51, 2701–2708.
- Nguyen, N.M., Tran, C.N., Phung, L.K., Duong, K.T., Huynh, H. e. A., Farrar, J., Nguyen, Q.T., Tran, H.T., Nguyen, C.V., Merson, L., Hoang, L.T., Hibberd, M.L., Aw, P.P., Wilm, A., Nagarajan, N., Nguyen, D.T., Pham, M.P., Nguyen, T.T., Javanbakht, H., Klumpp, K., Hammond, J., Petric, R., Wolbers, M., Nguyen, C.T., Simmons, C.P., 2013. A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. *J. Infect. Dis.* 207, 1442–1450.
- Nilsson, M., Kalaynov, G., Winqvist, A., Pinho, P., Sund, C., Zhou, X.X., Wähling, H., Belfrage, A.K., Pelcman, M., Agback, T., Benckestock, K., Wikström, K., Boothee, M., Lindqvist, A., Rydegård, C., Jonckers, T.H., Vandyck, K., Raboison, P., Lin, T.I., Lachau-Durand, S., de Kock, H., Smith, D.B., Martin, J.A., Klumpp, K., Simmen, K., Vrang, L., Terelius, Y., Samuelsson, B., Rosenquist, S., Johansson, N.G., 2012. Discovery of 4'-azido-2'-deoxy-2'-C-methyl cytidine and prodrugs thereof: a potent inhibitor of Hepatitis C virus replication. *Bioorg. Med. Chem. Lett.* 22, 3265–3268.
- Nomura, M., Shuto, S., Tanaka, M., Sasaki, T., Mori, S., Shigeta, S., Matsuda, A., 1999. Nucleosides and nucleotides. 185. Synthesis and biological activities of 4'-alpha-C-Branches-Chain sugar pyrimidine nucleosides. *J. Med. Chem.* 42, 2901–2908.
- O-Yang, C., Wu, H.Y., Fraser-Smith, W.B., Walker, K.A.M., 1992. Synthesis of 4'-cyanothymidine and analogs as potent inhibitors of HIV. *Tetrahedron Lett.* 33, 37–40.
- Ohrui, H., 2006. 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity. *Chem. Rec.* 6, 133–143.
- Ohrui, H., Kohgo, S., Hayakawa, H., Kodama, E., Matsuoka, M., Nakata, T., Mitsuya, H., 2007. 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine: a nucleoside reverse transcriptase inhibitor with highly potent activity against wide spectrum of HIV-1 strains, favorable toxic profiles, and stability in plasma. *Nucleos Nucleot. Nucleic Acids* 26, 1543–1546.
- Ohta, Y., Sueki, K., Kitta, K., Takemoto, K., Ishitsuka, H., Yagi, Y., 1980. Comparative studies on the immunosuppressive effect among 5'-deoxy-5-fluorouridine, ftorafur, and 5-fluorouracil. *Gann* 71.
- Olsen, D.B., Eldrup, A.B., Bartholomew, L., Bhat, B., Bosserman, M.R., Ceccacci, A., Colwell, L.F., Fay, J.F., Flores, O.A., Getty, K.L., Grobler, J.A., LaFemina, R.L., Markel, E.J., Migliaccio, G., Prhac, M., Stahlhut, M.W., Tomassini, J.E., MacCoss, M., Hazuda, D.J., Carroll, S.S., 2004. A 7-deaza-adenosine analog is a potent and selective inhibitor of hepatitis C virus replication with excellent pharmacokinetic properties. *Antimicrob. Agents Chemother.* 48, 3944–3953.
- van Oosterom, A.T., ten Bokkel Huinink, W.W., van der Burg, M.E., Vermorken, J.B., Willemse, P.H., Neijt, J.P., 1991. Phase II clinical trial of doxifluridine in patients with advanced ovarian cancer. *Eur. J. Cancer* 27, 747–749.
- Orlov, A.A., Drenichev, M.S., Oslovsky, V.E., Kurochkin, N.N., Solyev, P.N., Kozlovskaya, L.I., Palyulin, V.A., Karganova, G.G., Mikhailov, S.N., Osolodkin, D.I., 2017. New tools in nucleoside toolbox of tick-borne encephalitis virus reproduction inhibitors. *Bioorg. Med. Chem. Lett.* 27, 1267–1273.
- Owen, G.R., Verheyden, J.P.H., Moffatt, J.G., 1976. 4'-Substituted nucleosides. 3. Synthesis of some 4'-fluorouridine derivatives. *J. Org. Chem.* 41, 3010–3017.
- Pankiewicz, K.W., 2000. Fluorinated nucleosides. *Carbohydr. Res.* 327, 87–105.
- Pankiewicz, K.W., Watanabe, K.A., 1993. Synthesis of 2'-beta-fluoro-substituted Nucleosides by a Direct Approach, vol. 64 Elsevier, Amsterdam, PAYS-BAS.
- Park, B., Kitteringham, N., O'Neill, P., 2001. Metabolism of fluorine-containing drugs. *Annu. Rev. Pharmacol. Toxicol.* 41, 443–470.
- Perigaud, C., Gosselin, G., Imbach, J.L., 1992. Nucleoside analogues as chemotherapeutic agents: a review. *Nucleoside Nucleotides* 11, 903–945.
- Pertusati, F., Serpi, M., McGuigan, C., 2012. Medicinal chemistry of nucleoside phosphonate prodrugs for antiviral therapy. *Antivir. Chem. Chemother.* 22, 181–203.
- Peters, H.L., Jochmans, D., de Wilde, A.H., Posthuma, C.C., Snijder, E.J., Seley-Radtke, K.L., 2015. Design, synthesis and evaluation of a series of acyclic fleximer nucleoside analogues with anti-coronavirus activity. *Bioorg. Med. Chem. Lett.* 25, 2923–2926.
- Pierra, C., Amador, A., Benzaria, S., Cretton-Scott, E., D'Amours, M., Mao, J., Mathieu, S., Moussa, A., Bridges, E.G., Standing, D.N., Sommadossi, J.P., Storer, R., Gosselin, G., 2006. Synthesis and pharmacokinetics of valopicitabine (NM283), an efficient pro-drug of the potent anti-HCV agent 2'-C-methylcytidine. *J. Med. Chem.* 49, 6614–6620.
- Plunkett, W., Gandhi, V., 1993. Cellular pharmacodynamics of anticancer drugs. *Semin. Oncol.* 20, 50–63.
- Pockros, P.J., Nelson, D., Godofsky, E., Rodriguez-Torres, M., Everson, G.T., Fried, M.W., Ghalib, R., Harrison, S., Nyberg, L., Shiffman, M.L., Najera, I., Chan, A., Hill, G., 2008a. R1626 plus peginterferon Alfa-2a provides potent suppression of hepatitis C virus RNA and significant antiviral synergy in combination with ribavirin. *Hepatology* 48, 385–397.
- Pockros, P., Nelson, D., Godofsky, E., Rodriguez-Torres, M., Everson, G.T., Fried, M.W., Ghalib, R., Harrison, S., Nyberg, L., Shiffman, M.L., Chan, A., Hill, G., 2008b. High relapse rate seen at week 72 for patients treated with R1626 combination therapy. *Hepatology* 48, 1349–1350.
- Polak, M., Seley, K.L., Plavec, J., 2004. Conformational properties of shape modified nucleosides-fleximers. *J. Am. Chem. Soc.* 126, 8159–8166.
- Prince, H.N., Grunberg, E., Buck, M., Cleeland, R., 1969. A comparative study of the antitumor and antiviral activity of 1-beta-D-Arabinofuranosyl-5-fluorocytosine (FCA) and 1-beta-D-Arabinofuranosylcytosine (CA). *Proc. Soc. Exp. Biol. Med.* 130, 1080–1086.
- Qing, J., Luo, R., Wang, Y., Nong, J., Wu, M., Shao, Y., Tang, R., Yu, X., Yin, Z., Sun, Y., 2016. Resistance analysis and characterization of NITD008 as an adenosine analog inhibitor against hepatitis C virus. *Antivir. Res.* 126, 43–54.
- Quirk, S., Seley, K.L., 2005a. Substrate discrimination by the human GTP fucose pyrophosphorylase. *Biochemistry* 44, 10854–10863.
- Quirk, S., Seley, K.L., 2005b. Identification of catalytic amino acids in the human GTP fucose pyrophosphorylase active site. *Biochemistry* 44, 13172–13178.
- Reese, N.D., Schiller, G.J., 2013. High-dose cytarabine (HD araC) in the treatment of leukemias: a review. *Curr. Hematol. Malig. Rep.* 8, 141–148.
- Richardson, F.C., Kuchta, R.D., Mazurkiewicz, A., Richardson, K.A., 2000. Polymerization of 2'-fluoro- and 2'-O-methyl-dNTPs by human DNA polymerase alpha, polymerase gamma, and primase. *Biochem. Pharmacol.* 59, 1045–1052.

- Rios, A.C., Yu, H.T., Tor, Y., 2015. Hydrolytic fitness of N-glycosyl bonds: comparing the deglycosylation kinetics of modified, alternative and native nucleosides. *J. Phys. Org. Chem.* 28, 173–180.
- Roberts, R., Cooksley, W., Shaw, D., Berns, K.H., Brandl, T.M., Fettner, H.S., Hill, G., Ipe, D., Klumpp, K., Mannino, M., O'mara, E., Tuo, Y., Washington, B.C., 2006. Interim Results of a Multiple Ascending Dose Study of R1626, a Novel Nucleoside Analog Targeting HCV Polymerase in Chronic Hcv Patients, vol. 44.
- Roberts, S.K., Cooksley, G., Dore, G.J., Robson, R., Shaw, D., Berns, H., Hill, G., Klumpp, K., Najera, I., Washington, C., 2008. Robust antiviral activity of R1626, a novel nucleoside analog: a randomized, placebo-controlled study in patients with chronic hepatitis C. *Hepatology* 48, 398–406.
- Robson, R., Berns, H., Brandl, M., Fettner, S., Hill, G., Ipe, D., Love, J., Mannino, M., O'mara, E., Tu, Y., 2007. Safety, tolerability and pharmacokinetics of R1626, a novel nucleoside analog targeting hcv polymerase: results from a phase 1 single dose escalation trial in healthy subjects.: pii:41. *Clin. Pharmacol. Therapeut.* 81, S98.
- Rondia, R., Coats, S.J., McBrayer, T.R., Grier, J., Johns, M., Tharnish, P.M., Whitaker, T., Zhou, R.L., Schinazi, R.F., 2009. Anti-hepatitis C virus activity of novel beta-d-2'-C-methyl-4'-azido pyrimidine nucleoside phosphoramidate prodrugs. *Antivir. Chem. Chemother.* 20, 99–106.
- Saenger, W., 1984. Defining terms for the nucleic acids. In: Cantor, C.R. (Ed.), *Principles of Nucleic Acid Structure*. Springer-Verlag New York Inc., pp. 9–28.
- Salie, Z.L., Kirby, K.A., Michailidis, E., Marchand, B., Singh, K., Rohan, L.C., Kodama, E.N., Mitsuya, H., Parniak, M.A., Sarafianos, S.G., 2016. Structural basis of HIV inhibition by translocation-defective RT inhibitor 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EPfA). *Proc. Natl. Acad. Sci. U. S. A.* 113, 9274–9279.
- Schilsky, R.L., Williams, S.F., Ullmann, J.E., Watson, S., 1987. Sequential hydroxyurea-cytarabine chemotherapy for refractory non-Hodgkin's lymphoma. *J. Clin. Oncol.* 5, 419–425.
- Schul, W., Liu, W., Xu, H.Y., Flamand, M., Vasudevan, S.G., 2007. A dengue fever viremia model in mice shows reduction in viral replication and suppression of the inflammatory response after treatment with antiviral drugs. *J. Infect. Dis.* 195, 665–674.
- Seley, K.L., Schneller, S.W., Rattendi, D., Lane, S., Bacchi, C.J., 1997a. Synthesis and antitrypanosomal activities of a series of 7-deaza-5'-noraristeromycin derivatives with variations in the cyclopentyl ring substituents. *Antimicrob. Agents Chemother.* 41, 1658–1661.
- Seley, K.L., Schneller, S.W., Rattendi, D., Bacchi, C.J., 1997b. (+)-7-Deaza-5'-noraristeromycin as an anti-trypanosomal agent. *J. Med. Chem.* 40, 622–624.
- Seley, K.L., Schneller, S.W., Rattendi, D., Lane, S., Bacchi, C.J., 1997c. Synthesis and antitrypanosomal activity of various 8-aza-7-deaza-5'-noraristeromycin derivatives. *J. Med. Chem.* 40, 625–629.
- Seley, K.L., Schneller, S.W., Rattendi, D., Lane, S., Bacchi, C.J., 1997d. Synthesis and antitrypanosomal activity of various 8-Aza-7-deaza-5'-noraristeromycin derivatives. *J. Med. Chem.* 40, 625–629.
- Seley, K.L., Schneller, S.W., De Clercq, E., 1997e. A methylated derivative of 5'-noraristeromycin. *J. Org. Chem.* 62, 5645–5646.
- Seley, K.L., Schneller, S.W., Korba, B., 1997f. A 5'-noraristeromycin enantiomer with activity towards hepatitis B virus. *Nucleosides Nucleotides* 16, 2095–2099.
- Seley, K.L., Zhang, L., Hagos, A., 2001. "Fleximers". Design and synthesis of two novel split nucleosides. *Org. Lett.* 3, 3209–3210.
- Seley, K.L., Zhang, L., Hagos, A., Quirk, S., 2002. "Fleximers". design and synthesis of a new class of novel shape-modified nucleosides(1). *J. Org. Chem.* 67, 3365–3373.
- Seley, K.L., Quirk, S., Salim, S., Zhang, L., Hagos, A., 2003. Unexpected inhibition of S-adenosyl-L-homocysteine hydrolase by a guanosine nucleoside. *Bioorg. Med. Chem. Lett.* 13, 1985–1988.
- Seley, K.L., Salim, S., Zhang, L., 2005a. "Molecular chameleons". design and synthesis of C-4-substituted imidazole fleximers. *Org. Lett.* 7, 63–66.
- Seley, K.L., Salim, S., Zhang, L., O'Daniel, P.I., 2005b. "Molecular chameleons". design and synthesis of a second series of flexible nucleosides. *J. Org. Chem.* 70, 1612–1619.
- Seley-Radtke, K.L., 2018a. Flex-nucleoside Analogues. *Novel Therapeutics Against Filoviruses and Flaviviruses*.
- Seley-Radtke, K.L., 2018b. Design, Synthesis and Methods of Use of Acyclic Nucleoside Analogues Having Anti-coronavirus Activity.
- Seley-Radtke, K.L., Yates, M.K., 2018. The evolution of nucleoside analogue antivirals: a review for chemists and non-chemists. Part 1: early structural modifications to the nucleoside scaffold. *Antivir. Res.* 154, 66–86.
- Seley-Radtke, K.L., Zhang, Z., Wauchope, O.R., Zimmermann, S.C., Ivanov, A., Korba, B., 2008. Hetero-expanded purine nucleosides. Design, synthesis and preliminary biological activity. *Nucleic Acids Symp. Ser. (Oxf.)* 635–636.
- Shang, L., Wang, Y., Qing, J., Shu, B., Cao, L., Lou, Z., Gong, P., Sun, Y., Yin, Z., 2014. An adenosine nucleoside analogue NITD008 inhibits EV71 proliferation. *Antivir. Res.* 112, 47–58.
- Sheahan, T.P., Sims, A.C., Graham, R.L., Menachery, V.D., Gralinski, L.E., Case, J.B., Leist, S.R., Pyrc, K., Feng, J.Y., Trantcheva, I., Bannister, R., Park, Y., Babusis, D., Clarke, M.O., Mackman, R.L., Spahn, J.E., Palmiotti, C.A., Siegel, D., Ray, A.S., Cihlar, T., Jordan, R., Denison, M.R., Baric, R.S., 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci. Transl. Med.* 9.
- Shirasaka, T., Chokekijchai, S., Yamada, A., Gosselin, G., Imbach, J.L., Mitsuya, H., 1995. Comparative analysis of anti-human immunodeficiency virus type 1 activities of di-deoxynucleoside analogs in resting and activated peripheral blood mononuclear cells. *Antimicrob. Agents Chemother.* 39, 2555–2559.
- Siddiqi, S.M., Jacobson, K.A., Esker, J.L., Olah, M.E., Ji, X.D., Melman, N., Tiwari, K.N., Secrist, J.A., Schneller, S.W., Cristalli, G., 1995. Search for new purine- and ribose-modified adenosine analogues as selective agonists and antagonists at adenosine receptors. *J. Med. Chem.* 38, 1174–1188.
- Siegel, D., Hui, H.C., Doerfler, E., Clarke, M.O., Chun, K., Zhang, L., Neville, S., Carra, E., Lew, W., Ross, B., Wang, Q., Wolfe, L., Jordan, R., Soloveva, V., Knox, J., Perry, J., Perron, M., Stray, K.M., Barauskas, O., Feng, J.Y., Xu, Y., Lee, G., Rheingold, A.L., Ray, A.S., Bannister, R., Strickley, R., Swaminathan, S., Lee, W.A., Bavari, S., Cihlar, T., Lo, M.K., Warren, T.K., Mackman, R.L., 2017. Discovery and synthesis of a phosphoramidate prodrug of a pyrrolo[2,1-f][triazin-4-amino] adenine C-nucleoside (GS-5734) for the treatment of Ebola and emerging viruses. *J. Med. Chem.* 60, 1648–1661.
- Sizun, G., Pierra, C., Peyronnet, J., Badaroux, E., Rabeson, C., Benzaria-Prad, S., Surleraux, D., Loi, A.G., Musiu, C., Liuzzi, M., Seifer, M., Standring, D., Sommadossi, J.P., Gosselin, G., 2015. Design, synthesis and antiviral evaluation of 2'-C-methyl branched guanosine pronucleotides: the discovery of IDX184, a potent liver-targeted HCV polymerase inhibitor. *Future Med. Chem.* 7, 1675–1700.
- Smith, D.B., Martin, J.A., Klumpp, K., Baker, S.J., Blomgren, P.A., Devos, R., Grancyome, C., Hang, J., Hobbs, C.J., Jiang, W.R., Laxton, C., Le Pogam, S., Leveque, V., Ma, H., Maile, G., Merrett, J.H., Pichota, A., Sarma, K., Smith, M., Swallow, S., Symons, J., Vesey, D., Najera, I., Cammack, N., 2007. Design, synthesis, and antiviral properties of 4'-substituted ribonucleosides as inhibitors of hepatitis C virus replication: the discovery of R1479. *Bioorg. Med. Chem. Lett.* 17, 2570–2576.
- Smith, R.A., Gottlieb, G.S., Anderson, D.J., Pyrak, C.L., Preston, B.D., 2008. Human immunodeficiency virus types 1 and 2 exhibit comparable sensitivities to Zidovudine and other nucleoside analog inhibitors in vitro. *Antimicrob. Agents Chemother.* 52, 329–332.
- Smith, D.B., Kalaynov, G., Sund, C., Winqvist, A., Pinho, P., Maltseva, T., Morisson, V., Leveque, V., Rajyaguru, S., Le Pogam, S., Najera, I., Benkestock, K., Zhou, X.X., Maag, H., Cammack, N., Martin, J.A., Swallow, S., Johansson, N.G., Klumpp, K., Smith, M., 2009. The design, synthesis, and antiviral activity of 4'-azidothymidine analogues against hepatitis C virus replication: the discovery of 4'-azidoarabincytidine. *J. Med. Chem.* 52, 219–223.
- Sofia, M.J., Bao, D., Chang, W., Du, J., Nagarathnam, D., Rachakonda, S., Reddy, P.G., Ross, B.S., Wang, P., Zhang, H.R., Bansal, S., Espiritu, C., Keilman, M., Lam, A.M., Steuer, H.M., Niu, C., Otto, M.J., Furman, P.A., 2010. Discovery of a beta-d-2'-deoxy-2'-alpha-fluoro-2'-beta-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J. Med. Chem.* 53, 7202–7218.
- Sohl, C.D., Singh, K., Kasiviswanathan, R., Copeland, W.C., Mitsuya, H., Sarafianos, S.G., Anderson, K.S., 2012. Mechanism of interaction of human mitochondrial DNA polymerase γ with the novel nucleoside reverse transcriptase inhibitor 4'-ethynyl-2-fluoro-2'-deoxyadenosine indicates a low potential for host toxicity. *Antimicrob. Agents Chemother.* 56, 1630–1634.
- Sommadossi, J.P., Aubert, C., Cano, J.P., Gouveia, J., Ribaud, P., Mathe, G., 1983. Kinetics and metabolism of a new fluoropyrimidine, 5'-Deoxy-5-fluorouridine, in humans. *Cancer Res.* 43, 930–933.
- Stambaský, J., Hocek, M., Kocovsky, P., 2009. C-nucleosides: synthetic strategies and biological applications. *Chem. Rev.* 109, 6729–6764.
- Standring, D.N., Lanford, R., Cretton-Scott, E., Licklider, L., Larsson, M., Pierra, C., Gosselin, G., Perigaud, C., Surleraux, D., Mayes, B., Moussa, A., Selden, J., 2008. Potent antiviral activity of second generation nucleoside inhibitors, IDX102 and IDX184 in HCV-infected chimpanzees. *J. Hepatol.* 48.
- Stuyver, L.J., McBrayer, T.R., Whitaker, T., Tharnish, P.M., Ramesh, M., Lostia, S., Cartee, L., Shi, J., Hobbs, A., Schinazi, R.F., Watanabe, K.A., Otto, M.J., 2004. Inhibition of the subgenomic hepatitis C virus replicon in huh-7 cells by 2'-deoxy-2'-fluorocytidine. *Antimicrob. Agents Chemother.* 48, 651–654.
- Takamatsu, Y., Tanaka, Y., Kohgo, S., Murakami, S., Singh, K., Das, D., Venzon, D.J., Amano, M., Higashi-Kuwata, N., Aoki, M., Delino, N.S., Hayashi, S., Takahashi, S., Sukenaga, Y., Haraguchi, K., Sarafianos, S.G., Maeda, K., Mitsuya, H., 2015. 4'-modified nucleoside analogs: potent inhibitors active against entecavir-resistant hepatitis B virus. *Hepatology* 62, 1024–1036.
- Tanaka, M., Matsuda, A., Terao, T., Sasaki, T., 1992. Antitumor activity of a novel nucleoside, 2'-C-cyano-2'-deoxy-1-beta-D-arabinofuranosylcytosine (CNDAC) against murine and human tumors. *Cancer Lett.* 64, 67–74.
- Temburnikar, K., Seley-Radtke, K.L., 2018. Recent advances in synthetic approaches to C-nucleosides. *Beilstein J. Org. Chem.* 14, 772–785.
- Tevaearai, H.T., Givel, J.C., Laurent, P.L., Suardet, L., Eliason, J.F., Odartchenko, N., 1992. Interactions of interferon- α 2a with 5'-deoxy-5-fluorouridine in colorectal cancer cells in vitro. *Eur. J. Cancer* 28, 368–372.
- Thomas, S.O., Singleton, V.L., Lowery, J.A., Sharpe, R.W., Pruess, L.M., Porter, J.N., Mowat, J.H., Bohonos, N., 1956. Nucleocidin, a new antibiotic with activity against trypanosomes. *Antibiot. Annu.* 716–721.
- Toniutto, P., Fabris, C., Bitetto, D., Fumolo, E., Fornasiero, E., Pirisi, M.R., 2008. 1626, a specific oral NS5B polymerase inhibitor of hepatitis C virus. *IDrugs* 11, 738–749.
- Veal, G.J., Barry, M.G., Back, D.J., 1995. Zalcitabine (DDC) phosphorylation and drug-interactions. *Antiviral Chem. Chemother.* 6, 379–384.
- Vernachio, J.H., Bleiman, B., Bryant, K.D., Chamberlain, S., Hunley, D., Hutchins, J., Ames, B., Gorovits, E., Ganguly, B., Hall, A., Kolykhalov, A., Liu, Y., Muhammad, J., Raja, N., Walters, C.R., Wang, J., Williams, K., Patti, J.M., Henson, G., Madala, K., Aljarah, M., Gilles, A., McGuigan, C., 2011. INX-08189, a phosphoramidate prodrug of 6-O-methyl-2'-C-methyl guanosine, is a potent inhibitor of hepatitis C virus replication with excellent pharmacokinetic and pharmacodynamic properties. *Antimicrob. Agents Chemother.* 55, 1843–1851.
- Waga, T., Nishizaki, T., Miyakawa, I., Ohru, H., Meguro, H., 1993. Synthesis of 4'-C-methyl nucleosides. *Biosci. Biotechnol. Biochem.* 57, 1433–1438.
- Waga, T., Ohru, H., Meguro, H., 1996. Synthesis and biological evaluation of 4'-C-methyl nucleosides. *Nucleosides Nucleotides* 15, 287–304.
- Wang, G., Deval, J., Hong, J., Dyatkina, N., Prhavc, M., Taylor, J., Fung, A., Jin, Z., Stevens, S.K., Sebreyany, V., Liu, J., Zhang, Q., Tam, Y., Chanda, S.M., Smith, D.B., Symons, J.A., Blatt, L.M., Beigelman, L., 2015. Discovery of 4'-chloromethyl-2'-deoxy-3',5'-di-O-isobutyryl-2'-fluorocytidine (ALS-8176), a first-in-class RSV polymerase

- inhibitor for treatment of human respiratory syncytial virus infection. *J. Med. Chem.* 58, 1862–1878.
- Warren, T.K., Jordan, R., Lo, M.K., Ray, A.S., Mackman, R.L., Soloveva, V., Siegel, D., Perron, M., Bannister, R., Hui, H.C., Larson, N., Strickley, R., Wells, J., Stuthman, K.S., Van Tongeren, S.A., Garza, N.L., Donnelly, G., Shurtleff, A.C., Retterer, C.J., Gharaibeh, D., Zamani, R., Kenny, T., Eaton, B.P., Grimes, E., Welch, L.S., Gomba, L., Wilhelmsen, C.L., Nichols, D.K., Nuss, J.E., Nagle, E.R., Kugelman, J.R., Palacios, G., Doerfler, E., Neville, S., Carra, E., Clarke, M.O., Zhang, L., Lew, W., Ross, B., Wang, Q., Chun, K., Wolfe, L., Babusis, D., Park, Y., Stray, K.M., Trancheva, I., Feng, J.Y., Barauskas, O., Xu, Y., Wong, P., Braun, M.R., Flint, M., McMullan, L.K., Chen, S.S., Fearn, R., Swaminathan, S., Mayers, D.L., Spiropoulou, C.F., Lee, W.A., Nichol, S.T., Cihlar, T., Bavari, S., 2016. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature* 531, 381–385.
- Wauchope, O.R., Johnson, C., Krishnamoorthy, P., Andrei, G., Snoeck, R., Balzarini, J., Seley-Radtke, K.L., 2012a. Synthesis and biological evaluation of a series of thieno-expanded tricyclic purine 2'-deoxy nucleoside analogues. *Bioorg. Med. Chem.* 20, 3009–3015.
- Wauchope, O.R., Velasquez, M., Seley-Radtke, K., 2012b. Synthetic routes to a series of proximal and distal 2'-deoxy fleximers. *Synthesis (Stuttg.)* 44, 3496–3504.
- Wojtowicz-Rajchel, H., 2012. Synthesis and applications of fluorinated nucleoside analogues. *J. Fluorine Chem.* 143, 11–48.
- Yamada, K., Wahba, A.S., Bernatchez, J.A., Iliina, T., Martínez-Montero, S., Habibian, M., Deleavey, G.F., Götte, M., Parniak, M.A., Damha, M.J., 2015. Nucleotide sugar pucker preference mitigates excision by HIV-1 RT. *ACS Chem. Biol.* 10, 2024–2033.
- Yates, M.K., Raje, M.R., Chatterjee, P., Spiropoulou, C.F., Bavari, S., Flint, M., Soloveva, V., Seley-Radtke, K.L., 2017. Flex-nucleoside analogues - novel therapeutics against filoviruses. *Bioorg. Med. Chem. Lett.* 27, 2800–2802.
- Yates, M.K., Vallé, C., Martin, B., Aoudai, W., Selisko, B., Decroly, E., Canard, B., Day, C., Falat, A., Bassetto, M., Brancale, A., Seley-Radtke, K.L., 2018. Synthesis and biological evaluation of novel flexible nucleoside analogues that inhibit flavivirus replication in vitro. In preparation.
- Yeo, K.L., Chen, Y.L., Xu, H.Y., Dong, H., Wang, Q.Y., Yokokawa, F., Shi, P.Y., 2015. Synergistic suppression of dengue virus replication using a combination of nucleoside analogs and nucleoside synthesis inhibitors. *Antimicrob. Agents Chemother.* 59, 2086–2093.
- Yin, Z., Chen, Y.L., Schul, W., Wang, Q.Y., Gu, F., Duraiswamy, J., Kondreddi, R.R., Niyomrattanakit, P., Lakshminarayana, S.B., Goh, A., Xu, H.Y., Liu, W., Liu, B., Lim, J.Y., Ng, C.Y., Qing, M., Lim, C.C., Yip, A., Wang, G., Chan, W.L., Tan, H.P., Lin, K., Zhang, B., Zou, G., Bernard, K.A., Garrett, C., Beltz, K., Dong, M., Weaver, M., He, H., Pichota, A., Dartois, V., Keller, T.H., Shi, P.Y., 2009. An adenosine nucleoside inhibitor of dengue virus. *Proc. Natl. Acad. Sci. U. S. A.* 106, 20435–20439.
- Zhang, H.W., Zhou, L., Coats, S.J., McBrayer, T.R., Tharnish, P.M., Bondada, L., Detorio, M., Amichai, S.A., Johns, M.D., Whitaker, T., Schinazi, R.F., 2011. Synthesis of purine modified 2'-C-methyl nucleosides as potential anti-HCV agents. *Bioorg. Med. Chem. Lett.* 21, 6788–6792.
- Zhou, X.J., Pietropaolo, K., Chen, J., Khan, S., Sullivan-Bólyai, J., Mayers, D., 2011. Safety and pharmacokinetics of IDX184, a liver-targeted nucleotide polymerase inhibitor of hepatitis C virus, in healthy subjects. *Antimicrob. Agents Chemother.* 55, 76–81.
- Zhou, L., Amblard, F., Zhang, H., McBrayer, T.R., Detorio, M.A., Whitaker, T., Coats, S.J., Schinazi, R.F., 2013. Synthesis and evaluation of Janus type nucleosides as potential HCV NS5B polymerase inhibitors. *Bioorg. Med. Chem. Lett.* 23, 3385–3388.
- Zimmermann, S.C., Sadler, J.M., O'Daniel, P.I., Kim, N.T., Seley-Radtke, K.L., 2013. "Reverse" carbocyclic fleximers: synthesis of a new class of adenosine deaminase inhibitors. *Nucleos Nucleot. Nucleic Acids* 32, 137–154.
- Zimmermann, S.C., O'Neill, E., Ebiloma, G.U., Wallace, L.J., De Koning, H.P., Seley-Radtke, K.L., 2014. Design and synthesis of a series of truncated neplanocin fleximers. *Molecules* 19, 21200–21214.
- Zmurko, J., Marques, R.E., Schols, D., Verbeke, E., Kaptein, S.J., Neyts, J., 2016. The Viral polymerase Inhibitor 7-deaza-2'-C-Methyladenosine is a potent inhibitor of in vitro zika virus replication and delays disease progression in a robust mouse infection model. *PLoS Negl. Trop. Dis.* 10, e0004695.