HHS Public Access

Author manuscript

Chem Rev. Author manuscript; available in PMC 2020 December 04.

Published in final edited form as:

Chem Rev. 2016 December 14; 116(23): 14379–14455. doi:10.1021/acs.chemrev.6b00209.

Metabolism, Biochemical Actions, and Chemical Synthesis of Anticancer Nucleosides, Nucleotides, and Base Analogs

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Abstract

Nucleoside, nucleotide, and base analogs have been in the clinic for decades to treat both viral pathogens and neoplasms. More than 20% of patients on anticancer chemotherapy have been treated with one or more of these analogs. This review focuses on the chemical synthesis and biology of anticancer nucleoside, nucleotide, and base analogs that are FDA-approved and in clinical development since 2000. We highlight the cellular biology and clinical biology of analogs, drug resistance mechanisms, and compound specificity towards different cancer types. Furthermore, we explore analog syntheses as well as improved and scale-up syntheses. We conclude with a discussion on what might lie ahead for medicinal chemists, biologists, and physicians as they try to improve analog efficacy through prodrug strategies and drug combinations.

Graphical Abstract

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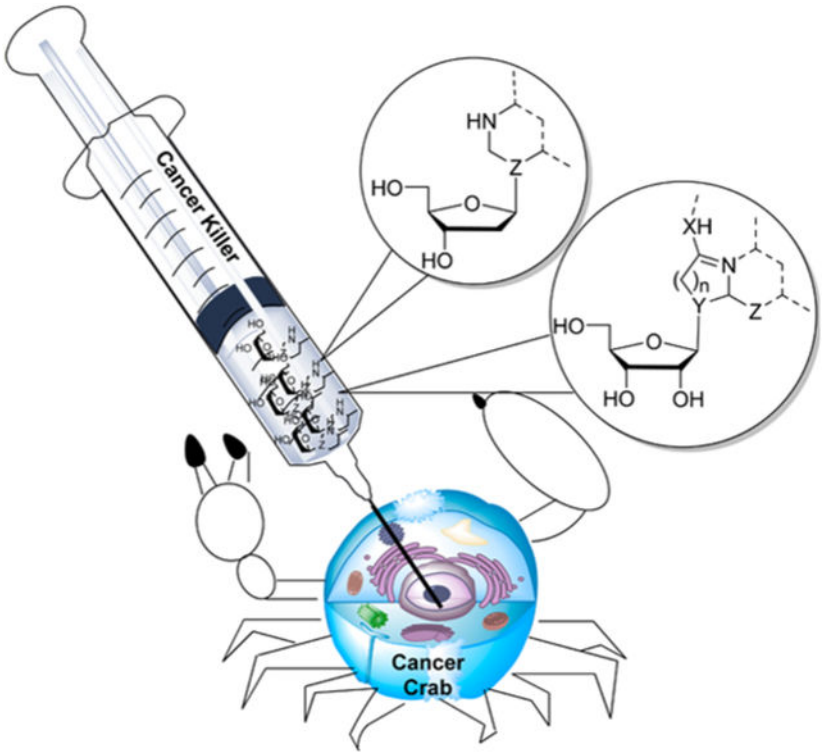
The authors declare no competing financial interest.



1. INTRODUCTION AND MOTIVATION

Cancer is the second leading cause of death in the United States, accounting for 1 in 4 deaths annually. Importantly, cancer incidence continues to increase worldwide.[1] Cancer encompasses a broad range of diseases in which host cells escape their normal cell cycle regulation. This phenomenon can be linked to several factors, including gender, ethnicity, age of onset, and lifestyle,[2] but cancer can also be caused by cellular transformation linked to viral infection, chemical exposure, or radiation exposure, or the cause can be unknown (spontaneous) in nature.[3–5] Advances in oncology treatments have increased the number of cancer survivors from 3 million in 1971 to more than 13 million in 2012.[6] This has led to a 39% improvement in 5-year survival rates for different cancers and stages of disease as indicated by the 2010 data, and rates should continue to improve with recent advances in new therapies.[1,7]

The primary standard-of-care treatment for cancer includes surgery, radiation, chemotherapy, hormone therapy, immuno-therapy, and targeted therapy.[1] Cutting-edge therapies may include one or more of these procedures listed above depending upon the type and stage of the cancer being treated. Sometimes surgery and radiation may be a second tier treatment, with chemotherapy being used first to reduce the tumor burden. Chemotherapies are divided into several main drug classes: alkylating agents,[8] antimetabolites, anti-tumor antibiotics,[9] topoisomerase inhibitors,[10] mitotic inhibitors,[11,12] and corticosteroids.[13] Recently, antibodies directed against programmed cell death 1 (PD-1) protein, PD-1 ligands 1 and 2, and cytotoxic T-lymphocyte-associated protein 4 (CLTA-4) have been found to

activate the immune system to attack tumors, and they are therefore used with varying success to treat some types of cancer.[14–16]

Chemotherapeutic nucleoside, nucleotide, and base analogs, herein referred to as "nucleoside analogs", are antimetabolites. They are chemically modified analogs of natural nucleosides, nucleotides, and bases, which are endogenous metabolites involved in many essential cellular processes, such as DNA and RNA synthesis, and purinergic signaling. Nucleoside analogs have been a cornerstone of anticancer and antiviral chemotherapy for decades. Currently, there are 15 FDA-approved nucleoside analogs used to treat various cancers, and they account for a large percentage of the current cancer chemotherapeutic arsenal.[17] Whereas chemotherapeutic nucleoside analogs alone seldom lead to a cure for cancer, they do provide a valuable treatment option for cancer patients. In addition, many other nucleoside analogs are currently being investigated in clinical trials as monotherapy or combination therapy for multiple cancers. These studies attempt to increase the potency or bioavailability of these agents while diminishing associated dose-limiting toxicity.

In this review, we focus on both the biology and chemical syntheses of the current FDA-approved anticancer nucleoside analogs (Figure 1), investigational anticancer nucleoside analogs in development since 2000 or analogs used outside of the United States (Figure 2), and nucleoside analogs in development since 2000 that have stalled in clinical trials (Figure 3). Additionally, we present an overview of the cellular metabolism, the biochemical mode of action, and the types of cancers treated with these agents, as well as focusing on current methodologies for their chemical synthesis.

2. GENERAL BIOLOGICAL ACTIVITY OF ANTICANCER NUCLEOSIDE ANALOGS

The two main routes for administering nucleoside analogs are by intravenously infusion and oral formulation. Once inside the body, the analogs can face a major bottleneck in cellular uptake. Both protein facilitated diffusion using either concentrative nucleoside transporters (CNT or SLC28; three members) and/or equilibrative nucleoside transporters (ENT or SLC29; four members) are involved in the cellular uptake for the majority of agents.[18,19] Passive diffusion is limited in regard to cellular uptake for parent nucleoside analogs, with troxacitabine **48** being the exception, requiring very high extracellular concentrations in order to occur.[20] Certain nucleoside analog prodrugs, such as NUC-1031 **32**, NUC-3373 **37**, or elacytarabine **51**, however, can enter the cell independent of transporters.

Nucleoside analogs are prodrugs and require cellular metabolism to be converted to their active metabolites. This is accomplished by the cellular salvage pathway—a group of enzymes that can phosphorylate nucleoside and nucleotide substrates. After transport across the plasma membrane, nucleoside analogs undergo an initial phosphorylation—or if a base analog, ribosylation followed by phosphorylation—to generate nucleoside-5′-monophosphate forms,[21] which is often the rate-limiting step within the cell in the process to generate active metabolites (Figure 4).[22] The cell primarily uses four kinases for nucleoside-5′-monophosphate phosphorylation: 2′-deoxycytidine kinase, thymidine kinase 1, thymidine kinase 2, and deoxyguananine kinase.[23] Each kinase has multiple substrates

and can have feedback inhibition to regulate their activities.[24,25] The initial phosphorylation is often performed by 2′-deoxycytidine kinase. Cancer cells usually express 2′-deoxycytidine kinase at a 3- to 5-fold higher protein level than most normal cells, affording some degree of selectivity.[26] Phosphoramidate prodrugs such as NUC-1031 **32** or NUC-3373 **37**, however, enter the cell as masked monophosphates and pass through a different enzymatic pathway in order to be converted to their nucleoside-5′-monophosphate forms.

Nucleoside-5′-monophosphate analogs are converted to nucleoside-5′-diphosphate and nucleoside-5′-triphosphate forms by various cellular kinases. Nucleoside-5′-triphosphate analogs are substrates for DNA polymerases and can be incorporated into DNA during replication or DNA excision repair synthesis, which gives rise to stalled replication forks and chain termination. These events activate various DNA damage sensors, which stimulate DNA repair, halt cell progression, and often lead to apoptosis.[27,28] Since most cancer cells replicate their genome more frequently than a majority of normal adult cells, which are quiescent and not actively synthesizing their DNA, this phenomenon allows for a degree of cancer cell selectivity.[17] Moreover, certain nucleoside-5′-triphosphate analogs can be incorporated into RNA, leading to transcriptional termination, and messenger RNA (mRNA) and ribosomal RNA (rRNA) instability.[29,30]

Nucleoside and nucleotide (mono-, di-, or triphosphates) derivatives can also inhibit key cellular enzymes, providing a secondary mode of action that inhibits cell growth. Such enzymes include ribonucleotide reductase,[31] which removes the 2′-OH group from the ribose sugar in order to generate *de novo* 2′-deoxyribonucleoside diphosphate,[32] purine nucleoside phosphorylase,[33,34] which is involved in purine metabolism catalyzing the reversible phosphorolysis of purine nucleosides,[35] and thymidylate synthase,[36] discussed below (see Section 5.1.1.1). A few newer nucleoside analogs, however, exert their anticancer activities as adenosine receptor antagonists, or by inhibiting cellular enzymes that are not involved in nucleic acid synthesis. These cellular enzymes include NEDD8-activating enzyme, which is involved in the ubiquitin-proteasome degradation system and has an important role in cellular processes associated with cancer cell growth,[37] and DOT1L (histone-lysine N-methyltransferase, H3 lysine-79 specific), which is involved in post-translational gene modification.[38] Furthermore, the enzymatic activity of DOT1L represents an oncogenic driver of MLL-r leukemia.[39]

Enzymes that dephosphorylate nucleotides are also present in the cell. 5′-Nucleotidases (5′-NTs) can dephosphorylate noncyclic nucleoside-5′-monophosphates to nucleosides and inorganic phosphates, leading to the regulation of cellular nucleotide and nucleoside levels.[40,41] There are seven intracellular 5′-NTs: cN-IA, cN-IB, cN-II, cN-III, cN-II-like, cdN, and mdN in the cell,[42,43] and they have been reported to modulated antiviral and anticancer nucleotide levels.[41] Their cellular functions involve cell–cell communication and signal transduction,[44] as well as nucleic acid repair.[45] Other enzymes involved in nucleotide degradation include eN (CD73), an ecto-5′-nucleotidase that converts AMP to adenosine plus phosphate,[43] and ectonucleoside triphosphate diphosphohydrolase-1 (CD39), which can hydrolyze nucleoside-5′-triphosphates into nucleoside-5′-monophosphate and nucleoside-5′-diphosphate products. Tumor expression of these two enzymes, in an animal

model, appears to enhance tumor growth by negatively regulating the immune response.[46,47] Furthermore, alkaline phosphatases are also present and can be targets for anticancer treatment, but they might have a lesser impact on antimetabolite metabolism.[48] Recently, a sterile alpha motif and histidine/aspartic acid domain containing protein 1 (SAMHD1) was discovered to be a dGTP/GTP-dependent deoxynucleotide triphosphohydrolase,[49,50] generating 2′-deoxynucleosides and inorganic triphosphates from the cellular canonical 2′-deoxyribonucleoside-5′-triphosphates. Clofarabine-5′-triphosphate has been shown to be a substrate for SAMHD1.[51] Furthermore, the SAMHD1 gene was shown to be mutated in CLL cancer,[52] and the protein level downregulated in lung cancer.[53]

Cancer chemotherapy is limited in effectiveness by off target toxicity and drug resistance, with the latter leading to a decreased ability to deliver an adequate concentration of drug to cancer cells. Drug resistance can arise from pre-existing intrinsic properties of cancer cells or by acquired traits, which cancer cells develop in response to limited drug exposure.[54] Cancer cells can acquire resistance to nucleoside analogs by several different mechanisms. First, many studies using cell lines exposed to nucleoside analogs have shown transporter mutations, which generally do not occur in cancer patients. Although a decrease in nucleoside transport in patients was detected, it was dependent on cell differentiation state: myeloblasts versus lymphoblasts.[55,56] For example, high expression of hENT1 was a potential co-determinant of poor clinical response to 5-FU **2** in cells *ex vivo* from colorectal cancer patients,[57] or using RNA1 to downregulate hENT1 in the pancreatic cell line.[58] In contrast, high hENT1 expression was reported as a biomarker for survival in patients with pancreatic cancer treated with gemcitabine.[59] Second, modulation of phosphorylating enzymes, such as 2′-deoxycytidine kinase, has been reported when using cancer cell lines.[60] In patients, however, resistance to nucleoside analogs by phosphorylating enzymes has been reported to be linked to decreases in mRNA levels and a decrease in enzyme activity.[61,62] Third, resistance to nucleoside analogs can also occur by augmentation of nucleotide excision repair machinery.[63]

An increase in cellular nucleopside analog efflux provides another mechanism of resistance.[64] Cellular nucleoside analog efflux is performed by several ATP-Binding Cassette (ABC) transporters, such as ABCB1 (MDR1), ABCC4 (MRP4), ABCC5 (MRP5), and ABCC11 (MRP8).[65,66] Furthermore, metabolic inactivation of nucleoside analogs can occur by the actions of cellular enzymes such as adenosine deaminase, cytidine deaminase, and purine nucleoside phosphorylase.[67–69]

3. GENERAL SYNTHETIC APPROACHES TO ANTICANCER NUCLEOSIDE ANALOGS

Syntheses highlighted in this review use three general approaches to access anticancer nucleoside analogs: divergent, convergent, and trans-glycosylation (Figure 5).[70] The divergent approach begins with an intact nucleoside that is subsequently modified at either the sugar or base. A major advantage of this method is that the stereochemistry, especially of the key glycosidic bond (β-anomer), is already fixed. A drawback of this approach is the

presence of two or three hydroxyl groups, which are relatively similar in chemical reactivity, and often require multiple protection/deprotection steps.

The convergent approach couples a nitrogenous base with a modified sugar in a glycosylation reaction. This method allows for more synthetic diversity, and it is more widely used, although the stereochemistry of the glycosylation can be an issue. The reaction is most often performed under Silyl–Hilbert–Johnson (Vorbrüggen) conditions or metal salt coupling conditions.

Benzoyl-protected furanose sugars are often used in glycosylations performed under Vorbrüggen conditions, because the stereochemistry of the glycosylation reaction is almost completely selective. Anchimeric assistance of the 2′-*O*-benzoyl group, protected furanose sugar **II**, leads to intermediate **III**, which effectively has its α-face blocked. This allows for selective formation of the protected β-anomer **IV**. Difficulties arise when dealing with 2′-deoxy furanose sugars. The absence of a 2′-*O*-benzoyl group precludes formation of an intermediate with a sterically hindered α-face, often leading to an anomeric mixture of products **VII**, and many times requires laborious chromatographic separation.

The metal salt glycosylation generally couples a furanose sugar possessing a leaving group (often halogens) with α-stereochemistry and a salt of a nitrogenous base. Conditions are sought to make the reaction predominantly (or exclusively if possible) S_N2 in nature, in an attempt to elude anomerization of the leaving group or avoid the formation of an oxonium ion intermediate similar to **VI**, favoring a S_N1-like reaction.

Enzyme-mediated base exchange is an addition method for nucleoside synthesis. Purine nucleoside phosphorylase or uridine phosphorylase can be used to replace one nucleoside base with another in a trans-glycosylation reaction. Overall, enzymes can selectively generate the desired β-anomer nucleoside.

4. PURINE AND ACYCLIC ANALOGS

4.1. Thiopurines

4.1.1. Thiopurines Biology.—In the early 1950s, Elion's group, while at the Wellcome Research Laboratories, discovered that hypoxanthine and guanine substituted with sulfur at the 6 position led to purine synthesis inhibition, which in turn decreased growth of *Lactobacillus casei*.[71] Subsequently, 6-mercaptopurine (6-MP, **1**) and 6-thioguanine (6-TG, **3**) were demonstrated to have potent anticancer activities against a wide variety of rodent tumors. The more promising 6-MP **1** was rapidly advanced into clinical trials; in 1953 it received FDA approval for treating pediatric acute lymphocytic leukemia (ALL), a rapid-developing cancer of the blood and bone marrow in which immature lymphocytes are overproduced.

Currently, 6-MP **1** and 6-TG **3** have various clinical applications. They can effectively treat hematologic cancers (childhood and adult leukemias).[72] 6-TG **3** is used to treat acute myelogenous leukemia, a cancer involving abnormal white blood cells of myeloid linage that rapidly divide and interfere with normal white blood cell production, whereas 6-MP **1** is

used in combination with other chemotherapeutic agents and remains part of the standard of care for acute lymphocytic leukemia.[73] These compounds are also being used to treat autoimmune disorders, such as rheumatoid arthritis, psoriasis, and ulcerative colitis,[74–76] and they have also been employed to prevent solid organ transplant rejection.[30,77,78]

Thiobases require further metabolism by cellular enzymes to reach their active forms (Figure 6).[79] 6-MP **1** is converted by hypoxanthine/guanine phosphoribosyl transferase to 6-thioinosine-5′-monophosphate **III** and then converted to 6-thio-guanosine-5′-monophosphate **VI**, which is also the product of 6-TG **3** and hypoxanthine/guanine phosphoribosyl transferase. Further phosphorylation leads to 6-thio-guanosine-5′-triphosphate **VIII**, which can be incorporated into RNA.[29] However, Nelson et al. have reported that 6-MP **1** activity was not directed toward specifically inhibiting RNA synthesis, suggesting that 6-MP-5′-triphosphate **VIII** incorporation into RNA was not a major mechanism for its anticancer activity *in vitro*.[80]

A major active metabolite of both 6-MP **1** and 6-TG **3** is 6-thio-2′-deoxyguanosine-5′-triphosphate **XI**, which is produced from ribonucleotide reductase-mediated deoxygenation of 6-TG-5′-diphosphate **VII**, followed by phosphorylation.[17] 6-Thio-2′-deoxyguanosine-5′-triphosphate **XI** can be incorporated into DNA. DNA polymerases continue polymerization after 6-thio-2′-deoxyguanosine-5′-monophosphate incorporation, resulting in multiple thiobase derivatives embedded in the newly synthesized DNA strand. The reactive thiol group of the incorporated active metabolite is susceptible to nonenzymatic methylation, leading to incorporated 6-methylthioguanine-5′-monophosphate **IX**, which preferentially base-pairs with a thymine during DNA replication.[81] The 6-methylthioguanine:-thymine base-pairings resemble DNA replication errors, which are recognized by cellular mismatch repair enzymes. It is hypothesized that 6-methylthioguanine:thymine base-pairing leads to a futile cycle of repair, which ultimately generates cytotoxic DNA damage.[81]

Another major active metabolite of 6-MP **1** is methylthioino-sine-5′-monophosphate **IV**, which is formed by the methylation of thioinosine-5′-monophosphate **III** by thiopurine S-methyltransferase.[82] Metabolite **IV** inhibits phosphoribosylpyrophosphate (PRPP) amidotransferase, the first enzyme in the *de novo* purine biosynthesis pathway.[83] PRPP amidotransferase inhibition leads to a decrease in purine nucleotide pools, which can disrupt DNA synthesis and repair, ultimately leading to cell death *in vitro*.[84] 6-Thioguanine-5′-monophosphate VI can also be methylated by S-methyltransferase to form methylthioguano-sine-5′-monophosphate **IX**. However, this metabolite is a weak inhibitor of PRPP amidotransferase.

Two enzymes involved in the detoxification of 6-MP **1** and 6-TG **3** are thiopurine methyltransferase and xanthine oxidase. The former directly methylates 6-MP **1** and 6-TG **3**, to metabolites **II** and **V**, respectively. Xanthine oxidase catabolizes 6-MP **1** to the 6-thiouric acid derivative **I**. However, because xanthine oxidase expression is low in hematopoietic cells, thiopurine S-methylation is considered to be the predominant pathway of detoxification of 6-MP **1** and 6-TG **3**.[79] In addition, six different variant alleles have been identified from clinical samples that lead to lower thiopurine methyltransferase activity.[79]

Thiopurine resistance can arise due to the action of ATP-Binding Cassette (ABC) transporters. 6-MP-5′-monophosphate **III** and 6-TG-5′-monophosphate **IV** are substrates for ABC transporters: MRP4 and MRP5, for active transport out of cancer cells.[85,86] Additional mechanisms of resistance identified using cell lines are the up regulation of P-glycoprotein[87] and decreased hypoxanthine-guanine phosphoribosyltransferase activity.[88] A genomic approach revealed that overexpression of the ATM/p53/p21 pathway, overexpression of TNFRSF10D, and overexpression of CCNG2 may also contribute to thiopurine resistance in cell lines.[89] Besides the natural mutations in thiopurine S-methyltransferase gene,[79] mutations in NT5C2 transporter gene led to an increase in nucleotidase activity and resistance to 6-mercaptopurine **1** and 6-thioguanine **3** in ALL patients.[90,91] Over 170 clinical trials evaluating thiopurines as a cancer treatment are listed at ClinicalTrials.gov.

4.1.2. Thiopurines Synthesis.—Elion and Hitchings reported the preparation of 6-MP **1** in 1952 (Scheme 1).[92] For their studies, they developed a multigram-scale synthesis of hypoxanthine **57** starting from 4-amino-6-hydroxy-5-nitrosopyrimidine-2-thiol **54**.[93] The heterocycle 54 was reduced, dethiolated, and cyclized, forming **57**, which was subsequently treated with P_2S_5, furnishing the desired 6-MP **1** Conversion of hypoxanthine **57** to 6-MP **1** using P_2S_5 has also been used in a recent process synthesis, which was done in toluene on a 200-g-scale with a yield of 93%.[94] Additionally, this transformation has been effected on a gram-scale using the crystalline and storable P_4S_{10}-pyridine complex reagent **58**.[95]

Elion and Hitchings synthesized 6-thioguanine (6-TG, **3**) in 1954 (Scheme 2).[96] Guanine **59** was converted to 6-TG **3** by treatment with P_2S_5 in refluxing pyridine. Diacylated guanine **60** has also been used to produce **3**. Sulfuration with P_2S_5, followed by deprotection, furnished the product on 100-g-scale (Scheme 2).[97] In addition, the sulfuration of **60** has been effected by treatment with Lawesson's reagent.[98]

4.2. GS-9219

4.2.1. GS-9219 Biology.—Several acyclic nucleotide derivatives, such as 9-(2-phosphonylmethoxyethyl)guanine (PMEG), 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP), and 9-(2-phosphonylmethoxyethyl)adenine (PMEA), display antiproliferative activities in cancer cells (Figure 7).[99] The derivatives bear a phosphonate group, which is a bioisostere of a phosphate, but metabolically and chemically more stable.[100] The acyclic nucleotide-5′-diphosphates are structural analogs of naturally occurring 2′-deoxyribonucleoside-5′-triphosphates and can be used as substrates for cellular DNA polymerases, acting as absolute chain terminators.[101]

PMEG has been shown to be the most cytotoxic among the acyclic derivatives studied.[99] Within the cell, PMEG is converted to its diphosphate form, which is the active metabolite. The PMEG-5′-diphosphate potently inhibits DNA polymerases α, δ, and ε (DNA chromosomal replication enzymes), leading to inhibition of DNA synthesis and repair.[102] The 3′ → 5′ exonuclease activity of DNA polymerase can remove PMEG incorporated during DNA synthesis.[99] However, if not excised from the growing DNA strand, the

PMEG-5′-monophosphate, which lacks a 3′-OH moiety, acts as an absolute DNA synthesis chain terminator.

Unfortunately, PMEG displays poor cellular permeability properties, in addition to kidney and gastrointestinal tract associated toxicities.[102] Therefore, researchers at Gilead developed GS-9219 (**53**, VDC-1101), a double prodrug of PMEG, in an attempt to preferentially target lymphoid tissues.[103] The N^6-cyclopropyl moiety was installed to increase specific intracellular activation as well as decrease plasma exposure to the toxic PMEG. The phosphorodiamidate prodrug was installed to increase lymphoid cell and tissue loading efficacy. Such prodrugs are also often employed to increase compound solubility, cellular penetration, and selective tissue accumulation.[104] GS-9219 **53** showed potent antiproliferative activity against activated lymphocytes and hematopoietic cancer cells *in vitro* and in canines.[103] It was proposed that GS-9219 **53** is intracellularly converted to PMEG-5′-diphosphate in three steps: enzymatic hydrolysis of the amino acid of the prodrug (involving cathepsin A), deamination of the cyclopropyl amino moiety (involving N^6-methyl-AMP-aminohydrolase), and phosphorylation of PMEG (Figure 7).[105]

Mutations in human adenosine deaminase-like proteins (H286R and S180N) have recently been found to promote resistance to GS-9219 **53** and to 9-(2-phosphonylmethoxyethyl)-N^6-cyclopropyl-2,6-diaminopurine, an intermediate in the conversion pathway of GS-9219 **53** to PMEG.[106] Furthermore, point mutations in human guanylate kinase (S35N and V168F) were shown to promote GS-9219 resistance in cell lines.[107]

Unfortunately, GS-9219 **53** has stalled in human clinical trials for treatment of non-Hodgkin's lymphoma, chronic lymphocytic lymphoma, and multiple myeloma due to an unacceptable safety profile (ClinicalTrials.gov identifier: NCT00499239—terminated—no results posted). However, the agent was FDA-approved for canine lymphoma in June 2013,[108,109] and an additional Phase II study was done evaluating the effectiveness in canine cutaneous T-cell lymphoma.[110] GS-343074 and GS-424044 are two additional prodrugs synthesized after GS-9219 **53** and are being evaluated as anticancer prodrugs, particularly for canine cancers. No structures have been revealed for these two compounds.

4.2.2. GS-9219 Synthesis.—In 2005, Gilead disclosed their discovery synthesis of GS-9219 **53** (Scheme 3).[111] 2-Amino-6-chloropurine **61** was selectively N^9-alkylated, and subsequent nucleophilic aromatic substitution with cyclopropylamine furnished alkyl diester **63**. TMSBr-mediated deprotection led to the phosphonic acid derivative **64**, which was sufficiently pure to require no chromatography after the reaction. Bis-amidate formation was effected by treatment of acid 64 with 2,2′-dithiodipyridine, triphenylphosphine, and D-alanine ethyl ester HCl.

Recently, Jansa *et al.* reported an improved route to bisamidate prodrugs.[112] These researchers surmised that the intermediate **65** would react directly with 2,2′-dithiodipyridine, triphenylphosphine, and the amino acid to produce the bisamidate (Scheme 4). This would obviate the need for the laborious purification step often needed to isolate and purity the intermediate diacid. The approach was successful and was used to synthesize GS-9219 **53**.[113] TMS bromide-mediated deprotection of the phosphonate diester **63**[113]

formed the bis(TMS)ester intermediate **65**, maintained by strictly avoiding any contact with air. The bis(TMS)ester intermediate was then directly converted to GS-9219 **53**.

5. PYRIMIDINE ANALOGS

5.1. Fluorinated Pyrimidines

5.1.1. Fluorouracil (5-FU), Prodrugs, and Combinations Biologies.

5.1.1.1. Fluorouracil (5-FU) Biology.: In 1954, Rutman and colleagues observed that rat liver tumor cells utilized more uracil **18** as compared to normal liver cells.[114] Thymidylate synthase can catalyze the conversion of deoxyuridine-5′-monophosphate to thymidine-5′-monophosphate using 5,10-methylenetetrahydrofolate as a methyl source. These observations led Heidelberger and colleagues to synthesize fluorouracil (5-FU, 2). Their hypothesis was that the 5-FU **2** would be converted to 5-fluorouracil-5′-monophosphate, and this product would selectively inhibit thymidylate synthase by forming a complex that could not breakdown due to prevention of elimination by the 5-fluorine (Figure 8).[36,115] Furthermore, they surmised that this would selectively target tumor cells, since 5-FU-5′-monophosphate would be found at higher concentrations in these cells. Moreover, the inhibition of thymidylate synthase and subsequent decrease of thymidine would be deleterious for cancer cells growth.

These researchers subsequently validated their hypothesis by showing that 5-FU **2** inhibited the production of thymine *in vitro*,[36] and soon thereafter reported that the agent inhibited tumor growth in animals.[116] Other reports have shown that the main mechanism of action for 5-FU **2** was the inhibition of synthesis,[117] although RNA metabolism was also influenced.[118]

5-FU **2** is used as a palliative treatment for colorectal, head and neck, and breast cancers.[119] Treatment can also include a biomodulator, such as leucovorin, which increases folate cofactors and the efficacy of 5-FU **2**.[120,121] Although introduced over 50 years ago, 5-FU **2** is still extensively studied, with over 2000 clinical trials listed at ClinicalTrials.gov.

Mechanisms of action for 5-FU **2** are complex, and an overview of its metabolism is shown in Figure 9. The agent is administered by intravenous infusion and is rapidly eliminated from the plasma, having a half-life of 8–20 min.[122] 5-FU **2** enters the cell by facilitated transport,[123] and subsequent ribosylation and phosphorylation occur by orotate phosphoribosyltransferase or in two steps via uridine phosphorylase and uridine kinase.[119,124] Nucleotide kinases convert 5-FU-5′-monophosphate **IV** to the active metabolite 5-FU-5′-triphosphate **VIII**, which may be incorporated into many species of RNA. This leads to several levels of RNA disruption, including rRNA maturation inhibition[101,125] and disruption of post-translational modifications of tRNAs,[126] which can lead to cell death.

5-FU-5′-diphosphate **VII** is also a substrate of ribonucleotide reductase, resulting in 2′-deoxyribose-5-FU-5′-diphosphate generation. It is further phosphorylated to 2′-deoxyribose-5-FU-5′-triphosphate **VI** (an additional active metabolite), which then can be incorporated into DNA by cellular polymerases. 2′-Deoxyribose-5-FU-5′-monophosphate embedded into DNA does not act as a chain terminator or delayed chain terminator as

compared to other antimetabolites. Instead, uracil glycosylase can excise the embedded 2′-deoxyribose-5-FU-5′-monophosphate from the DNA, or the activation of homologous recombination can occur.[127] This apyrimidinic site is recognized by apurinic/apyrimidinic endonuclease 1 and can promote a DNA single-strand break.[17] DNA repair enzymes recognize this break in the DNA, and a futile cycle of excision, repair, and nucleotide misincorporation ensues, which eventually causes cell death.

2′-Deoxyribose-5-FU-5′-triphosphate **VI** is converted by dUTPase to 2′-deoxyribose-5-FU-5′-monophosphate **II**, which then inhibits the enzyme thymidylate synthase. Inhibition of thymidylate synthase leads to a decrease in dTMP cellular concentration and an increase in dUMP concentration. As a result, less dTTP is available for DNA polymerases during DNA synthesis. This can promote the misincorporation of dUTP into newly synthesized DNA, which may result in the futile cycle mentioned above. Incorporation of dUTP into DNA has been shown to be mutagenic and promote genomic instability.[128–130]

There are several major obstacles for the effective use of 5-FU **2** as a chemotherapeutic. First, up to 80% of administered 5-FU **2** is catabolyzed by dihydropyrimidine dehydrogenase, which is abundantly expressed in the liver and acts as an innate resistance mechanism. Dihydropyrimidine dehydrogenase converts 5-FU **2** to dihydrofluorouracil **III** and is the rate-limiting enzyme in the catabolic pathway.[122] Secondly, 2′deoxy-5-FU-5′-monophosphate and 5-FU-5′-monophosphate metabolites (**II** and **IV**) can be exported from cancer cells via the ABC transporters MRP5 and MRP8, limiting the therapeutic impact of this compound *in vitro*.[131,132]

Several mechanisms for 5-FU **2** resistance have been reported. The miRNA-320a has been shown to downregulate PDCD4 (programmed cell death protein 4) gene expression, thereby promoting 5-FU **2** resistance *in vitro*.[133] Also, miRNA-21 induces resistance by regulating PTEN and PDCD4 levels *in vitro*, which correlate to cell survival and cell death pathways, respectively.[134] Overexpression of nicotinamide *N*-methyltransferase is common in many cancer cell lines and has been linked to 5-FU **2** resistance by regulating nitric oxide production *in vitro*. Reducing reactive oxygen species leads to inactivation of the ASK1-p38 mitogen-activated protein kinase (MAPK) pathway, leading to a reduction in 5-FU **2**-induced apoptosis *in vitro*.[135] Furthermore, putrescine released by macrophage has been shown to promote 5-FU 2 resistance *in vitro*, which might be important for regulating 5-FU **2** concentration in the microenvironment of solid tumors *in vivo*.[136] Overall, 5-FU **2** resistance appears to be a multifactorial event which includes transport mechanisms, metabolism, molecular mechanisms, protection from apoptosis, and resistance via cell cycle kinetics.

In patients, 5-FU **2** resistance has been linked to thymidylate synthase methylenetetrahydrofolate reductase, dihydropyrimidine dehydrogenase, and thymidine phosphorylase, which influence 5-FU **2** metabolism.[137,138] There have been over 1500 clinical trials performed that evaluated 5-FU **2** therapy listed at ClinicalTrials.gov.

5.1.1.2. Doxifluridine Biology.: 5-FU **2** is administered intravenously due to the high level of dihydropyrimidine dehydrogenase in the gut wall, which rapidly degrades the agent.

Researchers at Roche used prodrug strategies to develop an orally available prodrug that overcomes degradation in the gut leading to enhanced absorption and an improved pharmacokinetic profile.[139] This work led to the development of doxifluridine (5′-deoxy-5-fluorouridine) 23, an orally available prodrug which is converted to 5-FU **2** in tumors by either thymidine phosphorylase or pyrimidine-nucleoside phosphorylase.[140,141]

High expression levels of pyrimidine-nucleoside phosphorylase have been reported for esophageal squamous cell carcinoma and colorectal cancer patients.[142,143] Furthermore, thymidine phosphorylase is expressed at relatively high levels in tumor tissues such as esophageal, breast, cervical, pancreatic, and hepatic,[144,145] which increase drug specificity. However, high thymidine phosphorylase expression is also found in the human intestinal tract. Therefore, doxifluridine treatment can result in dose-limiting toxicity (diarrhea) in some individuals.[146] In addition, the most frequent adverse effects for doxifluridine **23** were neurotoxicity and mucositis, whereas leukopenia and nausea were reported for 5-FU **2** in patients.[147] Several clinical trials have been done using doxifluridine **23** as a monotherapy or in combination therapy with mitomycin-C and cisplatin (administered intravenously).[148]

5.1.1.3. Capecitabine Biology.: As mentioned above, doxifluridine **23** treatment had gastrointestinal toxicity in patients. This led Roche to develop capecitabine **10** (5′-deoxy-5-fluoro-*N*-[(pentyloxy)carbonyl]cytidine), a third-generation prodrug of 5-FU **2** that was FDA-approved in 1998.[149,150] Capecitabine **10** is almost 100% orally bioavailable and is not a substrate for thymidine phosphorylase.[151] After passage through the intestinal mucosa, carboxylesterases in the liver cleave the N^4-pentyl carbamate. Next, cytidine deaminase, in either the liver or tumor, catalyses the formation of doxifluridine **23**, which is subsequently converted by thymidine phosphorylase to 5-FU **2**.[152] Capecitabine **10** was highly effective in HCT116 human cancer xenograft models and showed better selective tumor delivery as compared to either doxifluridine **23** or 5-FU **2**.[153] In addition, it was found that radiation therapy stimulated thymidine phosphorylase activity, and combination therapy with capecitabine **10** enhanced tumor cell selectivity.[154]

Drug resistance to capecitabine **10** appears to involve the interplay between dihydropyrimidine dehydrogenase and thymidine phosphorylase, which are involved in the conversion of capecitabine **10** to 5-FU **2** and the degradation of 5-FU **2** within tumors.[155] In addition to these resistance mechanisms, drug efflux is associated with gene polymorphisms of the ABCB1 transporter in patients.[66]

Currently, capecitabine **10** is FDA-approved for treatment of metastatic colorectal and breast cancers. Moreover, several clinical trials are underway using capecitabine **10** in combination therapy. A Phase II study for capecitabine **10** and bendamustine (DNA alkylating agent) in women with pretreated locally advanced or metastatic Her2-negative breast cancer (MBC-6) is being conducted (ClinicalTrials.gov identifier: NCT01891227—active status—not recruiting patients). A Phase III study is being conducted that examines capecitabine **10** maintenance therapy following treatment with capecitabine **10** in combination with docetaxel (an antimitotic taxane) in treatment of mBC (CAMELLIA study) (ClinicalTrials.gov identifier: NCT01917279—recruiting patients). Capecitabine **10** is also being evaluated as a follow up therapy for efficacy of capecitabine **10** metronomic

chemotherapy to triple-negative breast cancer (SYSUCC-001 study) (ClinicalTrials.gov identifier: NCT01112826—recruiting patients). It is also under examination for maintenance therapy: a Phase II study of maintenance capecitabine **10** to treat resectable colorectal cancer (CAMCO study) (ClinicalTrials.gov identifier: NCT01880658—recruiting patients).

5.1.1.4. NUC-3373 Biology.: In 2011 a novel 5-FU **2** prodrug NUC-3373 **37** (aka. NUC-3073 and FUDR) was reported having potent anticancer activity *in vitro*.[156,157] NUC-3373 **37**, a phosphoramidate prodrug of floxuridine **5**, was shown to be resistant to thymidine phosphorylase activity, and showed (in comparison to **5**) a significantly decreased loss of potency in CEM cells deficient in hENT1 transporters.[157] The authors also proposed a mechanism for the prodrug decomposition sequence including enzyme-mediated carboxyl ester hydrolysis, spontaneous cyclization with concomitant loss of the aryloxy leaving group, water-mediated opening of the mixed anhydride, and phosphoramidase-mediated P–N bond cleavage, resulting in 2′-deoxyribose-5-FU-5′-monophosphate **II** (Figure 10).

NUC-3373 **37** is a mixture of phosphoramidate diastereomers, and it has been shown to have many impressive prodrug attributes, overcoming the major 5-FU **2** resistance mechanisms. The agent retains activity in thymidine kinase deficient cells, is resistant to thymidine phosphorylase activity, and maintains activity in hENT1 deficient cells by entering independently of nucleoside transporters. It is resistant to dihydropyrimidine dehydrogenase catabolism, shows lower toxicity than 5-FU **2** in a variety of cell lines, and achieves high intracellular levels of 2′-deoxyribose-5-FU-5′-monophosphate **II**. Furthermore, the agent rapidly distributes to tissues following bolus infusion, shows low plasma levels of degradation metabolites, and reduces tumor weigh in HT29 colorectal xenografts significantly more than 5-FU **2**.[157] This impressive profile has led to NUC-3373 **37** entering a Phase I clinical trial in late 2015 for treatment of advanced solid tumors (ClinicalTrials.gov identifier: NCT02723240—recruiting patients).

5.1.1.5. Floxuridine and 5′-Fluoro-2′-deoxycytidine with Tetrahydrouridine Biologies.: The National Cancer Institute was an early developer of floxuridine **5** (5-fluoro-2′-deoxyuridine, FdUrd), which has been reported to be 10–100 times more effective at inhibiting tumor cell proliferation *in vitro* than 5-FU **2**.[158] Floxuridine **5** gained FDA approval in 1970, and was marketed by Roche.[159] It is approved, but not widely used, for colon and colorectal cancers that have metastasized to the liver, kidney, and stomach.[160]

Floxuridine **5** is a hydrophilic compound, and is likely transported into cells by pyrimidine nucleoside transporters.[161] Thymidine kinase converts floxuridine 5 to floxuridine-5′-monophosphate **II**, which, as mentioned above, inhibits thymidylate synthase (Figure 9).

In the intestine, a significant amount of floxuridine **5** is converted to 5-FU **2** by thymidine phosphorylase. Therefore, hepatic arterial infusion has been employed to bypass the intestine and deliver more intact floxuridine **5** to the liver.[162] Furthermore, the agent exhibits two properties associated with drugs that are best fit for hepatic arterial infusion: a high hepatic extraction and a short plasma half-life.[160] These properties can lead to increased hepatic levels of floxuridine **5** and decreased systemic levels of the compound, which can

decrease overall toxicity. This procedure has shown improvements for floxuridine **5** treatment for colorectal cancers.[163–165]

Several mechanisms of floxuridine resistance have been reported. Studies have shown a decrease in orotate phosphoribosyltransferase activity and thymidine kinase activity *in vitro*, as well as an increase of thymidylate synthase mRNA expression *in vitro*.[166] In patients, an increase in thymidylate synthase levels has been correlated with floxuridine resistance.[167]

Several clinical trials that include floxuridine **5** are currently underway. These include hepatic arterial infusion with floxuridine **5** and the anti-inflammatory and immunosuppressive steroid derivative dexamethasone (ClinicalTrials.gov identifier: NCT01862315—recruiting patients). Another trial examines hepatic arterial infusion with floxuridine **5** and dexamethasone in combination with gemcitabine hydrochloride **9** or the antiangiogenic monoclonal antibody bevacizumab (ClinicalTrials. gov identifiers: NCT01938729—recruiting patients and NCT00200200—active status—not yet recruiting patients). Recent results from a Phase I trial using floxuridine **5** with modified oxaliplatin, 5-fluorouracil **2**, and leucovorin (m-FOLFOX6) were reported.[164]

A major problem for anticancer cytidine analogs (gemcitabine, cytarabine **4**, azacytidine **11**, decitabine **14**, capecitabine **10**, and 5-fluoro-2′-deoxycytidine **38**) is that they are substrates for cytidine deaminase and can be rapidly converted to uridine analogs, which are often inactive metabolites.[168] For example, the liver expresses high levels of cytidine deaminase that provide a microenvironment to protect cancer cells from decitabine **14** treatment.[169] In attempts to overcome the problem of deamination, cytidine deaminase inhibitors, tetrahydrouridine (**39**, NSC-112907) and (4*R*)-2′-deoxy-2′,2′-difluoro-3,4,5,6-tetrahydrouridine (Figure 11), are being investigated with nucleoside analogs as potential combination therapies.

Tetrahydrouridine **39** has been investigated since the 1970s and been evaluated in cell lines and mice with cytarabine **4**, gemcitabine, azacytidine (**11**, and decitabine **14**.[170–174] How ever, clinical data has shown that patients with the cytidine deaminase gene G208A polymorphism had several adverse reactions to gemcitabine treatment.[175,176] In addition, the cytidine deaminase gene K27Q polymorphism promotes greater catalytic activity toward deoxycytine and cytarabine **4**.[177] Collectively, these data suggest prescreening patients for polymorphisms would be helpful in guiding treatment options.

Currently 5-fluoro-2′-deoxycytidine (FdCyd, **38**) is being studied in combination with tetrahydrouridine **39** in mice.[168,174] Coadministration of **38** with tetrahydrouridine **39** led to higher levels of FdCyd-5′-monophosphate, produced by 2′-deoxycyti-dine kinase in mice.[178] Once in the cell, FdCyd **38** can be converted to FdUrd by the action of cytidine deaminase, and subsequently, phosphorylation leads to FdUrd-5′-monophosphate, which inhibits thymidylate synthase. Furthermore, FdCyd-5′-monophosphate can be phosphorylated to FdCyd-5′-triphosphate and incorporated into DNA. Once incorporated into DNA, FdCyd **38** has been shown to inhibit DNA methyltransferase with comparable activity to azacytidine **11** (see Section 5.2.1).[179] Therefore, FdCyd **38** exposure can lead to the following direct and indirect cytotoxic activities in cells: (1) FdCyd-5′-monophosphate

inhibition of DNA methylation, (2) 2′-deoxyribose-5-FU-5′-monophosphate **II** inhibition of thymidylate synthase, and (3) 2′-deoxy-5-FU-5′-triphosphate **VI** and 5-FU-5′-triphosphate **VIII** incorporation into DNA and RNA, respectively.[180]

Both preclinical and clinical trials are currently underway using FdCyd **38** with tetrahydrouridine **39**. In 2016, a preclinical evaluation of FdCyd **38** and tetrahydrouridine **39** in pediatric brain tumors was reported,[181] and a methods development report was published evaluating a combination treatment of tetrahydrouridine **39** and FdCyd **38** from human serum using HPLC-MS analysis.[182] In 2015, Newman *et al.* reported a Phase I trial that examined the toxicity, plasma exposures, peak response, and dosing for the two compounds.[183] Additional clinical studies are underway for patients with advanced non-small cell lung cancer, breast cancer, bladder cancer, head and neck cancer, and solid tumors (ClinicalTrials.gov identifiers: NCT00978250—recruiting patients and NCT01534598—recruiting patients). Recently, the oral and intravenous pharmacokinetics of FdCyd **38** and tetrahydrouridine **39** were reported for cynomolgus monkeys and humans trial.[184]

(4*R*)-2′-Deoxy-2′,2′-difluoro-3,4,5,6-tetrahydrouridine is a newer cytidine deaminase inhibitor being evaluated in preclinical studies.[185] The agent has an IC_{50} of 0.4 μM for cytidine deaminase and exhibited enhanced acid stability at its *N*-glycosyl bond as compared to tetrahydrouridine **39**. Rhesus monkeys cotreated with (4*R*)-2′-deoxy-2′,2′-difluoro-3,4,5,6-tetrahydrouridine and decitabine **14** had higher detectable levels of 14 in the serum.

5.1.1.6. Tegafur-Uracil, TS-1, Carmofur, and Flucytosine Biologies.: Tegafur **19**, TS-1, carmofur **24**, and flucytosine **31** are other examples of prodrugs of 5-FU **2** (Figure 9). The tegafururacil combination, TS-1 (a triple drug combo including tegafur **19**, chloro dihydropyridine **20**, and potassium oxonate **21**), and carmofur (**24**, mifurol), are used globally in cancer treatments.[186] In 1983, tegafur–uracil (**18** + **19**) was approved for use in Japan. The orally bioavailable combination is given in a 4:1 mol ratio of tegafur **19** and uracil **18**. Tegafur **19** is metabolized to 5-FU **2**, which can be converted to the inactive dihydrofluorouracil mainly by dihydropyrimidine dehydrogenase in the liver.[187] The coadministration of uracil **18** inhibits this enzyme, thus helping maintain high levels of 5-FU **2** in the liver and in the circulation. Tegafur-uracil (**18** + **19**) is approved in Japan and Taiwan for various advanced gastrointestinal cancers. Patients are still being recruited for a Phase II clinical trial using metronomic therapy with tegafur–uracil (**18** + **19**) to treat head and neck cancer (ClinicalTrials.gov identifier: NCT00855881—recruiting patients). While a Phase II study in combination with sorafenib (kinase inhibitor) for advanced hepatocellular carcinoma has been terminated in Jan 2015, with no results posted (ClinicalTrials.gov identifier: NCT01539018—terminated in 2015—no results posted).

TS-1 includes tegafur **19**, chlorodihydropyridine (gimeracil) **20**, and potassium oxonate **21** used in a respective molar ratio of 1:0.4:1.[188] TS-1 (**19** + **20** + **21**) is an orally administered triple combination drug regiment, which was approved in Japan in 1999. Chlorodihydropyridine **20** is a more potent inhibitor of dihydropyrimidine dehydrogenase than uracil **18**.[188,189] Potassium oxonate **21** may decrease gastrointestinal toxicity by selectively inhibiting 5-FU **2** activity within the intestinal lumen. TS-1 (**19** + **20** + **21**) is

approved for use and is currently undergoing multiple clinical trials. Some of these studies include coadministration with cisplatin (DNA alkylating agent) for treatment of advanced non-small-cell lung cell cancer (ClinicalTrials.gov identifier: NCT01874678—completed status—with no results), a Phase II study for combination treatment of advanced hepatocellular carcinoma with the DNA alkylating agent oxaliplatin (ClinicalTrials.gov identifier: NCT01429961–unknown status–no updates for 2 years), and a Phase II study for monotherapy treatment of advanced metastatic breast cancer (ClinicalTrials.gov identifier: NCT01492543—unknown status—no updates for 2 years). In addition, Phase I clinical studies using combination therapy of TS-1 (**19** + **20** + **21**) and TAS-114, a first-in-class dual inhibitor of dUTPase (deoxyuridine-5′-triphosphate nucleotide hydro-lase) and dihydropyrimidine dehydrogenase,[190] is being conducted (ClinicalTrials.gov identifier: NCT01610479—active status—not recruiting).

Product marketing for carmofur **24** started in 1981. Carmofur **24** is a lipophilic-masked analog of 5-FU **2** that can be administered orally. The carbamoyl moiety of the drug is removed *in vivo* to release 5-FU **2**. Carmofur **24** has been used to treat colorectal cancer in China, Japan, and Finland for many years.[186] However, the agent has been shown to induce delayed leukoencephalopathy, characterized by progressive damage to white matter in the brain with stroke-like symptoms.[191–193] There are currently no open clinical trials for this agent. A trial treating patients with stage II hepatocellular carcinoma was suspended prematurely, because 56% of the treated patients had unacceptable side effects and offered no survival advantage for certain cancers in stage 1 and 2 of the disease.[194] This may be a reason why carmofur **24** was never pursued for FDA-approval in the US.

Flucytosine **31** is another prodrug of 5-FU **2** in clinical trials. It is converted to 5-FU **2** by cytosine deaminase, which is not encoded by the human genome. Therefore, flucytosine **31** is used in combination with gene therapy of cancer cells in an attempt to have drug-specific targeting.[195] Engineered mesenchymal stromal cells have been examined to convert flucytosine **31** into its active compound,[196] and modified neural stem cells are currently being tested for the same purpose (ClinicalTrials.gov identifiers: NCT02015819—recruiting status, and NCT01172964—completed status—no results posted). Flucytosine 31 is currently in a Phase I/II clinical trial in combination with maltose and APS001F, a recombinant anaerobic bacteria *Bifidobacterium* engineered to express the cytosine deaminase gene, for treatment of solid tumors (ClinicalTrials.gov identifier: NCT01562626—recruiting status).

The progression of fluoropyrimidine analogs shows the importance of a biological observation–chemical modification interplay. The intravenously-administered 5-FU **2** and floxuridine **5** was followed by the orally-bioavailable carmofur **24** and flucytosine **31**. The next step in the progression involved drug combinations TS-1 (**19** + **20** + **21**), tegafur–uracil (**18** +**19**), and the recently FDA-approved TAS-102 (**15** and **16**), in order to decrease 5-FU **2** degradation. Further chemical modifications led to double (doxifluridine **23**) and triple (capecitabine **10**) prodrugs, which showed improvement in toxicity and pharmacokinetics profiles. Currently, NUC-3373 **37** appears to overcome both innate and drug-derived resistance mechanisms. Newer analogs could be used as combination therapy with DNA

damaging agents such as mitomycin-C and cisplatin, and immunotherapies—antiCTLA-4 and anti-PD-1, to improve patient survival rates.

5.1.2. Fluorouracil (5-FU), Fluorouracil Prodrugs, and Combinations Syntheses.

5.1.2.1. Fluorouracil (5-FU) Synthesis.: In 1957, a synthesis of 5-FU **2** was reported in which the compound was prepared from pseudourea salts and α-fluoro-β-keto ester enolates.[197–199] Twenty years later, Robins *et al.* reported a two-step synthesis of 5-FU **2** from uracil **18** (Scheme 5).[200] Trifluoromethyl hypofluorite was used to introduce a fluorine atom into the uracil ring, and the resulting fluorinated adduct **66** underwent base-catalyzed elimination of MeOH, generating 5-fluoro-pyrimidine **2**. An alternative synthesis used the cheap and readily available ortic acid (vitamin B13, **67**) as the starting material.[201] Fluorination of **67** with fluorine gas and trifluoroacetic acid furnished acid **68**, which in turn was decarboxylated to produce 5-FU **2** on a gram-scale.

Baasner et al. reported a 100-g-scale preparation of 5-FU **2**.[202] This procedure used selective fluorine/chlorine exchange and chlorine hydrogenolysis reactions (Scheme 6). Tetrafluoropyr-imidine **72**, which can be prepared in four steps from urea **69** and diethylmalonate **70** via 2,4,5-trichloropyrimidine **71**, was treated with HCl gas forming the dichlorinated intermediate **73**. Dechlorination by selective palladium-catalyzed hydrogenolysis was followed by hydrolysis with aqueous sodium hydroxide to complete the synthesis.

5.1.2.2. Doxifluridine and Capecitabine Syntheses.: The first synthesis of capecitabine **10** was reported in the mid-1990s (Scheme 7).[150,203] Compound **79** is a key intermediate and is synthesized in six steps from D-ribose through a cyclization, mesylation, iodidation, reductive dehalogenation, hydrolysis, and cyclization/acetylation sequence. 5-Fluorocytosine, prepared by treating cytosine with trifluoromethyl hypofluorite in MeOH,[204] was glycosylated with protected sugar **79** in good yield. N^4-Amino acylation followed by selective deprotection of the 2′,3′-acetyl groups by treatment with aqueous sodium methoxide afforded crude capecitabine **10**. Finally, pure capecitabine **10** was precipitated as a white solid from ethyl acetate/hexanes on a 100 gram-scale.

Two large-scale process syntheses of capecitabine **10** were reported by Hoffman-La Roche[205] and Gore *et al.*[206] Both syntheses use 5′-deoxy-5-fluorocytidine **85** as a key intermediate (Scheme 8). 5-Fluorocytidine **81**, synthesized by treating protected cytidine with CF_3 OF/CCl_3 F,[200] was protected, tosylated, and subsequently converted to 5′-iodo **83**.[207] Reductive dehalogenation followed by deprotection produced intermediate **85**.[208] This same sequence can also be employed to prepare doxifluridine **23**, although using 5-fluorouridine as the starting material.

The Hoffmann-La Roche synthesis converted **85** to the trisacylated intermediate **86**, using 3 equiv of *n*-pentyl chloroformate in cold dichloromethane and pyridine. Selective removal of 2′,3′-ester groups was accomplished by treatment with aqueous sodium hydroxide in cold methanol, and precipitation from cold ethyl acetate afforded pure capecitabine **10**.

The Gore *et al.* approach converted **85** directly to the product **10** by selective N^4 acylation. In refluxing THF, intermediate **85** was reacted with pentyloxycarbonyl-1-hydroxybenzotriazole **87**, which can be easily made from pentyl chloroformate and HOBt. This was followed by aqueous workup and precipitation to afford **10**. Other acylating agents, such as pentyloxycarbonyl-imidazole, pentyloxycarbonyl-4-nitrophenoxy, and pentyloxycarbonyl-pentafluorophenoxy were also used, and produced similar yields.

The Jamison group used continuous flow chemistry to prepare capecitabine **10**.[209] The reaction exhibited impressive yields, "green" conditions, and very short reaction times (Scheme 9). The glycosylation reaction between triacetate intermediate **88** and silylated pyrimidine **90**, while under BrØnsted acid-catalyzed conditions, was completed in 20 min. This procedure does not require aqueous workup. Mixing conditions were carefully monitored to form the N^4-carbamate intermediate. Deprotection with a mixture of NaOH/MeOH/H_2O occurred at room temperature in only 2 min. The overall process produced milligrams of capecitabine **10** in less than 1 h and a 72% yield from starting materials **88** and **90**.

In the same report,[209] doxifluridine **23** was also synthesized using protected sugar **88**, which was condensed with silylated 5-FU **2** in a glycosylation reaction. Deprotection of the resulting intermediate with sodium methoxide furnished the product. This one-flow, two-step process produced doxifluridine **23** in 89% yield in 10 min.

5.1.2.3. NUC-3373 Synthesis.: In 2011, McGuigan and co-workers reported the synthesis of NUC-3373 **37**.[156] The agent was prepared by reaction of phosphochloridate **93** (produced using L-alanine benzyl ester salt and 1-naphthyl-dichlorophosphate **92**) with floxuridine **5** in THF in the presence of *t*-BuMgCl (Scheme 10). The synthesis produces milligrams of the final product and requires column chromatography to produce the purified mixture of diastereomers. Furthermore, no large-scale syntheses of NUC-3373 **37** have been reported.

5.1.2.4. Floxuridine, 5-Fluoro-2′-deoxycytidine, and Tetrahydrouridine Syntheses.: In the late 1950s, floxuridine **5** was originally prepared enzymatically and later converted to 5-fluoro-2′-deoxycytidine **38** via chemical synthesis.[210–212] Robin's method (see Sections 5.1.2.1) was used to prepare both compounds in only two steps from 2′-deoxyuridine **94a** and 2′-deoxycytidine **94b**, with the key ring fluorination and sugar deprotection occurring in one pot (Scheme 11). This approach has the advantage of starting from a deoxynucleoside, which already has the needed stereochemistry fixed.

Surprisingly few syntheses of tetrahydrouridine **39** have been reported. In 1967, Hanze reported two approaches to the compound (Scheme 11).[213] Cytidine **96** was "overreduced" to tetrahydrocytidine **97** in one step using rhodium on aluminum in water, and subsequent hydrolysis afforded tetrahydrouridine **39**. Uridine **98** was reduced to 5,6-dihydrouridine **99** also using rhodium and aluminum, and subsequent treatment with sodium borohydride furnished the target compound. The syntheses suffered from complex reaction mixtures, and no yields were reported.

Aoyama reported a gram-scale synthesis of floxuridine **5**,[214] in which the key step was the condensation of 5-fluoro-2,4-bis(trimethylsilyloxy)pyrimidine **102** with 3,5-bis(*O*-*p*-chorobenzoyl)-2-deoxy-α-D-ribofuranosyl chloride **101**. Chlorosugar **101** was made as the pure α anomer in three steps from 2′-deoxyribose (Scheme 12).[215,216] The condensation was performed in the presence of *p*-nitrophenol, which led, interestingly, to the stereoselective production of the desired β-anomer **103**, whereas the combination of *p*-nitrophenol and pyridine as catalysts led to formation of the α-anomer. Recrystallization from acetic acid gave the pure β-anomer, which was deprotected with methanolic ammonia, concluding the synthesis.

5.1.2.5. Tegafur, Carmofur, and Flucytosine Syntheses.: In 1976, an original synthesis of tegafur **19** was reported that coupled 2-acetoxytetrahydrofuran with silylated 5-FU **2** in the presence of sodium iodide in 95% yield.[70,217] Five years later, Miyashita *et al.* reported an alternative preparation (Scheme 13).[218] The synthesis started with a one-pot condensation of urea **69**, triethyl orthformate **104**, and methyl malonate **105** under solvent-free conditions. The resulting methyl ureidomethylenemalonate **106** was then cyclized in methanolic sodium methoxide to give 5-methoxycarbonyluracil **107**. Condensation of **107** with 2,3-dihydrofuran followed by ring fluorination and subsequent hydrolysis concluded the synthesis of **19**.

Carmofur **24** has been synthesized by treating 5-FU **2** with phosgene and hexylamine.[219,220] An alternative approach also utilized 5-FU **2** as starting material but used aqueous formaldehyde to effect N-formylation. This was followed by oxidation with potassium bromate and finally condensation with hexylamine to conclude the synthesis of **24** (Scheme 13).[221]

Flucytosine **31** was synthesized by reacting cytidine **110** with CF_3OF/CCl_3F in methanol for 5 min followed by addition of triethylamine in water (Scheme 14).[200]

5.1.2.6. Gimeracil and Oteracil Syntheses.: In 1953, Kolder and Hertog reported a synthesis of the TS-1 additive gimeracil **20**, which was completed in seven steps using 4-nitropyridine *N*-oxide as starting material.[222] Later, Yano *et al.* reported an alternative gram-scale synthesis (Scheme 15).[223] The one-pot, three component condensation of malononitrile **111**, 1,1,1-trimethoxyethane, and 1,1-dimethyoxytrimethylamine generated the dicyano intermediate **112**, which was into 2(1*H*)-pyridinone **113**.[224] Selective chlorination of **113** was followed by acid-mediated demethylation, hydrolysis, and decarboxylation, to afford gimeracil **20**. Interestingly, Xu *et al.* found that treatment of intermediate **113** with sulfuryl chloride resulted in dichloro **115** formation, which could still be converted to gimeracil **20** by treatment with sulfuric acid.[225]

Poje *et al.* reported a two-step, gram-scale preparation of the TS-1 additive oteracil **21** (Scheme 16).[226] Iodine-mediated-oxidation of uric acid **116** produced dehydroallantoin **117** as the major product, and subsequent treatment with potassium hydroxide resulted in the rearranged product oteracil **21**.[227]

5.1.3. Trifluorothymidine and Tipiracil Hydrochloride (TAS-102) Biology.—In 1964, Heidelberger and co-workers synthesized trifluorothymidine (TFT, **15**) and demonstrated that it had promising anticancer activity.[228–231] Initial animal studies showed

that TFT **15** was degraded to trifluorothymidine and catabolite 5-carboxyuracil, in the liver, spleen, and intestines.[230] The agent showed reductions in some tumor sizes during a clinical trial but, like other antimetabolites, exhibited a plasma half-life of 15 min in cancer patients.[232] Research on TFT **15** was discontinued due to inadequate information on the pharmacokinetics and toxicity profile.[233,234]

TFT **15** is a substrate for thymidine kinase, generating TFT-5′-monophosphate (an active intracellular metabolite). Similar to 5-FU-5′-monophosphate **II**, TFT-5′-monophosphate inhibits thymidylate synthase (Figure 8).[235] In contrast to 5-FU-5′-monophosphate **II**, TFT-5′-monophosphate does not form a ternary complex with thymidylate synthase but inhibits it by binding to the active site of the enzyme.[236] Furthermore, TFT-5′-monophosphate is a reversible inhibitor of thymidylate synthase, and removal of the agent results in rapid recovery of enzyme activity, whereas inhibition caused by formation of the 5-FU-5′-monophosphate **II** ternary complex has prolonged effects.[237] TFT-5′-monophosphate can be further phosphorylated to its triphosphate form (another active metabolite) and then incorporated into DNA to promote single-strand breaks.[238] DNA-incorporated TFT-5′-monophosphate is resistant to DNA glycosylase,[239] and incorporated TFT-5′-monophosphate can promote DNA instability and double-stranded breaks.[240,241]

The major drawback of TFT **15** monotherapy is its rapid degradation within the body, primarily by the action of thymidine phosphorylase, providing a natural TFT resistance mechanism.[242] However, coadministration of TFT **15** and a thymidine phosphorylase inhibitor improved the pharmacokinetic profile in animal models and antitumor activity in cell lines and animal models.[243] Currently, Taiho Pharmaceuticals is developing TAS-102 (**15** + **16**) as a combination therapy that uses TFT **15** with tipiracil hydrochloride **16**, a thymidine phosphorylase inhibitor with an IC of 35 nM,[243] in a respective molar ratio of 2:1. This combination was shown to greatly decreased the biodegradation of TFT **15** *in vitro*.[240] Interestingly, tipiracil hydrochloride **16** has also been shown to have antiangiogenic activity.[228]

TAS-102 (**15** + **16**) was FDA-approved in Sept 2015 for treatment in patients with colorectal cancer,[244] and it is also currently being evaluated in clinical trials for the treatment of advanced solid tumors and metastatic refractory colorectal cancer[233] (ClinicalTrials.gov identifier: NCT01607957—active status, not recruiting). A study was initiated evaluating TAS-102 (**15** + **16**) plus nivolumab in patients with microsatellite stable refractory metastatic colorectal cancer ClinicalTrials.gov identifier: NCT02860546—recruiting patients). In addition, TFT **15** has been pursued as an antiviral agent (registered as Viroptic) for use against the herpes simplex virus.[245,246]

5.1.4. Trifluorothymidine and Tipiracil Hydrochloride (TAS-102) Syntheses.—

Heidelberger *et al.* reported the first synthesis of TFT **15** (Scheme 17), featuring an enzyme-mediated transglycosylation of 5-trifluoromethyluracil **126** with thymidine.[229] The trifluoro base **126** was prepared in an eight-step sequence. The synthesis began with treatment of trifluoroacetone **118** with hydrogen cyanide to produce cyanohydrin **119**, and subsequent acetylation and ester pyrolysis afforded alkene **120**. Treatment of **121** with anhydrous HBr and urea provided ureidoamide **123**, which was then refluxed in acid to generate 5,6-

dihydro-5-trifluoromethyluracil **124**. This intermediate was subjected to a bromination–dehydrobromination sequence, furnishing 5-trifluoromethyluracil **126**.

Komatsu et al. have reported a more modern and enzyme-free preparation of TFT **15**.[247] The preparation featured a green glycosylation reaction performed on the 100-g-scale using the TMS-protected analog of thymine **127** and the corresponding chlorosugar **101**, made in two steps from 2′-deoxyribose. Conditions were sought to increase the rate of glycosylation (e.g., **101**α to **128**) and decrease the rate of chlorosugar anomerization (e.g., **101**α to **101**β), thereby increasing the yield of the β-glycosylation product 128 (Scheme 18).[248]

Nonpolar solvents were used to decrease chlorosugar anomerization, and temperature and reactant concentrations were explored empirically. Optimized large-scale conditions included condensation of silylated trifluorothymidine with an equimolar amount of chlorosugar **101** in minimal amounts of anisole (96% w/w of **101**) at 50 °C, producing an 85:15 β/α anomeric mixture of **128**/**129** in 71% yield. Deprotection with NaOMe at 4 °C, precipitation and filtration, and finally washing with AcOBu (to remove residual methyl 4-chlorobenzoate) produced pure trifluorothymidine **15**.

A concise 100-g-scale synthesis of tipiracil hydrochloride **16** has been reported (Scheme 19).[249] Amination of **134** with methanolic ammonia led to the cyclized intermediate 2-iminopyrrolidine **135**.[250] In parallel, 6-chloromethyl uridine, which can be prepared in four steps from urea **69** and ethyl 2-acetoacetate **130** via intermediate **131**,[251] was chlorinated at the 5-position to furnish the dichloromethyl uracil derivative **133**.[252] Finally, condensation of **133** with **135** produced tipiracil hydrochloride **16**.[249]

5.2. Azanucleosides

5.2.1. Decitabine, Azacytidine, CP-4200, and SGI-110 Biologies.—

The azanucleosides, decitabine (Dacogen, **14**), and azacytidine (Videza, **11**), were first developed as cytostatic agents nearly half a century ago.[253,254] These cytostatic agents were later found to inhibit DNA methylation in human cell lines and were developed as epigenetic drugs.

Decitabine **14** is a 2′-deoxy-5-azanucleoside analog of cytidine that enters the cell by ENT-1.[255] It is subsequently converted to decitabine-5′-monophosphate by 2′-deoxycytidine kinase. Further phosphorylation leads to the active metabolite decitabine-5′-triphosphate, which is a substrate for DNA polymerase α and is incorporated into the DNA.[256] The incorporated decitabine-5′-monophosphate cannot be methylated, which can influence epigenetic gene regulation.[257] DNA methylation usually involves the transfer of a methyl group from *S*-adenosyl-L-methionine, catalyzed by DNA methyl transferases (DNMTs), to the 5-position of a cytosine base within a cytosine-phosphate-guanosine dinucleotide.[258,259] Furthermore, DNA hypermethylation often silences tumor suppressor genes in hematopoietic malignancies,[260] and inhibition of DNA methylation at regions of decitabine-5′-monophosphate incorporation in the DNA most likely restores the expression of some of these genes.[261–264]

The ability of decitabine **14** to inhibit DNA methylation is often attributed to the formation of a complex between the azacytosine-guanine dinucleotide and DNMT1.[265] This

maypossibly occur via the covalent trapping paradigm (Figure 12).[266] A covalent bond is formed between DNMT1 and the C6-position of the azacytosine base. Methylation of the nitrogen at position 5 of the base then occurs; however, β-elimination cannot occur due to the lack of a hydrogen atom at this position. As a consequence, DNMT1 remains covalently bound to the aza-base and is unable to continue its methylation activity. The covalent complex also triggers DNA damage signaling to result in DNMT1 degradation.[267] A recent study, however, indicated that decitabine **14** could induce degradation of methyltransferases without the formation of the covalent complex, suggesting that the agent may work by additional mechanisms as well.[268]

Decitabine **14** suffers from some chemical stability issues. The triazine ring of the drug is prone to hydrolytic opening and deformylation, whereas the sugar is susceptible to anomerization. Several groups have reported a half-life of decitabine **14** ranging from 3.5 to 21 h at physiological pH and temperature,[269] whereas the *in vivo* half-life of decitabine **14** has been reported to be 15–20 min.[270] Once the prodrug is metabolized to release decitabine **14** in the liver or cells, the high levels of cytidine deaminase protein will generate an inactive nucleoside byproduct,[169] which in turn limits the intracellular concentration and toxicity of decitabine **14**. Finally if patients have reduced levels of 2′-deoxycytidine kinase activity, this may also contribute to natural decitabine resistance, as observed with decitabine.[271]

At low concentrations, decitabine **14** has the ability to inhibit DNA methyltransferase 1, but it is cytotoxic at high concentrations *in vitro*.[272,273] Optimal treatment with decitabine **14** has been tested using continuous treatment at a low concentration in patients.[274] However, the agent has poor oral bioavailability (currently administered by intravenous infusion) and is also a substrate for cytidine deaminase, which provides a natural resistance mechanism. Additionally, *ex vivo* studies suggest that reduction in phosphorylation by 2′-deoxycytidine kinase may also contribute to decitabine resistance.[271] Attempts to overcome these problems include the preparation of a decitabine mesylate salt,[275] designed to increase the oral bioavailability, as well as coadministration of oral decitabine **14** with oral tetrahydrouridine **39** in patients.[276]

Azacytidine **11**, a riboside analog of decitabine **14**, is given by injection and enters cells by the uridine/cytidine transport system.[277] After phosphorylation by uridine-cytidine kinase,[278,279] the active metabolite azacytidine-5′-triphosphate is incorporated into RNA to disrupt RNA metabolism and protein synthesis.[279] Azacytidine-5′-diphosphate can also be reduced by ribonucleotide reductase to form 2′-deoxyazacytidine-5′-diphosphate and then follows the mechanism of action of decitabine **14**.

After years of clinical research and dosage refinement, conditions were finally produced to effectively treat myelodysplastic syndrome, with azacytidine **11** and decitabine **14** being FDA-approved in 2004 and 2006, respectively.[280,281] Myelodysplastic syndrome, also known as preleukemia, is a group of related diseases originating in the bone marrow in which hematopoietic stem cells produce ineffective myeloid cells.[282] A genetic polymorphism in the cytidine deaminase gene (79A>C) and promoter depletion (–31delC) can lead to a rapid-deaminator phenotype and an increase in mRNA expression, leading to increased toxicity in patients treated with azacytidine **11**.[283,284] Sorting patients based on

cytidine deaminase genotypes remains difficult due to genotype to phenotype relationships, whereas developing a functional cytidine deaminase assay would be beneficial.[285]

Azacytidine **11** and decitabine **14** treatments are being evaluated in numerous clinical studies. An oral formulation of azacytidine (CC-486, Celgene) is currently in Phase I clinical trials (alone and in combination) for treatment of refractory solid tumors and Japanese myelodysplastic syndrome (ClinicalTrials. gov identifiers: NCT01478685—completed—no results posted, and NCT01908387—terminated status—no results posted). A Phase I/II Study of azacitidine **11** and with capecitabine **10** and oxaliplatin (DNA alkylating agent) is underway (ClinicalTrials. gov identifier: NCT01193517–active status–not recruiting). A Phase II study is examining the kinase inhibitor sorafenib with azacitidine **11** for primary response and secondary toxicity profile (ClinicalTrials.gov identifier: NCT02196857–actively recruiting patients). For decitabine **14** and tetrahydrouridine **39** clinical trials, one is currently recruiting patients— adjuvant oral decitabine **14** and tetrahydrouridine **39** with or without celecoxib in people undergoing pulmonary metastasectomy (ClinicalTrials.gov identifiers: NCT02839694–recruiting patients). Four additional studies have yet to start recruiting patients with refractory/relapsed lymphoid malignancies, pancreatic cancer, and non-small cell lung cancer using different combination treatments (ClinicalTrials.gov identifiers: NCT02846935–not yet recruiting patients, NCT02847000–not yet recruiting patients, NCT02795923–not yet recruiting patients, and NCT02664181–not yet recruiting patients).

Clavis Pharma is developing CP-4200, an elaidic acid derivative of azacytidine, which has strong epigenetic modulatory potency in human cancer cell lines (Figure 13).[286] The aim of this drug is to circumvent therapy resistance in patients with MDS/AML due to decrease in drug uptake by hENT1, because the agent enters the cell by a hENT1-independent mechanism.[287] CP-4200 has not yet moved past pre-clinical development. CP-4200 might have an acceptable bioavailability profile for oral application.

Potential hurdles for CP-4200 include the following. First, upon prodrug metabolism to release azacytidine **11** in the liver or tumor cells, high levels of cytidine deaminase activity will generate an inactive nucleoside byproduct.[288] Second, patients with reduced levels of 2′-deoxycytidine kinase activity may have some level of natural azacytidine resistance, as observed with decitabine **14**.[271] Third, two other elaidic acid derivative prodrugs: elacytarabine (**51**, Phase I trial) and CP-4126 (**49**, two Phase III trials) have not been successful in clinical trials.

Astex Pharmaceuticals has developed the dinucleotide SGI-110 **22**, a second-generation prodrug of decitabine **14**, which couples the agent with deoxyguanosine via a phosphodiester bond.[289] It was synthesized in order to increase the *in vivo* exposure and efficacy of decitabine **14** by protecting it from cytidine deaminase activity.[290,291] SGI-110 **22** is being evaluated as a hypomethylation agent with an epigenetic mechanism of action in tumors.[292,293] In a Phase I/II dose-escalating study, SGI-110 22 showed a favorable pharmacokinetic profile.[294] The dinucleotide was efficiently converted to decitabine **14** *in vivo*. Moreover, it exhibited a longer apparent half-life, lower C_{max}, and prolonged plasma exposures of decitabine **14** than equivalent amounts of IV-administered decitabine **14**.

Furthermore, SGI-110 **22** exhibited a greater stability, and lower doses of the agent achieved equal or better methylation inhibition than decitabine **14** monotherapy. SGI-110 **22** is currently in several Phase I/II studies (as monotherapy and combination therapy) for treatment of ovarian cancer, metastatic colorectal cancer, myelodysplastic syndrome, acute myelogenous leukemia, and advanced hepatocellular carcinoma (ClinicalTrials.gov identifiers: NCT01696032–active status–not recruiting patients, NCT01896856–recruiting patients, NCT01261312–active status–not recruiting patients, NCT01752933–completed in 2015–no results posted, and NCT01966289–recruiting patients).

5.2.2. Azacytidine, Decitabine, and SGI-110 Syntheses.—In 1964, Piskala *et al.* reported the first synthesis of azacytidine **11** (Scheme 20).[295] Chlorination of the 1′-position of tetraacetate riboside **136** followed by treatment with silver isocyanate produced 1-glycosyl isocyanate **137**. Conversion of **137** to isobiuret **138** using 2-methylisourea was followed by cyclization with triethyl orthoformate and deprotection with methanolic ammonia to give azacytidine **11**.

In 2006, Ionescu and Blumbergs reported a large-scale synthesis of azacytidine (11, Scheme 21).[296] Cyanoguanidine **139** was hydrolyzed and subsequently cyclized to produce the highly water-sensitive triazine **141**. The glycosylation reaction between silylated triazine **142** and 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose was performed in cold dichloromethane, with CF_3SO_3H added at 0 °C, followed by reaction warming to room temperature. Cooled solutions were used during the quenching/extraction step, which also minimized decomposition of the triazine base. Deprotection with a sodium methoxide/methanol solution, followed by recrystallization from DMSO/MeOH afforded azacytidine **11** on kilogram-scale. The last three steps, from glycosylation to recrystallization, were also performed in one pot starting with 5 g of the triazine, producing azacytidine **11** in 41% yield.

An additional process synthesis of azacytidine **11** using triflic acid in the glycosylation was reported.[297] Detailed empirical studies of the reaction found that a 1:1.2:0.95 ratio (silylated triazine **142**:triflic acid:1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose) produced the highest yield. Exposure times to water or acid during the workup were minimized in order to protect the triazine base of intermediate **143**. Further empirical studies found optimized conditions for acetyl group deprotection of **143**, which involved *n*-butylamine in refluxing methanol. Recrystallization of the crude azacytidine **11** from DMSO/toluene gave the pure product in 99.9% purity on a kilogram-scale.

In 1964, the first synthesis of decitabine **14** was reported, which used a multistep sequence similar to the preparation of azacytidine **11** shown in Scheme 20. However, no yield was reported, as the *α*/*β*-anomers were not separated.[298] Several years later, Winkley and Robins reported a synthesis of pure *β* decitabine (**14**, Scheme 22).[299] Triacetyl protected sugar **144** was converted to 3′,5′-di-*O*-acetyl-2′-deoxy-D-ribofuranosyl chloride and then treated with the silylated 5-azacytosine **142**, forming **145** as a mixture of diastereomers. Deprotection with ethanolic ammonia followed by *α*/*β* anomer separation, involving fractional crystallization followed by preparative layer chromatography on silica gel, gave decitabine

14 in 7% yield. Unfortunately, the undesired α-anomer was the predominant product, forming in 52% yield.

Two difficulties for large-scale synthesis of decitabine **14** are the lack of stability of the triazine base and the absence of anomeric selectivity during the glycosylation step. Indeed, when conjugated to a carbohydrate, 5-azacytosine is sensitive to hydrolytic decomposition under acidic, basic, and neutral conditions.[300] Furthermore, coupling of the silylated triazine with a 2′-deoxyribosugar generally leads to a 1:1 mixture of α/β anomers.

Henschke *et al.* recently performed detailed studies under various conditions of the glycosylation step in attempts to improve anomeric selectivity (Scheme 23).[301] Solvent choice, reaction temperature, and ratio of the silylated triazine to the protected ribosugar were all found to affect anomeric selectivity, producing glycosylation products ranging from an anomeric ratio of 1:1 to 1:3 (α:β). Optimal large-scale conditions included a 1:1 molar ratio of the protected sugar to silylated triazine **142** with the addition of 1.05 equiv of TMSOTf in cold DCM. Kilograms of protected decitabine **147** were produced in a 1:2.7 α/β ratio in up to 55% yield. Furthermore, it was discovered that immediate quenching of the reaction with an organic amine (usually a primary amine) was needed in order to preserve the α/β-ratio. Since they had observed isomerization of the β-anomer under different conditions, the authors hypothesized that prolonged reaction standing led to the resultant decrease of the β-anomer. Moreover, the decrease in the resultant was a result of its anomerization to the α-product and not due to decomposition of the triazine base of protected decitabine. The synthesis of decitabine **14** was concluded by deprotection of a finely milled **147**; high quality of this intermediate was required for reaction success, in methanol with or without sodium methoxide. Subsequent filtration and recrystallization from DMSO and methanol afforded kilograms of API-grade decitabine **14**.

Kolla *et al.* have recently reported a process synthesis of decitabine (**14**, Scheme 24).[302] Bis-acetyl protected sugar **149**, formed by treating 2′-deoxy-α/β-D-ribose **100** with acetyl chloride in methanol followed by acetyl chloride in pyridine/DCM, was condensed with silylated 5-azacytosine in the presence of TMSOTf, giving a 1:1 α/β mixture of acylated **150**. Deprotection with methanolic ammonia treatment followed by crystallization afforded decitabine **14** in an HPLC purity of 90–99%. The resulting solid was dissolved in DMSO, filtered, washed with MeOH/EtOAc, and suction dried to produce crystalline decitabine **14** at 99.8% purity on a gram-scale.

Recently, Redkar reported an approach to SGI-110 **22** which increased the scale of compound production from 500 mg to 1 kg (Scheme 25).[303] Decitabine **14** was selectively protected at the N^4- and 5′-positions by treatment with DMF dimethyl acetal and then vinyl acetate in the presence of Lipozyme RM IM. Conversion to the 3′-phosphoramidite **151** was followed by coupling with 3′,N^2-protected guanosine **152**, and subsequent oxidation by *tert*-butyl hydrogen peroxide furnished dinucleotide **154**. Deprotection in methanolic ammonia followed by hydrolysis produced SGI-110 **22**, which precipitated as a sodium salt.

5.3. Ribosugar-Modified Cytidine Analogs

5.3.1. Gemcitabine and Gemcitabine Prodrugs Biologies.

5.3.1.1. Gemcitabine Biology.: Hertel and colleagues at Eli Lilly originally synthesized gemcitabine (2′,2′-difluoro-2′-deoxycytidine) hydrochloride **9** as an antiviral nucleoside analog agent.[304] However, it was also discovered to be very effective at inhibiting cancer proliferation in cell culture.[304] Gemcitabine was originally screened against hematological cancers, but was also surprisingly shown to have activity in solid tumors. The agent is the only FDA-approved cytidine analog to be effective in solid tumors.[305,306]

Gemcitabine is imported into the cell by hENT-1,[307] and to a lesser extent by hCNT-1 and hCNT-3.[308–310] The agent is initially phosphorylated by 2′-deoxycytidine kinase, which is the rate-limiting step in its activation. Subsequent phosphorylations lead to the active metabolites: gemcitabine-5′-triphosphate and gemcitabine-5′-diphosphate. Gemcitabine-5′-triphosphate inhibits DNA synthesis by being incorporated into the newly synthesized DNA strand. Once incorporated into DNA, one additional nucleotide is inserted past gemcitabine-5′-monophosphate before chain elongation is inhibited.[311,312] This “masked chain termination” cannot be detected by exonucleases and eventually causes cell cycle arrest primarily in the S phase, resulting in apoptosis.[313] Alternatively, gemcitabine-5′-triphosphate can also be incorporated into DNA without chain elongation termination,[17,314] and is recognized by a DNA-dependent protein kinase/p53 protein complex, which coincides with cellular apoptosis.[27] Gemcitabine was also shown to have anticancer activity by being incorporated into RNA.[315] Furthermore, synergistic interaction of gemcitabine and sphingomyelin can induce ceramide-mediated apoptosis through Fas-induced cytotoxicity.[316]

Gemcitabine has self-potentiation mechanisms.[317,318] Gemcitabine-5′-diphosphate is a major active metabolite that inhibits ribonucleotide reductase, leading to one self-potentiation mechanism. Blocking ribonucleotide reductase activity decreases the natural deoxyribonucleotide pools, promoting more gemcitabine-5′-triphosphate to be incorporated into genomic DNA by DNA polymerase.[31,319,320] Furthermore, inhibition of ribonucleotide reductase leads to an indirect self-potentiation mechanism involving the regulation of 2′-deoxycytidine kinase. The enzyme is inhibited by a high dCTP concentration by a negative feedback loop regulation, and gemcitabine-induced ribonucleotide reductase inhibition decreases the dCTP and dATP concentrations.[321] This in turn leads to an increase in the activity of 2′-deoxycytidine kinase and more gemcitabine-5′-monophosphe accumulation in the cell. Gemcitabine-5′-triphosphate induces another direct potentiation mechanism involving the inhibition of deoxycytidylate, an enzyme that deaminates deoxycytidine-5′-monophosphate as well as gemcitabine-5′-monophosphate, preventing deamination of gemcitabine-5′-monophosphate into 2′,2′-difluoro-2′-deoxyuridine-5′-monophosphate, an inactive metabolite.[322]

There are several mechanisms of resistance for gemcitabine reported from tissue culture studies using cell lines, and some (but not all) of the proteins and pathways being discovered include the following. The deaminated byproduct of gemcitabine, 2′,2′-difluoro-5′-deoxyuridine, is a competitive inhibitor for its uptake by the hENT transporter,[323] and

mutations in nucleotide transporters and 2′-deoxycytidine kinase prevent the uptake and initial phosphorylation of gemcitabine in the cell.[60,324] Intracellularly, gemcitabine-5′-monophosphate can be deaminated to form 2′,2′-difluoro-2′-deoxycytidine-5′-monophosphate, and high gene expression of NT5C3 has been correlated with lower levels of phosphorylated gemcitabine metabolites.[325] Furthermore, upregulation of ABCC5 and hENT1 mRNA or protein was detected in gemcitabine resistance cell lines but has not been directly linked to promoting drug resistance in patients.[326] Additional studies have shown that histone methyltransferase G9a,[327] p38MAPK,[328] and Nutlin-3[329] might be correlated with gemcitabine resistance. Furthermore, gemcitabine resistance can arise from changes in high expression of drug efflux pumps and in nucleotide metabolism enzymes, inactivation of the apoptosis pathway, changes in miRNA expression, and modulation of Hedgehog, Wnt, and Notch pathways.[330]

In patients, gemcitabine deamination appears to be a major mechanism limiting drug efficacy. Gemcitabine can be deaminated at the N^4 position of the cytidine base in the serum to the inactive 2′,2′-difluoro-5′-deoxyuridine metabolite by cytidine deaminase,[331] or gemcitabine-5′-monophoshate can be deaminated by deoxycytdine deaminase in the cell. This metabolism is responsible for the very short half-life of the drug, thus limiting availability at the tumor.[332] Changes in ribonucleotide reductase subunit protein levels or polymorphisms, which regulate dNTP cellular concentrations, have been demonstrated.[333–335] Furthermore, collective cell line data suggest that modulation of mRNA expression, cell survival pathways, and tumor suppressor genes all might contribute to gemcitabine resistance. These mechanisms for gemcitabine resistance will need to be validated in the future using *ex vivo* models to determine their importance in patient populations. Since gemcitabine resistance is polygenic, combination treatments with alkylating agents, topoisomerase inhibitors, mitotic inhibitors, and biologicals (such as anti-PD-1 and anti-CTLA-4) appear to be a logical choice for new investigatory treatments.

Gemcitabine hydrochloride **9** is currently being used to treat non-small cell lung, pancreatic, breast, bladder, and ovarian cancers.[336–338] The agent is also being used to treat pancreatic cancer; however, treatment with gemcitabine hydrochloride **9** monotherapy showed only a modest benefit.[339,340] Currently, various clinical trials studying using gemcitabine hydrochloride **9** in combination therapy are underway. Some of these trials include: gemcitabine-cisplatin (DNA alkylating agent) combination therapy for advanced non-small-cell lung cancer,[341] Hodgkin's lymphoma, and non-Hodgkin's lymphoma,[342] gemcitabine-erlotinib (tyrosine kinase inhibitor) regimen to treat locally advanced and metastatic pancreatic cancers,[343] and gemcitabine-oxaliplatin (DNA alkylating agent) treatment for biliary adenocarcinoma,[344] pancreatic cancer,[345] hepatocellular cancer,[346] testicular cancer,[347] and epithelial ovarian cancer.[348] Additional combination treatments of gemcitabine with necitumumab (a monoclonal antibody which binds the epidermal growth factor receptor) and radiation treatments are under investigation (ClinicalTrials.gov identifiers: NCT01606748–active status–not recruiting patients and NCT02254681–recruiting patients).

5.3.1.2. LY2334737 Biology.: As an N^4-valproic acid prodrug, LY2334737 **52** was designed to improve the oral bioavailability of gemcitabine hydrochloride **9**, primarily by blocking the site of deamination and decreasing first-pass metabolism.[349,350] The prodrug

passes through the intestinal epithelium and enters the systemic circulation largely intact,[350] thereby reducing gut exposure to gemcitabine. LY2334737 **52** is hydrolyzed by slow systemic cleavage via the actions of carboxyesterase 2, liberating gemcitabine and valproic acid, and leads to prolonged gemcitabine exposure *in vitro*.[351] Preclinical studies showed that low-dosing of LY2334737 **52** was efficient for human colon and lung tumor xenograft models.[352] Moreover, elevated carboxyesterase 2 activity and ENT1 expression may enhance LY2334737 tumor response.

In a clinical study to determine the maximum tolerated dose and dose-limiting toxicities of LY2334737 **52**, stable disease was achieved in 22 of the 65 patients with advanced solid tumors.[353] In 2012, a Phase I clinical trial was conducted in persons with solid and metastatic tumors (ClinicalTrials.gov identifier: NCT01648764–completed in 2014–no study results were posted). However, in 2013 Eli Lilly discontinued the development of LY2334737 **52**,[354] due to results from a Japanese study that showed hepatic toxicities in patients.[355]

5.3.1.3. CP-4126 Biology.: CP-4126 (**49**, CO-1.01) is an elaidic acid ester derivative of gemcitabine developed by Clavis Pharma. The lipophilic side chain was installed to increase the ability of gemcitabine to diffuse through the plasma membrane. This modification makes CP-4126 **49** a transporter-independent analog as compared to gemcitabine, which enters the cell primarily by hENT1.[356] Once in the cell, cleavage to gemcitabine is believed to occur via the action of carboxyesterases, and subsequent phosphorylation to gemcibine-5′-monophosphate is performed by deoxycytidne kinase.[357] Furthermore, resistance studies showed that CP-4126 **49** and gemcitabine can arise by down regulation of 2′-deoxycytidine kinase *in vitro*.[324]

CP-4126 **49** has been assessed in several Phase I/II clinical trials for safety and efficacy as monotherapy or combination therapy,[357] involving non-small-cell lung cancer, metastatic pancreatic adenocarcinoma, and other advance solid tumors. The results demonstrated no marked difference in overall survivals of individuals treated with CP-4126 **49** *versus* gemcitabine hydrochloride **9** having pancreatic ductal adenocarcinoma and a low hENT1 protein expression level.[358] Two Phase I clinical trials have been completed (ClinicalTrials.gov identifiers: NCT01392976–completed in 2013–results were published). For one clinical trial, CP-4126 **49** was poorly absorbed and was subject to metabolism before systemic exposure. Overall, this led to further testing of CP-4126 **49** in patients by intravenous administration.[359] Another Phase I clinical trial had CP-4126 **49** given intravenously and reported positive results (ClinicalTrials.gov identifiers: NCT00778128–completed in 2010–results were published).[360] Another Phase I trial testing CP-4126 **49** and cisplatin was stopped (ClinicalTrials.gov identifiers: NCT01641575–terminated in 2013–no results posted). As a result of these studies, Clavis suspended development of CP-4126 **49**.

We have not covered all the potential prodrug combinations or technologies under development for gemcitabine. Other unique modifications of the agent include mesoporous silica nanoparticles,[361] amino acid monoester prodrugs,[362] Hoechst conjugates,[363] and further base modifications.[364]

5.3.1.4. NUC-1031 Biology.: The ProTide concept has been widely used in the development of antiviral and anticancer nucleoside-5′-monophosphate prodrugs.[104,365] Protides were designed as protected nucleotide-5′-monophosphate isomers to increase cellular penetration as well as bypass the often rate-limiting monophosphorylation of the nucleoside analog.[104] Furthermore, they are also believed to improve the delivery of the parent compound to the liver.[366] NUC-1031 (Acelarin, 32) is a 5′-phosphoramidate prodrug of gemcitabine.[367] This agent is able to overcome gemcitabine resistance mechanisms: decreased uptake by cellular transporters, decreased activity of 2′-deoxycytidine kinase (by mutation), and cytosine deamination.[368] However, it would still be sensitive to gemcitabine-5′-monophosphate deamination by deoxycytidine deaminase. Like gemcitabine, NUC-1031 **32**, is administered by intravenous injection.

A Phase I study in people with advanced solid tumors has been launched in 2012.[368,369] The agent could be given at four times the maximum dose of gemcitabine, and over half the patients achieved stable disease, with a few patients having a reduction in tumor size. In addition, administration of NUC-1031 **32** produced a 13-fold increase in intracellular gemcitabine concentration as compared to gemcitabine intravenous injection. An additional study funded by NuCana BioMed Limited examining combination treatment of NUC-1031 **32** with carboplatin (DNA alkylating agent) for ovarian cancer is planned (ClinicalTrials.gov identifiers: NCT01621854–completed June 2015–no results posted, and NCT02303912–recruiting patients).

5.3.2. Gemcitabine and Gemcitabine Prodrugs Syntheses.

5.3.2.1. Gemcitabine Syntheses.: Hertel and co-workers reported the discovery synthesis of gemcitabine hydrochloride **9** (Scheme 26).[370] It commenced with a Reformatsky reaction involving ethyl bromodifluoroacetate and aldehyde **155**, which is easily prepared by isopropylidene protection and subsequent oxidative cleavage of D-mannitol, forming **156** as a mixture of diastereomers (3:1 *anti*/*syn*) which were separated by HPLC. Acid-mediated deprotection and cyclization were followed by 3′,5′-*O*-silylation, affording the protected lactone **157**. Reduction, mesylation, and glycosylation under Vorbrüggen conditions produced protected sugar **158** as a 4:1 mixture of α/β anomers. Deprotection allowed for reverse phase HPLC separation of the anomers. The unfortunate α/β anomeric ratio produced under these conditions gave gemcitabine hydrochloride 9 in only 10% yield from intermediate **158**.

In 2010, Chang *et al.* reported a large-scale synthesis of gemcitabine **163** using 2,2-fluoro-α-ribofuranosyl bromide **162** as a key intermediate to increase the β-anomeric selectivity in the glycosylation reaction (Scheme 27).[371] The stereoisomers of **156** were protected with *p*-phenylbenzyl chloride and subjected to ester hydrolysis under basic conditions. Fortunately, the reaction volume reduction led to selective precipitation of the desired potassium *erythro*-pentonate **159**, and filtration furnished the pure diastereomer, representing a marked increase in efficiency when compared to the laborious diastereomeric separations of other syntheses (e.g., HPLC purification in the Hertel method). Deprotection, cyclization, and 5′-O-benzoylation of intermediate 159 were followed by lactone reduction and phosphorylation of the crude anomeric mixture, directly furnishing intermediate 161 (1:10.8 α/β ratio).

Subsequent bromination led to a 10.8:1 α/β mixture, and recrystallization from isopropanol afforded the pure α-anomer **162**, with the phenylbenzoyl protecting group being necessary for recrystallization.

In order to maximize the S_N2-displacement of α-bromide **162**, the glycosylation conditions were optimized. Therefore, non-polar solvents were used, and *in situ* generated TMSBr was removed via continuous distillation, in an attempt to decrease anomerization of **162** or formation of the oxocarbenium ion. Indeed, this led to an improved β/α anomeric mixture of 5.5:1. Deprotection with methanolic ammonia, followed by isolation of the pure β-diastereomer by simple extraction, evaporation, and crystallization from water, produced the hemi- or dihydrate of gemcitabine, depending on workup conditions.

Jiang *et al.* also recently reported a process preparation for gemcitabine hydrochloride **9** on a 100-g-scale (Scheme 28).[372] A diastereomeric mixture of difluoro lactone **164** was acylated with cinnamoyl chloride, and when the solution was cooled, stereospecific crystallization afforded the desired lactone **165**. Reduction and tosylation gave a 1:1 mixture of anomeric tosylates **166**, which was found to crystallize in ethyl acetate/petroleum ether. Subsequent glycosylation of the anomeric mixture **166** with *N*-acetyl cytosine in the presence of TMSOTf, followed by direct treatment of the reaction mixture with sodium carbonate, allowed for the precipitation of the undesired α-nucleoside analogue. Deprotection of β-isomer **167** with methanolic ammonia at room temperature gave crude gemcitabine **163**, which was acidified with HCl and crystallized in acetone/H_2O, affording the target salt in 99.9% purity.

5.3.2.2. LY2334737, CP-4126, and NUC-1301 Syntheses.: The gemcitabine prodrug LY2334737 **52** was formed by directly coupling valproic acid with gemcitabine hydrochloride **9**, using peptide coupling conditions (EDC/HOBt/NMM).[350] Prodrug LY2334737 **52** is not crystalline; therefore, it was purified as a *p*-toluenesulfonic acid (pTSA) salt co-crystal (Scheme 28).

CP-4126 **49** was formed by direct esterification of the 5′-OH of gemcitabine **163** using elaidic acid chloride as reagent (Scheme 29).[373] NUC-1301 **32** was prepared by reaction of phosphochloridate **169**, which was produced by treating L-alanine benzyl ester hydrochloric salt **168** with phenyl-dichlorophosphate **92**, with gemcitabine **163** in THF/pyridine in the presence of *N*-methyl imidazole.[374]

An improved synthesis for NUC-1031 **32** has recently been reported, which affords the agent on a 20-gram-scale.[375] Boc protection of the 3′-OH and N^4-amino group of gemcitabine **163** was followed by reaction with phenyl-dichlorophosphate **92** and L-alanine benzyl ester hydrochloride **168**, which installed the protide. Subsequent Boc deprotection completed the synthesis.

5.3.3. Sapacitabine Biology.—In the early 1990s, Matsuda *et al.* published the synthesis and anticancer activity of 2′-*C*-cyano-2′-deoxy-1-β-D-*arabino*-pentofuranosylcytosine (CNDAC) along with the rationale for the structure of the agent.[376] Since a cyanoethyl group beta to a phosphate diester of a nucleoside was reported to undergo

β-elimination under alkaline conditions,[377] it was hypothesized that a 2′-β-*C*-cyano nucleoside when incorporated into DNA could lead to β-elimination of the 3′-phosphodiester moiety and produce DNA strand breaks (Figure 14). This mechanism of anticancer activity would be similar to that of radiation therapy, which was shown to produce similar DNA strand breaks that were believed to result in tumor cell death.[378,379]

Sapacitabine **25** (Cyclacel Pharmaceuticals) is an N^4-palmitoyl derivatized analog of CNDAC and is orally bioavailable. It is primarily metabolized in the plasma, gut, and/or liver to CNDAC, and eventually transported into cells.[380] CNDAC is phosphorylated by 2′-deoxycytidine kinase and further phosphorylated by other cellular kinases to generate the triphosphate form.[381] DNA polymerases incorporate the CNDAC-5′-triphosphate into the growing DNA strands, and further elongation of the chain is sluggish due to the steric effects of CNDAC analog.[26] Upon incorporation of CNDAC-5′-monophosphate into DNA, β-elimination of the 3′-phosphodiester moiety can result in a severed DNA strand that is terminated with 2′-*C*-cyano-2′,3′-didehydro-2′,3′-dideoxycytidine-5′-monophosphate (Figure 14).[382] This represents a unique anticancer mechanism of a nucleoside derivative, and results in cell cycle arrest at the G_2 phase, which is different from the majority of other anticancer nucleoside analogs that cause arrest in the S phase.[383]

Sapacitabine **25** is a weak substrate for cytidine deaminase, whereas CNDAC is a substrate and is converted into the inactive uracil derivative: 2′-*C*-cyano-2′-deoxy-1-β-D-*arabino*-(pentofuranosyl)uridine. In addition, unlike other nucleoside analogs in their corresponding di- or triphosphates forms, CNDAC-5′-diphosphate does not effectively inhibit ribonucleotide reductase, and CNDAC-5′-triphosphate does not act as a feedback inhibitor of 2′-deoxycytidine kinase.[26]

Cells deficient in nucleotide excision repair (NER) of endonuclease XPF are 4- to 5-fold more sensitive to CNDAC exposure,[384] suggesting that the NER pathway is used to excise the agent from the DNA strand. If DNA single-strand breaks caused by CNDAC exposure are not repaired, they become more lethal DNA double-stranded breaks during the following round of DNA replication.[385] These CNDAC-induced DNA double-stranded breaks can occur when the replication fork encounters a DNA single-stranded break or after collapse of a stalled replication fork,[386] which are repaired primarily by homologous recombination. Cells with defects or mutations in the homologous recombination pathway (ATM, RAD51, Brac2, Xrcc3) are up to 100-fold more sensitive to CNDAC exposure.[387] In addition to DNA incorporation, CNDAC exposure was also shown to inhibit RNA synthesis to a limited extent *in vitro*.[26]

Sapacitabine **25** is in several clinical trials (Phase I–III) either as a monotherapy or in combination with other drugs for treatment of acute myelogenous leukemia, chronic lymphocytic leukemia, non-small cell lung cancer, and some solid tumors.[349,387,388] However, it failed to achieve end point efficacy in a Phase III study using oral sapacitabine **25** in elderly patients with newly diagnosed acute myelogenous leukemia (ClinicalTrials.gov identifier: NCT01303796–unknown, >2 years since reported). In 2013, Cyclacel Pharmaceuticals announced that sapacitabine **25** had activity against 75% of primary ovarian cancer samples isolated from individuals. DFP-10917 (**26**, intravenously administered

hydrochloride salt form of CNDAC) is in Phase I/II studies to treat relapsed or refractory acute leukemia (ClinicalTrials.gov identifier: NCT01702155–recruiting patients).

5.3.4. Sapacitabine Synthesis.—The discovery synthesis of CNDAC is shown in Scheme 30.[376,389] N^4-Acylation of cytidine **96** followed by 3′,5′-TIPS protection and 2′-OH oxidation provided ketosugar **170**. Treatment of **170** with sodium cyanide followed by reaction with phenyl chlorothionocarbonate produced an epimeric mixture of 2′-cyanohydrins **171**. Subsequent deoxygenation under standard Barton conditions produced **172** diastereoselectively, presumably due to steric hindrance on the β-face. Silyl deprotection followed by N^4-acetyl removal with HCl in MeOH afforded DFP-10917 (**26**, the hydrochloride salt of CNDAC). Interestingly, attempted deacetylation of **172** with NH_3/MeOH produced mainly cytosine, adding further evidence for the increased acidity of the 2′-α of **26**.

Soon after the discovery synthesis of CNDAC hydrochloride (DFP-10917, **26**), a process synthesis for sapacitabine **25** was reported.[390] The N^4-palmitoyl group was installed in the first step, and the rest of the synthesis followed a route similar to that of Scheme 30, with a few variations in reagent choice (i.e., pyridinium dichromate for the 2′-oxidation) producing sapacitabine **25** in nine steps in 20% overall yield.

More recently, Westwood *et al.* reported a kilogram preparation of sapacitabine **25**.[391,392] The synthesis followed a similar route as the two above-mentioned preparations with some variation in reagent use and synthetic order. Deoxygenation was performed using the economical lauroyl peroxide with collidine on a substrate similar to intermediate **171**, except with a less-bulky ethyl xanthate ester. Additionally, 3′,5′-OH silyl protection came before N^4-acylation, generating a solid intermediate that was easy to purify and use in subsequent steps. The final step of the preparation involved N^4-acylation with palmitic anhydride, producing sapacitabine **25** on a kilogram-scale in nine steps and 37% overall yield.

5.3.5. TAS-106 Biology.—Many antineoplastic agents exert their cytotoxic effects during the S phase (DNA synthesis) of the cell cycle. This poses a problem for more slow growing solid tumors, which have decreased DNA replication rates as compared to leukemias, making the incorporation of 2′-deoxynucleoside-5′-triphosphate analogs very inefficient. However, RNA synthesis occurs throughout the cell cycle except in the M phase. Therefore, a drug that targets both DNA and RNA was hypothesized to offer a broader interval in the cell cycle to exert anticancer effects.[26]

Drawing on the observation that CNDAC-5′-triphosphate (see Section 5.3.3) was shown to have some RNA synthesis inhibition, TAS-106 **46** (ECyd; [1-(3-C-ethynyl-β-D-ribopentofuranosyl)cytosine]) was developed by Taiho Pharmaceuticals in hopes that the TAS-106–5′-triphosphate analog would inhibit RNA polymerase.[382,393] Indeed, the main mechanism of action of TAS-106 **46** was shown to be inhibition of RNA polymerases I, II, and III, which essentially blocks RNA synthesis to cause apoptosis.[394] In addition, TAS-106 **46** was shown to inhibit DNA repair proteins (BRAC2 and Rad51) in the homologous repair pathway, suggesting it might also act via some of the mechanisms of sapacitabine **25**.[395]

TAS-106 **46** is phosphorylated to the monophosphate form by uridine/cytidine kinase, which is preferentially expressed in tumor cells versus normal cells.[396] It is subsequently converted to the TAS-106–5′-diphosphate and TAS-106–5′-triphosphate forms by uridine/cytidine monophosphate kinase and nucleoside diphosphate kinase, respectively.[397] TAS-106–5′-triphosphate remains in cells for a prolonged period of time even after short-term exposure to TAS-106 **46**, producing significant exposure of cells to the active form of the drug *in vitro*.[398] TAS-106 **46** is a poor substrate for cytidine deaminase, although the deaminated uridine analog of the agent also exhibited potent cytotoxicity *in vitro*.[399,400] TAS-106–5′-diphosphate was anticipated to also block activity of the enzyme due to the precedent that other 2′-deoxyribonucleoside anticancer agents are ribonucleotide reductase inhibitors. However, contrary to the original hypothesis, TAS-106–5′-diphosphate was not found to inhibit ribonucleotide reductase. Additional investigational studies have examined duplex drug linking of 5FdU(5′−5′)TAS-106 against gastric adenocarcinoma cell lines[401] and 5-FdU(3′−5′)TAS-106 exposure to hepatoblastoma cells lines.[402] Both studies showed positive cytotoxic results *in vitro*, but further studies are needed to examine the full potential of using these duplex analogs.

TAS-106 **46** therapy has produced modest results in clinical trials. In a Phase II clinical trial for salvage metastatic or recurrent head and neck squamous cell cancers and nasopharyngeal cancer, TAS-106 **46** treatment was reasonably well tolerated for platinum-failure patients, but had myelosuppression as its main toxicity.[403] However, the agent showed no anticancer efficacy (ClinicalTrials.gov identifier: NCT00737360–terminated in 2012–limited number of patients). TAS-106 **46** treatment in combination with carboplatin, a DNA alkylating agent, recently underwent a Phase I dose-escalating study in subjects with solid tumors.[404] The combination was well tolerated, and a few individuals experienced stabilized disease, in which the cancer did not progress (>4 months). There are no current clinical trials underway for TAS-106 **46**.

5.3.6. TAS-106 Synthesis.—The discovery synthesis of TAS-106 **46** is shown in Scheme 31.[399] The chosen starting material was 1,2-*O*-isopropylidene-*α*-D-xylofuranose **173**, which was 5′-OH protected and then 3′-oxidized, forming intermediate **174**.[405] Stereoselective addition of lithiated (trimethyl)acetylene to **174** gave the tertiary alcohol **175** as the desired *β*-adduct. Subsequent desilylatation, selective 5′-OH benzoylation, isopropylidene deprotection, and 2′-OH benzoylation generated **178**. Glycosylation of **178** with cytosine under Vorbrüggen conditions followed by deprotection furnished the desired TAS-106 **46** in good overall yield.

In preparation for clinical trials, a large-scale synthesis of TAS-106 **46** was developed (Scheme 32).[406] Most of the intermediates were crystalline and required no chromatography. Selective 5′-OH protection of 1,2-*O*-isopropylidine-D-xylofuranose **173** with *p*-chlorobenzoyl chloride was followed by TEMPO-mediated oxidation of the 3′-OH group. Stereoselective introduction of a TMS-ethynyl group followed by protecting group manipulation set the stage for the key glycosylation reaction. Multiple leaving groups at the anomeric position were screened, and the isobutyryloxy leaving group proved to be most effective, affording the protected intermediate **184** in good yield and stereoselectivity. Final

deprotection and crystallization from aqueous methanol furnished 128 g of crystallized TAS-106 **46**.

5.3.7. Tezacitabine Biology.—Tezacitabine **45** [MDL 101,731; (*E*)-2′-deoxy-2′-(fluoromethylene)cytidine] was designed by Hoechst Marion Roussel Inc. as a mechanism-based irreversible ribonucleotide reductase inhibitor.[407] Such inhibitors are modified at the 2′-position, and often form covalent complexes with the enzyme. Tezacitabine **45** employs a 2′-fluorovinyl moiety, which was proposed to aid in the ribonucleotide reductase-catalyzed abstraction of the 3′-hydrogen atom from tezacitabine-5′-diphosphate, by resonance stabilization of the resultant 3′-radical (Figure 15).[408] The design was successful, as tezacitabine-5′-diphosphate was indeed found to be an irreversible inhibitor of ribonucleotide reductase.[409] Furthermore, tezacitabine-5′-triphosphate was found to be a DNA polymerase chain terminator.[410]

Although a cytidine nucleoside analog, tezacitabine **45**, is relatively resistant to cytidine deaminase activity,[411] upon entering the cell, the agent is phosphorylated by 2′-deoxycytidine kinase and then by other endogenous kinases to the active metabolites: tezacitabine-5′-diphosphate and tezacitabine-5′-triphosphate. Tezacitabine-5′-triphosphate is a substrate for DNA polymerase α, being incorporated into the growing DNA strand in the place of cytidine, and it prevents further chain elongation by DNA polymerases.[412]

In tissue culture tumor cell lines and mouse tumor models, tezacitabine **45** has been examined as a candidate for treatment of colorectal and hematological solid cancers, wherein hydroxyurea and cytarabine **4** have limited antitumor activities.[413,414] Furthermore, the radiosensitizing effects of tezacitabine **45** in the presence of zidovudine (a nucleoside analog inhibitor of reverse-transcriptase) were tested in the colon cancer cell line WiDr.[415] In addition, tezacitabine **45** treatment was also shown to have antiangiogenic activity in xenograft models.[416]

A phase I clinical study showed that tezacitabine **45** treatment caused myelotoxicity in 53% of the patient and 83% had febrile episodes.[412] An additional Phase I study showed that tezacitabine **45** plus 5-FU **2** treatment had activity in patients with advanced esophageal and other gastrointestinal carcinomas.[417] However, tezacitabine **45** treatment failed to have significant activity as a stand-alone therapy.[417] In 2004 Chiron reported that it had discontinued development of tezacitabine **45** after it failed to meet expectations in a Phase II trial (ClinicalTrials.gov identifier: NCT00051688–terminated in 2004).

5.3.8. Tezacitabine Synthesis.—McCarthy *et al.* reported a synthesis of tezacitabine **45**,[418] which proceeded in seven steps from uridine in 10% overall yield, requiring five column chromatography purifications. Two years later, an improved process was published that used cytidine as starting material, allowing for the preparation of multigram quantities of the compound in five steps with a 29% overall yield (Scheme 33).[419] A one-pot protection of the 3′-OH and 5′-OH of cytidine **96** by treatment with TIPSCl and of the N^4-amino group by the addition of *N*,*N*-dimethylformamide dimethyl acetal gave intermediate **185**. This derivative underwent swern oxidation to provide the keto intermediate **186**, which was worked up by a nonaqueous silica gel plug filtration in order to avoid ketone hydration.

A Horner–Wadsworth–Emmons reaction between **186** and the *in situ*-generated diethyl-1-fluoro-1-(phenylsulfonyl)-methylphosphonate produced a separable mixture of fluorovinyl sulfones (*Z*/*E* ratio 10:1, respectively). Deprotection of the N^4-amino group with methanolic ammonia yielded the corresponding α-fluorovinyl sulfone **187**, which readily crystallized from hexanes. Radical-mediated sulfone/stannane interchange provided **188** with retention of configuration, and treatment of the intermediate with cesium fluoride in refluxing methanol allowed for the simultaneously removal of the TIPS and tributyltin groups.

5.3.9. Troxacitabine Biology.—Troxacitabine **48**, β-L-dioxolane-cytidine (Troxaty, SGX Pharmaceuticals), was originally studied as an anti-HIV agent, and later it was shown to have potential as an anticancer agent.[420] Moreover, this agent is the first L-nucleoside studied for cancer.[421]

Troxacitabine **48** enters cancer cells by passive diffusion, possibly due to the lack of 3′-OH making it not a good substrate for facilitated transporters.[422] The agent is not a substrate for cytidine deaminase.[423] However, it is recognized by 2′-deoxycytidine kinase, suggesting more chiral specificity from the former enzyme and less from the latter.[424]

Troxacitabine-5′-monophosphate is further phosphorylated to the di- and triphosphate forms by cellular enzymes. In contrast to a majority of other anticancer nucleoside analogs, troxacitabine-5′-diphosphate is the predominant intracellular metabolite, and a large pool accumulates intracellularly.[311,425] Phosphoglycerate kinase converts troxacitabine-5′-diphosphate to troxacitabine-5′-triphosphate, the major intracellular active metabolite of troxacitabine **48**.[426]

Troxacitabine-5′-triphosphate is incorporated into newly synthesized DNA, being a substrate for DNA polymerases $\alpha, \beta, \delta, \gamma$, and ε, leading to polymerase inhibition by an absolute DNA chain termination mechanism.[427,428] The incorporated troxacitabine-5′-monophosphate is not readily excised from the DNA chain by proof reading $3' \rightarrow 5'$ exonuclease activities associated with DNA polymerases, due to the chiral specificity of the enzymes. However, removal of the incorporated troxacitabine-5′-monophosphate from DNA can occur by the APE-1 excision repair mechanism.[420] Resistance to troxacitabine **48** exposure can arise by decreased expression of 2′-deoxycytidine kinase *in vitro*.[429]

Troxacitabine **48** initially showed promising anticancer activity against solid tumors and leukemias.[424,427,430–432] Tumor cells with catalytically inactive p53 have increased sensitivity to troxacitabine **48** exposure.[429] In addition, pancreatic cell lines were shown to have increased sensitivity to troxacitabine prodrugs bearing N^4 single carbon chain amides to increase drug lipophilicity.[433] However, no additional information is available addressing preclinical efficacy study models for this compound.

Unfortunately, despite the promising preclinical results, troxacitabine **48** therapy had limited success in clinical trials for leukemias and neoplasms (administered by intravenous infusion)[434,435] and ended in the termination of multiple trials (ClinicalTrials.gov identifiers: NCT00129948–terminated in 2006, NCT00104468–terminated in 2007, and NCT00104286–terminated in 2006). In 2007, results were reported for a Phase II study

evaluating troxacitabine **48** therapy in relapsed or refractory lymphoproliferative neoplasms or multiple myeloma.[436] All the patients had one adverse event, with 62% with one serious adverse event. Overall, troxacitabine **48** treatment had limited benefit in patients with these advanced disorders.[436] Furthermore, the agent also exhibited significant toxicity issues, indicating that further development of the agent is unlikely.

5.3.10. Troxacitabine Synthesis.—An initial diastereoselective synthesis of troxacitabine **48** used L-ascorbic acid as starting material (Scheme 34).[437] Acid **189** was condensed with benzyloxyacetaldehyde dimethyl acetal in the presence of tosylic acid, affording dioxolane **190** as a mixture of diastereomers. Oxidative degradation followed by benzyltriethylammonium chloride-catalyzed Wolfe oxidation produced a mixture of carboxylic acid products **192**, which could be separated with flash chromatography. Oxidative decarboxylation followed by glycosylation, separation of the α and β isomers, and deprotection furnished troxacitabine **48**.

Kim *et al.* reported a gram-scale synthesis (Scheme 35)[438] starting from 2,3:5,6-di-*O*-isopropylidene-L-gulofuranose **194**, which is made by protecting and reducing L-gulono-6,3-lactone. Acid-mediated rearrangement of **194** to pyranose **195** followed by oxidative cleavage and reduction produced dioxolane **196**. Protecting group manipulation, oxidation, and $Pb(OAc)_4$-mediated decarboxylation furnished intermediate **198**. Glycosylation of benzoyl-protected cytosine with **198** followed by deprotection with methanolic ammonia afforded troxacitabine **48**.

5.3.11. Thiarabine Biology.—Thiarabine **47** was first prepared in the 1970s and showed significant anticancer activity. However, difficulty in its synthetic preparation limited further research.[439,440] Two decades later, Southern Research Institute drew upon this finding as well as the report that 4-thionucleo-sides showed resistance to purine nucleoside phosphorylase cleavage[441] to continue the development of this next-generation anticancer nucleoside analog.[442,443]

Thiarabine **47** (T-ara C; 4′-thio-arabinofuranosylcytosine; OSI-7836) is phosphorylated to the monophosphate form via 2′-deoxycytidine kinase, although the phosphorylation rate of thiarabine **47** is 1% as compared to cytarabine **4**.[444] The thiarabine-5′-monophosphate exhibits reduced susceptibility to deaminase activity yet is still a good substrate for uridine/cytidine monophosphate kinase, leading to thiarabine-5′-diphosphate.[445,446]

Thiarabine-5′-diphosphate is further converted to the triphosphate form (the active metabolite of thiarabine **47**). Thiarabine-5′-triphosphate accumulates slowly in the cell but was shown to have long intracellular retention,[447] and yet was still efficiently degraded to the nucleoside when using cellular extracts.[446]

Thiarabine-5′-triphosphate is a substrate for the nuclear DNA polymerase and is incorporated into DNA, inhibiting DNA synthesis and generating DNA lesions,[444,448] with the primary mechanism of action being disruption of DNA polymerase activity.[449] In addition, HCT 116 colon carcinoma cells exposed to thiarabine **47** led to cleavage of caspase

3 and PARP that promoted cell death via apoptosis.[447,450] Thiarabine **47** was also reported to have antiangiogenic properties.[442]

Preclinical studies for thiarabine **47** were promising, having broad spectrum activities against human solid tumors and leukemia/lymphoma xenografts in mice.[17,446] In addition, cross-resistance in multidrug resistant cell lines did not occur for thiarabine **47**, suggesting that it potentially could be used in combination drug therapy.[451,452] Previously, a Phase I/II clinical trial showed positive results when using clofarabine **12** and cytarabine **4**, having a proposed mechanism of increased cytarabine-5′-triphosphate concentration in cells.[453] These results led to a combination therapy of clofarabine **12** and thiarabine **47** study, which showed synergistic activity of **12** and **47** leading to delayed tumor growth in mice.[17]

A few clinical trials have been reported for thiarabine **47**. In 2006, two Phase I trials were reported for thiarabine **47**.[454,455] Study results indicated excessive fatigue reversible grade-3 lymphopenia. Changes in the schedule and duration of thiarabine **47** did not improve its tolerability. Development of the agent has stalled, although some have called for continued clinical evaluation of the agent for hematological and/or solid tumors.[449]

5.3.12. Thiarabine Synthesis.—The first synthesis of thiarabine **47** was accomplished by Whistler *et al.* (Scheme 36)[439,456–459] through a long and arduous sequence. The protected glucanofuranose **199**, made from glucose in two steps (isopropylidene installation followed by benzylation), was transformed into the protected tosyl sugar **200** in six steps after protecting group manipulation and tosylation. Subsequent treatment of **200** with sodium methoxide, sodium benzyloxide, and tosyl chloride furnished **201**, which was further converted to intermediate **202** by exposure to potassium thioacetate. Isopropylidene removal followed by oxidative cleavage gave the ring-opened aldehyde **203**, and subsequent cyclization, deprotection/protection, and bromination furnished **204** as a mixture of diastereomers. The glycosylation step afforded an anomeric mixture, with the desired β-adduct crystallizing from solution, and deprotection concluded the synthesis of thiarabine **47**.

Secrist *et al.* developed an improved gram-scale synthesis of thiarabine **47** in order to access sufficient quantities for biological study (Scheme 37).[441,443] L-Xylose **205** was converted into the benzyl-protected dithioacetal **208** by selective methoxylation, O-benzylation, and dithioacetalization. Treatment of **208** with a triphenylphosphine-iodine-imidazole reagent resulted in a single inversion cyclization at C-4, affording the desired 4-thiosugar **209** in the D-*arabino* configuration.[441] Coupling of **209** with silylated uracil led to a separable anomeric mixture of **210**; as compared to making the cytosine analog, this reaction has several advantages, including a higher β:α ratio, higher yield, and easier purification process. **210**β was converted to **212** via C-4 carbonyl activation and amination. Subsequent benzyl deprotection gave thiarabine **47**.[443]

5.3.13. RX-3117 Biology.—RX-3117 **29**, a fluorocyclopentenyl-cytosine nucleoside analog, was shown to have anticancer activity[460,461] with an IC_{50} range of 0.4 to >30 μM for 59 cell lines.[462] The agent was also shown to inhibit DNA and RNA synthesis and induce apoptotic cell death of tumor cells.[349] Interestingly, preclinical studies using nude mice

implanted with various tumors, including colon, lung, renal, and pancreatic, indicated that the ribose form and not the 2′-deoxyribose form was the active compound.[463]

Biological data were recently published on the metabolism and mechanism of action of RX-3117 **29**.[462] Cellular uptake of the agent occurs by hENT, and the agent is a poor substrate for cytidine deaminase, preventing its degradation within the cell.[462] Furthermore, uridine-cytidine kinase generates RX-3117–5′-monophosphate, which is subsequently phosphorylated to the di- and triphosphate forms by cellular kinases. RX-3117–5′-trisphophate is incorporated into RNA and inhibits RNA synthesis at high concentrations, but at IC_{50} values it does not affect RNA structural integrity. RX-3117–5′-diphosphate is a substrate for ribonucleotide reductase, and the 2′-deoxy-RX-3117–5′-triphosphate can be incorporated into DNA, potentially providing another mechanism of cancer cell growth inhibition. The 2′-deoxy-RX-3117–5′-triphosphate also downregulates DNA methyltransferase-1 protein (at IC_{50} values for specific cell line) and Cdc2 protein expression, which are both involved in the cell cycle division process.[462]

Preclinical and clinical studies of RX-3117 **29** showed potent efficacy in xenograph models.[464] In 2012, Rexahn Pharmaceuticals reported the completion of a Phase I clinical trial for RX-3117 **29** in Europe and indicated that the drug had 56% orally bioavailable, had a plasma half-life of 14 h, and was well tolerated in cancer patients. Currently, a Phase Ib trial is ongoing to test the dose-finding and safety of RX-3117 **29** as an oral monotherapy to treat advanced malignancies (ClinicalTrials.gov identifier: NCT02030067–recruiting patients).

5.3.14. RX-3117 Synthesis.—A synthesis of RX-3117 **29** is illustrated in Scheme 38.[463] Protection of D-ribose **213** followed by Wittig olefination, Swern oxidation, and vinyl Grignard addition afforded diene **217**. Silyl ether deprotection and selective primary alcohol benzylation, followed by ring-closing metathesis and oxidative rearrangement via a [3,3]-sigmatropic shift yielded benzylated cyclopentenone **221**. Subsequent iodination of the double bond, lactone reduction, and protection of the resulting lactol provided TBDPS ether **224**. Electrophilic vinyl fluorination followed by desilylation gave fluorocyclopentenol **225**, which was coupled with N^3-benzoyluracil under Mitsunobu conditions, providing protected nucleoside analogue **226**. Removal of the N^3-benzoyl group by methanolic ammonia and the 5′-benzyl group by BBr_3 generated **227**. Finally, triacetatyl intermediate **227** was converted to the cytidine analog RX-3117 **29** by a sequence involving formation of a 4-triazole intermediate and nucleophilic displacement with ammonia.

An alternative and slightly improved approach to RX-3117 **29** uses a trityl protecting group at the 5′-position of intermediate **214**, which avoids a deprotection/reprotection step, decreasing the total number of steps of the synthesis by two.[465] However, both processes include long and laborious syntheses, producing only small quantities of the final product in low yield. A large-scale synthesis is most likely needed for significant clinical treatment of RX-3117 **29**.

5.4. Cytarabine and Cytarabine Prodrugs

5.4.1. Cytarabine Biology.—In the 1940s, Werner Bergmann isolated two nucleosides from the marine sponge *Cryptotethya crypta*, which he termed spongothymidine and

spongouridine. These spongonucleosides were determined to have a 2′-OH in the *arabino* configuration. In the late 1950s, various unnatural nucleosides (including spongonucleosides) were prepared with the hypothesis that they would kill cancer cells by interfering with DNA replication, and indeed, many were found to inhibit DNA synthesis *in vitro*. In the 1960s, Seymour Cohen reported promising cytotoxic effects of a new spongonucleoside in cancer cells: cytarabine **4** (aka spongocytidine, cytosine arabinoside, or ara-C).[466] The anticancer agent rapidly progressed through rodent models and clinical trials to gain FDA approval as a cancer therapy.

Cytarabine **4** is transported into the cell primarily by hENT-1.[467,468] Cytarabine **4** is monophosphorylated by 2′-deoxycyti-dine kinase, which is the rate-limiting step in the formation of the active form: cytarabine-5′-triphosphate.[469] Cytarabine-5′-tri-phosphate competes with dCTP for incorporation into DNA and is a weak competitive inhibitor of DNA polymerases. Cytarabine-5′-triphosphate is also a substrate for DNA polymerase α and is incorporated into both the leading and lagging strands of DNA. This incorporation leads to delayed chain termination, allowing several additional nucleotides to be incorporated before the DNA polymerase stops extending and falls off the replication fork. Cell cycle arrest occurs with the DNA repair mechanism deployed. If the embedded cytarabine-5′-monophosphate is not removed, the cell will enter apoptosis.[470–472]

Various factors decrease the effectiveness of cytarabine **4**. The agent is deaminated by cytidine deaminase, and cytarabine-5′-monophosphate can be deaminated by deoxycytidylate deaminase, leading to the inactive uridine analogs.[473] In addition, cytarabine-5′-triphosphate is a feedback inhibitor of 2′- deoxycytidine kinase, limiting the formation of cytarabine-5′-monophosphate.[474]

Resistance to cytarabine **4** exposure is afforded by several different mechanisms, including nucleotide transporter and 2′-deoxycytidine kinase gene mutations and decreased expression levels,[475] and changes in cytosolic nucleotidase II,[476] cytidine deaminase, and deoxycytidylate deaminase activities.[477] Recently, a correlation was reported between increased expression of the ABC transporter MRP8 and a poor treatment response of individuals with acute myelogenous leukemia treated with cytarabine **4**.[478]

Cytarabine **4** has been FDA-approved since 1969 for the treatment of acute myeloid leukemia,[479] with the exception of acute promyelocytic leukemia.[480] It is usually given in combination with a topoisomerase II inhibitor such as daunorubicin, idarubicin, or mitoxantrone.[481–483] Several clinical trials are underway using cytarabine **4** alone or in combination treatment studies. (ClinicalTrials.gov identifiers: NCT01191541–recruiting patients, NCT01289457–recruiting patients, and NCT01756677–recruiting patients).

5.4.2. Elacytarabine Biology.—Elacytarabine (**51**, CP-4055), an oleic acid ester of cytarabine **4**, was designed to enter cancer cells independent of nucleoside transporters, which would also overcome the low expression level of hENT-1 at the plasma membrane or loss of function mutation in hENT-1.[484,485] The lipophilic prodrug diffuses through the phospholipid bilayer, being retained in a membrane fraction within the cell.[486] After the action of an unidentified esterase, cytarabine **4** is released into the cytosol.[349] In comparison

to cytarabine **4**, elacytarabine **51** exposure led to an increased intracellular level of active metabolite, which was also retained longer in cells after drug removal.[486]

Elacytarabine **51** showed promising results in both preclinical and clinical studies.[487–489] However, in April 2013, despite the initial success, Clavis Pharma announced that elacytarabine **51** did not perform better than the regular standard of care therapy in a pivotal Phase III trial[354] (ClinicalTrials.gov identifier: NCT01147939–completed in 2013). Moreover, patients with relapsed/refractory acute myeloid leukemia had no meaningful benefit from elacytarabine **51** when directly compared to seven other treatment regiments.[490] As a result, Clavis has suspended all further developmental work on elacytarabine **51**.

5.4.3. MB 07133 Biology.—Ligand Pharmaceuticals Inc. developed MB 07133 **34**, a phosphoramidate prodrug of cytarabine **4**. In 2003, MB 07133 **34** entered a phase I/II clinical trial as an intravenous infusion agent for the treatment of unresectable hepatocellular carcinoma (ClinicalTrials.gov identifier: NCT00073736–completed–no results posted). MB 07133 **34** was well tolerated with evidence of disease stabilization. In 2011, Ligand Pharmaceuticals granted the license to Chiva Pharmaceuticals to develop MB 07133 **34** in China.

5.4.4. Cytarabine, Elacytarabine, and MB 07133 Syntheses.—In 1959, cytarabine **4** and 3-β-D-arabinofuranosyluracil were originally synthesized by the Dekker group (Scheme 39).[491] Cytidine **93** was treated with polyphosphoryic acid and purified by gradient elution chromatography to afford the diphosphate intermediate **228** as a crystalline cyclohexylammonium salt. Subsequent enzymatic dephosphorylation with prostatic phosphatase furnished cytarabine **4**. In order to verify the hypothesis that the transformation proceeded via a cyclonucleoside-5′-diphosphate intermediate, O^2-2′-cyclocyti-dine **229** was isolated by ion-exchange fractionation. The intermediate could further be converted to cytarabine **4** by enzyme-mediated dephosphorylation and hydrolysis.

Two different process syntheses of cytarabine **4** used 1-β-D-arabinfuranosyl uracil **231** as a key intermediate, which can be synthesized on a large-scale in two steps from uridine **98** via 2,2′-anhydro-uridine **230** (Scheme 40).[492] One process treated **231** with HMDS under high pressure and high temperature, leading to the intermediate **232**, which upon treatment with MeOH provided cytarabine **4** on a 50-g-scale.[493] The other process converted **231** to the *O*-acyl-substituted, 1,2,4-triazole derivative **233**, and subsequent deprotection produced cytarabine **4** on a kilogram-scale.[494]

Elacytarabine **51** can be prepared in one step by enzyme-mediated esterification of the 5′-OH of cytarabine **4** with oleic anhydride in DMF,[495] whereas the cyclic protide MB 07133 **34** has been synthesized from cytarabine **4** in four steps (Scheme 41).[496,497] Selective 2′,3′-silyl protection followed by treatment with *N,N*-dimethylformamide dimethyl acetal generated intermediate **236**.[496] In parallel, *trans-p*-nitrophenylphosphate **239** was prepared in three steps from methyl 3-hydroxy-3-(pyridin-4-yl)-propanoate **237** via esterification (with L-*N,N*-dimethyl phenylalanine) and subsequent diastereomer separation, reduction with $LiAlH_4$, and coupling with *p*-nitrophenyl phosphorodichloridate. Condensation of

protected nucleoside **236** with *p*-nitrophenyl phosphorochloridate **239** followed by TBS deprotection furnished MB 07133 **34**.[497]

6. PURINE ANALOGS

6.1. Deoxypurines

6.1.1. Cordycepin Biology.—In 1951, cordycepin **27** was isolated from the fungus *Cordyceps militaris*[498] and exhibited anticancer, anti-inflammatory, and antifungal activities.[499,500] It is currently thought to be the active ingredient in various traditional Chinese medicines.[501] The mechanism of cellular uptake of this agent has not been fully elucidated. However, the 3′-OH has been shown to be required for efficient uptake of nucleosides and nucleoside analogs by hENTs, suggesting that cordycepin **27** does not efficiently use this mechanism for uptake.[502] Once in the cell, the agent is converted to the cordycepin-5′-monophosphate by adenosine kinase,[503] and then to the corresponding di- and triphosphate forms by other cellular kinases. Cordycepin-5′-triphosphate is incorporated into the poly A tail of the growing mRNA chain, presumably causing chain termination due to its lack of a 3′-OH.[504] Furthermore, cordycepin **27** can inhibit ribose-phosphate pyrophosphokinase and 5-phosphoribosyl-1-pyrophosphate amidotransferase *in vitro*.[505,506]

Cordycepin **27** exposure was shown to cause DNA double-stranded break in breast cancer cells.[507] A possible explanation for this observation is the inhibitory effect this agent has on poly(ADP-ribose) polymerase, an enzyme involved in the repair of DNA single-strand breaks.[28] Unrepaired DNA single-strand breaks could lead to more extensive DNA damage, such as double-stranded breaks. In addition, cordycepin **27** is a substrate for adenosine deaminase, which can lead to significant degradation *in vivo*. Other mechanisms of action for cordycepin **27** are binding A3 adenosine and DR3 receptors, activating caspase-8/−3 pathways for cell death, suppressing cyclin D1 and c-myc expression cell signal pathways, inhibiting cAMP formation, and decreasing glycogen synthase kinase-3 *β*/*β*-catenin activation.[508–511]

In 2008, Oncovista initiated a Phase I/II clinical trial of cordycepin **27** in combination with pentostatin **6** (adenosine deaminase inhibitor; see Section 6.1.5) for the treatment of refractory TdT-positive leukemia (ClinicalTrials.gov identifier: NCT00709215–unknown status >2 years without an update). Currently, no study updates have been reported. Earlier in 2000, a Phase I trial was conducted using cordycepin **27** plus pentostatin **6** in treating patients with refractory acute lymphocytic or chronic myelogenous leukemia (ClinicalTrials.gov identifier: NCT00003005–completed in 2001–no results reported). Development of cordycepin **27** seemed to have stalled, until Rainforest Pharmacal announced plans to reactivate the IND in 2016 and start a Phase I/II trial in TdT-positive refractory leukemia patients in 2017. However, issues with deamination will have to be addressed before cordycepin **27** can progress significantly in clinical trials.

6.1.2. Cordycepin Synthesis.—In 1961, Lee *et al*. reported a pioneering synthesis of cordycepin **27**, which involved desulfurization of 3′-ethylthioadenosine by treatment with Raney nickel in refluxing 2-methoxyethanol.[512] Later, Hansske and Robins used 2-acetoxyisobutyryl bromide (Mattock's bromide) to synthesize cordycepin (Scheme 42).[513]

Thus, reaction of adenosine **240** with 2-acetoxyisobutyryl bromide afforded the corresponding *trans*-bromoacetates (**241a** and **241b**). Cordycepin **27** was finally obtained after treatment with acidic resin to provide epoxide **242** and regioselective reduction with lithium triethylborohydride.

Aman *et al*. used a similar approach for a process synthesis of cordycepin **27** (Scheme 43).[514] Initially, the authors thought to use the epoxide **242** as a key intermediate, but they had difficulty controlling the regioselectivity of the subsequent reduction. They noticed, however, that *trans*-bromoacetate **243b** fortuitously precipitated selectively from solution. This intermediate was isolated and subsequently deprotected at the 2′- and 5′-positions in order to increase compound solubility and reactivity (decreased steric hindrance) in the final reduction reaction. The deprotection required a delicate balance of reaction temperature, acid concentration, and methanol concentration in order to fully hydrolyze **243b** to **244**, without leading to compound decomposition via depurination. Depurination also plagued the final dehalogenation step, and conditions had to be optimized. Finally, hydrogenation in the presence of 4–5 molar equivalent of sodium acetate in a 1:1 solvent mixture of ethanol and water effectively debrominated the hydrochloride salt of **244**. Ethyl acetate was used to extract crude cordycepin **27** from the reaction mixture, which had a high salt load. Subsequent addition of methanol to the organic solution followed by cooled agitation precipitated cordycepin **27**. The overall process required no chromatography and produced the product cordycepin **27** on a 100-g-scale at 99% purity.

6.1.3. Cladribine Biology.—Inherited adenosine deaminase deficiencies were reported to cause lymphopenia, an immunodeficiency in humans.[515] Furthermore, the accumulation of deoxyadenosine nucleotides had been implicated in the pathogenesis of this disorder. An increase in dATP levels was shown to kill lymphocytes.[516] Experimentally, a high dADP level in lymphoid cells led to negative feedback inhibition of ribonucleotide reductase, and the subsequent decrease in dNTP pools resulted in DNA synthesis inhibition and cell death.[517] It is now known that inhibition of adenosine deaminase causes an increase in both dATP and dADP levels.

Carson *et al*. reasoned that deoxyadenosine analogs, which are resistant to adenosine deaminase activity, may be phosphorylated into their triphosphate forms in the cell. Moreover, a deoxyadenosine analog that inhibits adenosine deaminase activity might be successful for the treatment of lymphocytic malignancies.[518] In collaboration with John Montgomery, Carson studied 2-chloro-2′-deoxyadenosine (cladribine, **8**), which was shown to have significant cytotoxicity in malignant T lymphocytes.[518] The presence of the C2 chlorine in the purine base was believed to make the agent resistant to adenosine deaminase activity.[519] Furthermore, cladribine 8 was shown to be converted to the cladribine-5′-triphosphate *in vitro*, and to exert significant cytotoxicity both *in vitro* and *in vivo*.[519–521]

Cladribine **8** is transported by both hCNT and hENT into cells.[522] It is phosphorylated by cytosolic 2′-deoxycytidine kinase[516] and mitochondrial deoxyguanosine kinase to generate cladribine-5′-monophosphate. This phosphorylation is the rate-limiting step in the activation of the parent nucleoside analog.[523] Subsequent phosphorylation generates cladribine-5′-diphosphate and cladribine-5′-triphosphate forms; both are active metabolites of cladribine

8. Cladribine-5′-triphosphate is readily incorporated into DNA.[524] Chain extension by DNA polymerase continues past a single DNA incorporated cladribine-5′-monophosphate, but elongation is terminated when the polymerase encounters three successive incorporated cladribine molecules in the growing DNA strand.[17] In addition, cladribine-5′-triphosphate inhibits ribonucleotide reductase activity.[525] Collectively, these actions can promote cell death by dNTP imbalance and DNA double-stranded breaks.[526,527] Furthermore, cladribine **8** was shown to interfere with methyltransferases and may promote hypomethylation *in vitro*.[528–530] Other effects of cladribine **8** include the induction of caspase-3 activation, thereby promoting cell apoptosis *in vitro*.[531] Interestingly, the agent has also been shown to impede mononuclear cells from crossing the blood brain barrier and to inhibit T cells and B cells in the central nervous system.[532]

Resistance to cladribine **8** exposure can arise from compound metabolism and detoxification as well as increased egress of the agent from cancer cells *in vitro*.[533] Interestingly, a recent report examining metabolism data for cladribine **8** treatment in animals and humans indicated the formation of 2-chlorodeoxyinosine and 2-chlorohypoxanthine (inactive metabolites), suggesting a sensitivity to adenosine deaminase degradation.[534] Furthermore, cladribine **8** was shown to be a substrate for the ABCG2 transporter, leading to cell egress.[535]

Cladribine **8** gained FDA approval in 2004 for use as a first-line monotherapy for hairy cell leukemia[536–538] and has a favorable toxicity profile in comparison to other lymphocytic drugs.[539] It has been used in combination treatment for chronic lymphocytic leukemia[35] and abnormal mast cells debulking.[540] Cladribine **8** has also been reported to kill immature and mature monocyte-derived dendritic cells.[537] In addition, the agent was shown to be effective for treatment of Langerhans cell histiocytosis.[541] Cladribine **8** therapy has also been investigated for relapsingremitting multiple sclerosis, but has not gained FDA approval for this disease due to lack of safety information.[542]

6.1.4. Cladribine Synthesis.—Interestingly, the first reported preparation of cladribine 8 was as an intermediate in a synthesis of 2′-deoxyguanosine.[543,544] In 1972, Christensen *et al*. reported a nonstereoselective fusion glycosylation synthesis of cladribine 8 from diprotected sugar **245**, made by treatment of 2′-deoxyribose in methanol with a catalytic amount of acid followed by *p*-toluoyl protection (Scheme 44).[545] The anomeric mixture of glycosylated product **246** was resolved by silica gel chromatography, and the β-anomer was treated with methanolic ammonia, to give cladribine **8**.

In 2006, Robins reported a new synthesis of cladribine **8** that focused on improving the yield of the glycosylation step between N^6-imidazolyl purine **248** and α−1′-chlorosugar **247**, which is formed by treating 2′-deoxyribose with HCl in methanol followed by tosylation and stereoselective crystallization of the α-anomer (Scheme 45).[546,547] The modified purine base exhibited improved solubility in organic solvents as well as better glycosylation regioselectivity, due to steric hindrance of the N^7 position of the base, leading to exclusive formation of the N^9 adduct. A binary solvent system was employed in the glycosylation in attempts to increase the solubility of the modified purine base as well as improve the stereoselectivity of the reaction. The rational for this system drew on a previously reported

observation that preferential solvation of reactants can lead to different free energies of activation, and therefore alter the ratios of two competitive pathways.[548] Indeed, a judicious solvent choice of cold dichloromethane and acetonitrile (1:1 mixture) allowed for the selective formation of the desired β-nucleoside in 95% yield. Furthermore, the use of cold solvent led to decreased anomerization of chlorosugar **247**. The synthesis was concluded by converting the imidazoyl group to an imidazolium group by treatment with *in situ*-generated benzyl iodide, and the resulting salt was heated in methanolic ammonia to give, after recrystallization, pure cladribine **8**, in >90% from chlorosugar **247**.

More recently, a kilogram-scale synthesis has been reported for cladribine **8** (Scheme 46).[549] The researchers chose to explore Vorbrüggen conditions for the glycosylation. Generally, when this reaction involves 2′-deoxy lactones, it usually gives a product ratio of roughly 50% α/β nucleosides. This was indeed found to be the case when the glycosylation of the 4-chlorobenzyl protected sugar **249** and silylated 2-chloroadenine was performed in solvents such as tetrahydrofuran and dichloromethane. However, when solvents such as acetonitrile or lithium hexamethyldisilazane were employed, the desired β-anomer fortuitously precipitated from solution. It was hypothesized that a dynamic equilibrium existed in the reaction, involving glycosylation and retroglycosylation of the α-anomer along with concomitant precipitation of the β-anomer, driving the equilibrium to the desired β-nucleoside product **253**. The reaction was complete within 1 h; however, an aging step at 60 °C was used to further increase the yield. Furthermore, only a true catalytic amount of the Lewis acid trimethylsilyl trifluoromethanesulfonate was needed, which greatly simplified the purification of the product by obviating the need for a catalyst quench and aqueous workup. Isolation was accomplished by direct filtration. Deprotection with 10–20 mol % of sodium methoxide in MeOH led to *in situ* crystallization of high purity cladribine **8**, in 99.8–99.9% HPLC purity with an overall yield of 43%.

6.1.5. Pentostatin Biology.—Pentostatin (6, [(*R*)-3-(2-deoxy-β-D-erythro-pento-furanosyl)-3,6,7,8-tetrahydro-imidazo-[4,5-*d*][1,3-diazepin-8-ol]) is an antibiotic isolated from a culture broth of *Streptomyces antibioticus*.[550] It is an irreversible, stoichiometric inhibitor of mammalian adenosine deaminase, with a K_i in the nanomolar range.[551,552] Importantly, pentostatin 6 directly inhibits this enzyme in order to truly mimic adenosine deaminase deficiencies, which is in contrast to cladribine 8, which was designed as an adenosine deaminase-resistant antimetabolite (see Section 6.1.3.). Pentostatin **6** is unique among anticancer nucleoside analogs in that it is active without metabolism (cellular phosphorylation). It has been proposed that the tetrahedral carbon at position 8 of the diazepine ring of pentostatin **6** mimics the purported transition state tetrahedral carbon in the deamination of adenosine to inosine.[553] In addition to this effect, pentostatin **6** also targets cellular methyltransferases, hindering the ability of the cell to methylate both DNA and mRNA.[554–556]

Pentostatin **6** is used to treat hairy cell leukemia and is effective against lymphoid malignancies with high adenosine deaminase activities.[557,558] The agent has also been used to prevent and to treat both acute and chronic graft-versus-host disease.[559] Pentostatin **6** is currently being tested in several clinical trials. An additional Phase I/II clinical trial uses

pentostatin **6** and cyclophosphamide (DNA-alkylating agent) cotreatment for low-intensity stem cell transplantation with multiple lymphocyte infusions and to treat advanced kidney cancer (ClinicalTrials.gov identifier: NCT00923845–active status–primary objective of trial was not achieved). A Phase II trial combines pentostatin **6**, cyclophosphamide (DNA alkylating agent), and rituximab (monoclonal antibody against B cell protein CD20) followed by lenalidomide (antiangiogenic/immunomodulatory agent) treatment for relapsed or refractory B cell chronic lymphocytic leukemia (ClinicalTrials.gov identifier: NCT00074282–unknown status–no updates in 2 years). Finally, a Phase I trial involves the combination of pentostatin **6**, bendamustine (DNA-alkylating agent), and ofatumumab (monoclonal antibody against B cell protein CD20) for the treatment of chronic lymphocytic leukemia (ClinicalTrials.gov identifier: NCT01352312–active status–not recruiting patients).

6.1.6. Pentostatin Synthesis.—Parke-Davis developed a large-scale industrial production of pentostatin **6** using fermentation cultures of *Streptomyces antibioticus* NRRL 3238. To date, this approach is the major form of production of the compound, although a few other syntheses have been reported.[560]

In 1982, the first synthesis of pentostatin **6** was reported by chemists at Warner-Lambert/Parke-Davis (Scheme 47).[561,562] Knoevenagel condensation of azole **254** with benzaldehyde produced styrylimidazole **255**, and subsequent benzylation formed regioisomers **256** and **257**. The mixture was subjected to ozonolysis, and **258** was separated from its isomeric product by differential precipitation. Carbon homologation of **258** was followed by nitro reduction, ring closure, and debenzylation, producing heterocycle **261**. Due to the instability of heterocycle **261**, glycosylation conditions had to be optimized. The use of tin(IV) chloride as Lewis acid and BSTFA as silylating agent were found to produce the desired product in a 1:1 mixture of α/β diastereomers. The mixture can be separated by preparative HPLC on a small-scale and by fractional crystallization on a larger-scale. Deprotection of the β-anomer **262** in the presence of sodium methoxide followed by ketone reduction produced a separable 1:1 mixture of pentostatin **6** and its stereoisomer. It is noteworthy that Zhang *et al.* improved the conclusion of the synthesis by using a chiral ruthenium catalyst for asymmetric transfer hydrogenation of ketone **262**.[563] This reaction was completely diastereoselective and gave a yield of 80%.

Phiasivongsa and Redkar reported a ring expansion of protected 2′-deoxyinosine **263** using diazomethane.[564] This was followed by deprotection and reduction to furnish a diastereomeric mixture of pentostatin **6**, which was separated by following the Warner-Lambert/Parke-Davis procedure in Scheme 48.[564] Unfortunately, no yields were reported.

6.1.7. Clofarabine Biology.—Clofarabine **12** is a second-generation purine nucleoside analog designed to overcome the limitations of fludarabine-5′-monophosphate **7** (see Section 6.2.3) and cladribine **8** (see Section 6.1.3),[565] which are both susceptible to glycosidic bond cleavage.[521] Indeed, the introduction of a C2′-fluorine in the *arabino* configuration in clofarabine **12** significantly increased the stability of the glycosidic bond in acidic solution and toward phosphorolytic cleavage.[566,567] Chlorine substitution instead of fluorine at the 2-position of the adenine base was chosen in order to avoid production of the

2-fluoroadenine, a precursor to the toxic 2-fluoro-adenosine-5′-triphosphate, should the glycosidic bond be cleaved.[568]

Clofarabine **12** is usually administered intravenously; however, the increased stability of the glycosidic bond also allows for oral administration.[566,569,570] Clofarabine **12** enters the cell via hENT1, hENT2, and hCNT2, and to a much lesser extent by passive diffusion.[429] 2′-Deoxycytidine kinase generates clofarabine-5′-monophosphate;[571] however, the enzyme has a lower K_m for clofarabine **12** than cladribine **8**.[572] Moreover, clofarabine resistance arises from decreased 2′-deoxycytidine kinase activity *in vitro*.[572] Formation of clofarabine-5′-diphosphosphate is generated by purine nucleotide monophosphate kinase, and is the rate-limiting step in the activation of the agent.[573] This is in contrast to many other nucleoside analogs, in which the rate-limiting step is the generation of clofarabine-5′-monophosphate.

Clofarabine-5′-diphosphate serves as an intracellular reservoir for the active-metabolite clofarabine-5′-triphosphate, which is retained in the cell longer than clofarabine-5′-triphosphate in cells from CLL and AML patients *ex vivo*.[572] Clofarabine-5′-triphosphate has several mechanisms of action in the cell. It inhibits ribonucleotide reductase (presumably by allosteric inhibition), which results in a decrease in dCTP and dATP concentrations.[520] The reduction in the amount of dCTP likely leads to DNA synthesis inhibition, and a decrease in the concentrations of dATP leads to greater DNA incorporation of clofarabine-5′-triphosphate (self-potentiation).[568] A low intracellular concentration of clofarabine-5′-triphosphate can be incorporated by DNA polymerases α and ε[574] into DNA and can promote polymerase arrest at the replication fork, inhibiting DNA repair and inducing strand breaks *in vitro*.[573,574] This DNA damage leads to the induction of cytochrome c-mediated apoptosis *in vitro*.[575] Studies have shown that clofarabine-5′-triphosphate can also be incorporated into RNA in cell lines.[573]

Clofarabine **12** is an FDA-approved drug for the treatment of relapsed or refractory pediatric acute lymphoblastic leukemias.[35,576] It is being evaluated for the treatment of acute myeloid leukemia in both children and the elderly.[577] The agent was examined for treatment of non-Hodgkin's lymphomas, myelodysplastic syndrome, and solid tumors.[520] Oral clofarabine **12** has been studied in relapse/refractory non-Hodgkin's lymphomas[578] and in high-risk myelodysplastic syndrome patients.[579] Currently clofarabine **12** in combination treatments is being investigated in over a dozen clinical trials for chronic lymphocytic leukemia, acute myelogenous leukemia, myelodysplastic syndrome, and mixed phenotype acute leukemia with nucleoside analogs such as cytarabine **4**, gemcitabine, and fludarabine-5′-monophosphate 7 and with other chemotherapies such as but not limited to busulfan, idarubicin, etoposide, and mitoxantrone.

6.1.8. Clofarabine Synthesis.—In the early 1990s, Montgomery *et al.* utilized differentially protected sugar **272** in order to access various nucleoside analogs, including clofarabine **12** (Scheme 49).[566,580,581] The synthesis began with the stereoselective fluorination of diprotected tosylate **267**, which can be formed by isopropylidene protection and tosylation of α-D-glucofuranose, followed by selective deprotection, leading to fluorinated sugar **268**. Benzoylation of the 5′-OH followed by acid-mediated deprotection afforded lactol **269**. Oxidative cleavage and subsequent rearrangement produced **270**, which

was eventually converted to bromosugar **272**. Condensation of 2,6-dichloropurine with **272** occurred in refluxing dichloroethane, and chromatographic separation of the anomeric isomers produced nucleoside derivative **273**. Three days of treatment of **273** in a steel bomb with ethanolic ammonia afforded a mixture of clofarabine **12** and the partially deprotected 5′-*O*-benzoyl derivative. Treatment of the mixture with LiOH in MeCN/H_2O removed the residual protecting group, and pure clofarabine **12** was produced by three crystallizations from water.

Twelve years later, Bauta *et al.* reported an improved and scalable process synthesis of clofarabine **12** (Scheme 50).[582] Studies were performed on bromosugar **279**,[583] with the desire to increase β-anomer formation in the glycosylation step. Compound **279** was easily prepared by first conversion of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **274** to protected sugar **276**[584] via formation of the benzoxonium ion intermediate and solvolysis. Subsequent installation of the imidazolylsulfonate leaving group, stereoselective deoxyfluorination using excess KHF_2, and finally bromination under acidic conditions completed the synthesis of **279**.[583] Empirical studies of the glycosylation step of 2-chloroadenine found potassium *tert*-butoxide to be the optimum base, with the potassium counterion being particularly important for both yield and β-selectivity. Solvent choice was guided by the precedent that low dielectric constants of a solvent favor the formation of the β-anomer in glycosylations involving bromosugar **279**. It is believed that this effect arises from suppression of the formation of the oxonium ion intermediate **280** and subsequent S_N1-type condensation, which would lead to both α- and β-glycosylation products. Nonpolar solvents would therefore be needed in order to favor a S_N2 reaction pathway. However, solubility in such solvents would also be an issue, particularly with the 2-chloroadenine base. In order to find a balance between the two conflicting requirements of anomeric selectivity and substrate solubility, a 1:2:1 mixture of MeCN/*tert*-amyl alcohol/DCE was eventually chosen after considerable experimentation. In addition, the additive CaH_2 was found to increase overall yield and anomeric ratio, due in part to removal of trace amounts of water from the solvent.

A number of challenges were overcome to separate protected clofarabine **281** from the heterogeneous reaction mixture. Excess 2-chloroadenine was filtered off. A subsequent solvent exchange to *n*-butyl acetate dissolved the mixture to allow for heptane-induced precipitation of crude **281**, which after two subsequent pH-maintained methanol slurries was isolated in a >50:1 β/α ratio. Finally, deprotection with a catalytic amount of sodium methoxide in methanol followed by recrystallization formed drug-pure clofarabine **12** on a 25-g-scale.

6.2. Arabinose Purine Analogs

6.2.1. Nelarabine Biology.—Arabinosylguanine (9-β-D-arabinofuranosylguanine; ara-G) was discovered in the 1960s, but was not significantly developed at that time. In the 1970s, studies on the rare autosomal disorder purine nucleoside phosphorylase deficiency rekindled interest in ara-G.[393,585] A deficiency in purine nucleoside phosphorylase, an enzyme that catalyzes the phosphate-mediated deribosylation of certain purine nucleosides, results in a profound dGTP-mediated imbalance in the endogenous nucleotide pools that

leads to T cell lymphopenia without concomitant decreases in B cell populations.[586] This sensitivity of human T cells (especially immature T cells) to purine nucleoside phosphorylase deficiency is hypothesized to arise from their relatively high ratio of kinase activity versus nucleotidase activity (in contrast to other cell types) which results in a high accumulation of intracellular dGTP.[587] Deoxyguanosine, but not ara-G, is a substrate for purine nucleoside phosphorylase.[588,589]

Nelarabine (**13**, 2-amino-9-β-D-arabinosyl-6-methoxy-9H-guanine; 506U78) is a prodrug of ara-G and has a 10-fold higher solubility as compared to ara-G.[590,591] Nelarabine **13** is converted to ara-G in the serum by adenosine deaminase.[592] Ara-G is phosphorylated by either cytosolic 2′-deoxycytidine kinase or mitochondrial deoxyguanidine kinase into ara-G-5′-monophosphate[593] and, subsequently, into ara-G-5′-diphosphate and ara-G-5′-triphosphate by cellular kinases. The active metabolite ara-G-5′-triphosphate is a substrate for DNA polymerases and is incorporated into DNA. This incorporation leads to inhibition of the DNA polymerases, causing inhibition of DNA replication and resulting in cell death via apoptosis.[594] Moreover, it was believed that ara-G-5′-triphosphate would accumulate to high intracellular levels and would promote cytotoxic effects similar to those created by high dGTP concentrations. When T cell lymphoma cell lines were exposed to nelarabine, this resulted in FasL-mediated cytotoxicity, whereas myeloid and B cells did not accumulate ara-G-5′-triphosphate, but were stopped in the S phase of the cell cycle.[595] High concentrations of ara-G-5′-triphosphate accumulated in malignant T cells from human bone marrow when tested in a rodent model.[596]

In 2005, nelarabine **13** was approved by the FDA for the treatment of relapse T cell acute lymphocytic leukemia and relapse T cell lymphoblastic lymphoma.[597] Additional clinical trials are being conducted studying pharmacokinetic and pharmacodynamics properties in addition to efficacy in combination with etoposide (topoisomerase inhibitor) and cyclophosphamide (DNA-alkylating agent) treatment in lymphomas (ClinicalTrials.gov identifiers: NCT01094860–recruiting patients and NCT00981799–terminated status for Phase I/II in 2015).

6.2.2. Nelarabine Synthesis.—

An early synthesis of nelarabine 13, shown in Scheme 51,[598] goes through a key enzyme catalyzed trans-glycosylation between 1-β-D-arabinosyl uracil **231** and 2-amino-6-methoxy-*9H*-purine **282**. The reaction uses uridine phosphorylase and purine nucleoside phosphorylase and is completely regio- and stereoselective, furnishing only the N^9, β-anomer nelarabine **13** (Scheme 51).

A more recent process synthesis for nelarabine **13** was reported by Zong *et al.* (Scheme 52).[599] Glycosylation of 2-amino-6-chloropurine **61** with 2,3,5 tri-*O*-benzyl-arabifuranyl **283β** (see Section 6.2.4 for preparation) followed by methoxy displacement of the chlorine at position 6 of the purine base furnished benzylated intermediate **285**.[600,601] Debenzylation by catalytic transfer hydrogenation, using ammonium formate as the *in situ* hydrogen source, afforded nelarabine **13** on a 30-g-scale at 99.8% purity after HPLC.

6.2.3. Fludarabine and Fludarabine-5′-monophosphate Biologies.—

Arabinofuranosyladenine (ara-A; vidarabine) has been around since the 1960s and was

originally studied for its antiviral effects.[602] The agent was also tested for anticancer effects, but showed limited success due to its rapid degradation by adenosine deaminase. In order to avoid adenosine deaminase-mediated deactivation, a fluorine was installed at the 2-position of the purine base of ara-A, producing fludarabine **291**.[603] This agent, however, had limited solubility and difficulties with its formulation. Therefore, the fludarabine-5′-monophosphate prodrug **7** (Fludara or fludarabine phosphate) was subsequently explored in clinical trials, which led to its FDA approval in 1991. Fludarabine-5′-monophosphate **7** is a commonly used drug against chronic lymphoid leukemia and hairy cell leukemia.[538]

The negatively charged fludarabine-5′-monophosphate **7** is dephosphorylated in the plasma by ecto-5′-nucleotidase, and fludarabine **291** is transported into cells by nucleoside transporters hENT1, hENT2, and hCNT3.[429,592] Although fludarabine-5′-monophosphate is adenosine deaminase resistant,[604] the drug is susceptible to glycosidic bond cleavage, which results in the formation of 2-fluoroadenine, a precursor to the toxic 2-fluoroadenosine-5′-triphosphate (see section 6.1.7).[605] After entering the cell, fludarabine is initially phosphorylated by cellular 2′-deoxycytidine kinase, although it is a relatively poor substrate for the enzyme.[606,607] Deoxyguanosine kinase has also been reported to phosphorylate fludarabine.[608] Fludarabine-5′-monophosphate **7** is then phosphorylated to the fludarabine-5′-diphosphate and fludarabine-5′-triphosphate by adenylate kinase and nucleoside diphosphate kinase, respectively, in the cell.

Fludarabine-5′-triphosphate is the active cytotoxic agent. It is a ribonucleotide reductase inhibitor, which leads to a decrease in cellular dNTP concentrations, and therefore negatively influences DNA synthesis. As the concentrations of dNTP diminish during cell division, this results in more fludarabine-5′-triphosphate being incorporated into the host DNA (self-potentiation).[572,592] Furthermore, the incorporated fludarabine-5′-monophosphate prevents further DNA chain elongation by restricting the addition of more dNTPs, leading to effective chain termination.[603] Moreover, fludarabine-5′-triphosphate can inhibit DNA polymerase, DNA primase, and DNA ligase, causing DNA strand breaks, which may lead to apoptosis.[592,609] Fludarabine-5′-triphosphate was also shown to be incorporated into RNA and can inhibit RNA synthesis.[312,610] In addition, fludarabine **291** exposure can also suppress the excision repair enzyme ERCC1 and led to cytotoxic synergy with SJG-136 (DNA minor groove binding agent) in chronic lymphocytic leukemia cells.[611] However, the side effects of fludarabine-5′-monophosphate **7** treatment are becoming an increasing concern, because secondary myelodysplastic syndrome and leukemia have been reported in at least 3% of persons treated with fludarabine-5′-monophosphate **7** (Fludara).[609]

Several different mechanisms have been reported that lead to fludarabine resistance *in vivo*. Changes in the expressions of miRNA-29a, miRNA-181a, and miRNA-221 have been reported.[612] The amplification of *MYC* gene and its transcript level miRNAs can promote fludarabine resistance.[613] Differential overexpression of sulfatase was correlated to fludarabine resistance.[613]

Fludarabine-5′-monophosphate **7**, cyclophosphamide (DNA-alkylating agent), and rituximab (monoclonal antibody against B cell protein CD20) combination therapy is

generally considered the standard treatment for younger fit patients with chronic lymphocytic leukemia.[614] A combination treatment of fludarabine-5′-monophosphate **7**, bendamustine (DNA-alkylating agent), and rituximab for chronic lymphocytic leukemia is being investigated, in addition to the fludarabine-5′-monophosphate **7**, clofarabine **12**, SAHA (vorinostat, histone deacetylase inhibitor), and busulfan (antineoplastic alkylating agent) combination for acute leukemia (ClinicalTrials.gov identifiers: NCT01096992–active status–not recruiting patients yet, and NCT02083250–recruiting patients).

6.2.4. Fludarabine and Fludarabine-5′-Monophosphate Syntheses.—In 1969, Montgomery and Hewson reported the discovery synthesis of fludarabine **291** (Scheme 53).[615] Key chlorosugar **283** was prepared from D-ribose by first cyclization in methanolic sulfuric acid to methyl-β-D-ribofuranoside, followed by 3′,5′-O-benzylation, and subsequent hydrolysis, *p*-nitrobenzoylation, and chlorination of the anomeric position.[616,617] The glycosylation step between chlorosugar **283** and 2,6-dichloropurine **286** gave a column-chromatography-separable mixture of α/β diastereomers, with the desired β-anomer **287** formed in only 11%. Treatment with sodium azide followed by catalytic reduction furnished the diaminopurine intermediate **289**. Due to solubility issues of **289**, a mixed solvent system was employed for the modified Balz–Schiemann reaction, which introduced the N^2-fluorine. Debenzylation concluded the synthesis, producing fludarabine **291**.

Montgomery later reported a gram-scale preparation of fludarabine **291**, which utilized the same basic route, although the glycosylation reaction was performed using a silylated or acylated derivative of 2,6-diaminopurine.[618] Soon thereafter, he reported a gram-scale preparation of fludarabine-5′-monophosphate **7** by treatment of fludarabine **291** with phosphoryl chloride and triethyl phosphate (Scheme 53).[619]

Blumbergs *et al.* developed a kilogram-scale preparation of fludarabine-5′-monophosphate **7**, which followed a similar route used by Montgomery (Scheme 54).[620,621] Yields were improved, allowing the synthesis of the drug in five steps from chlorosugar **283α** (12% yield). Improvements included the use of 2,6-di(methoxyacetamido)purine **293** during the glycosylation, which increased base solubility and greatly reduced the volume of solvent (ethylene dichloride) required for the reaction. Furthermore, chlorosugar **283α**, which was produced almost exclusively as the α-anomer by treating the *p*-nitrophenol intermediate **294** with hydrogen chloride in dichloromethane,[617] was used in the glycosylation step, which allowed for higher yield of the desired β-anomer of **295**.

Another highlight of the synthesis involved the use of hydrochloric acid in the debenzylation step to avoid the partial-defluorination problem associated with Montgomery's catalytic hydrogenation, and to simplify the purification procedure. Furthermore, the phosphorylation step yield was significantly increased when using scrupulously dried nucleoside as the starting material. Recently, another publication effected the debenzylation by transfer hydrogenation, using ammonia formate as an *in situ* hydrogen donor, to produce kilograms of fludarabine **291** at 99.8% purity in 90–95% yield.[622]

Farina *et al.* prepared fludarabine **291** through enzymatic transglycosylation involving *Enterobacter aerogenes*, 2-fluoroade-nine, and 9-β-D-arabinofuranosyl-uracil (Scheme 55).

[623] The crude product was treated with acetic anhydride, forming 2′,3′,5′-tri-*O*-acetyl-9-*β*-D-arabinofuranosyl-2-fluoroadenine, which was hydrolyzed and recrystallized to the pure fludarabine **291**. Subsequent phosphorylation afforded fludarabine-5′-monophosphate **7**.

6.3. Base Modified Purine Nucleosides

6.3.1. 8-Chloro-adenosine and Tocladesine Biologies.—In the 1980s, 8-chloro-adenosine **28** and tocladesine (**50**; 8-chloroadenosine 3′,5′-monophosphate; 8-Cl-cAMP) were reported to have potent anticancer activities.[624–627] Tocladesine 50 is converted extracellularly to 8-chloro-adenosine **28**,[628] which is then transported by hCNT1 and hCNT2 into the cell.[629] Once in the cell, this agent is converted via adenosine kinase to 8-chloro-adenosine-5′-monophosphate, and ultimately to the active metabolite 8-chloro-adenosine-5′-triphosphate by cellular kinases. 8-Chloro-adenosine-5′-triphosphate accumulates to almost millimolar concentration in the cell.[630] 8-Chloroadenosine is a poor substrate for adenosine deaminase, and therefore, deamination of the agent is minimal.[603]

The 8-chloro-adenosine-5′-triphosphate affects cellular energetics by decreasing the levels of intracellular ATP, leading to RNA synthesis inhibition. Furthermore, RNA polymerase II incorporates 8-chloro-adenosine-5′-triphosphate into mRNA, and inhibits polyadenylation of full-length mRNA transcripts.[631,632] A decrease in these short-lived mRNA transcripts can also lead to a reduction in protein levels, which promote growth and survival of tumor cells. 8-Chloro-adenosine **28** exposure has been found to deplete the cyclin E level in breast cancer cell lines,[624] decrease the level of the receptor tyrosine kinase MET in multiple myeloma cell lines,[633] and depress the level of Mcl-1 in chronic lymphocytic leukemia cells.[630] Collectively, all of these mechanisms promote apoptosis.

Although many effects of 8-chloro-adenosine **28** are RNA-directed, 8-chloro-adenosine-5′-triphosphate has also been found to inhibit topoisomerase II and induce DNA double-stranded breaks in human myelocytic leukemia K562 cells.[634] 8-Chloro-adenosine **28** exposure also inhibits the rate of DNA synthesis and decreases dATP pools in mantle cell lymphoma cell lines.[635] Furthermore, decrease in cancer cell proliferation by 8-chloro-adenosine **28** exposure has been linked to AMP-activated protein kinase and p38 mitogen-activated protein kinase inhibitions *in vitro*.[636,637]

Glucose-6-phosphate dehydrogenase has been linked to 8-chloro-adenosine **28** resistance in multiple myeloma and chronic lymphocytic leukemia cell lines.[638] Also, a report indicated that the agent influences glucose consumption, leading to autophagy activation in multiple myeloma cells.[639]

In 2008, the M.D. Anderson Cancer Center sponsored a Phase I clinical trial of a 8-chloro-adenosine **28** therapy to study dosing tolerance in subjects with chronic lymphocytic leukemia (ClinicalTrials.gov identifier: NCT00714103–active status–not recruiting patients yet). In June 2015, the City of Hope Medical Center began a Phase I/II clinical trial evaluating 8-chloro-adenosine **28** in treating patients with relapsed or refractory acute myeloid leukemia (ClinicalTrials.gov identifier: NCT00004902–currently recruiting participants). Tocladesine **50** was evaluated in two clinical trials. In 2000, it was evaluated in a Phase II clinical trial for the treatment of persons with recurrent or refractory multiple

myeloma (ClinicalTrials.gov identifier: NCT00004902–completed status). In 2001 the agent was studied in a Phase I trial for the treatment of progressive metastatic colorectal cancer (ClinicalTrials.gov identifier NCT00021268–unknown status–greater than 2 years since updated). No trial results have been reported.

6.3.2. 8-Chloroadenosine and Tocladesine Syntheses.—Synthesis of 8-chloroadenosine **28** has been achieved in one step from adenosine **240** by using either tetrabutylammonium iodotetrachloride as the chlorinating reagent[640] or *m*-CPBA and acid (HCl or acetyl chloride) in aprotic solvents (Scheme 56).[641,642] Additionally, the synthesis of 8-chloroadenosine **28** has been achieved by direct coupling of 8-chloroadenosine **28** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide. However, the major product of the reaction is the N^3 glycosylated product and not the desired N^9 product.[643] Tocladesine **50** can be prepared by treatment of 8-chloroadenosine **28** with phosphoryl chloride in triethyl phosphate.[642]

6.3.3. Forodesine Hydrochloride Biology.—Forodesine hydrochloride (**36**, (1*S*)-1-(9-deazaguanin-9-yl)-1,4-dideoxy-1–4-imino-D-ribitol; BCX-1777; Immucillin H) was developed as a purine nucleoside phosphorylase inhibitor (see Section 6.2.1). Transition state analysis of the purine nucleoside phosphorylasecatalyzed phosphorolysis of inosine was used as a model system in the design of the agent (Figure 16).[33,34] The protonated nitrogen of the iminoribitol ring of forodesine exhibits a similar charge distribution to the ribosyl moiety oxocarbenium ion of the transition state. Furthermore, the 9-deazapurine of forodesine can be protonated at N7, which provides additional interaction with a residue of the purine nucleoside phosphorylase active site.[644] In addition, instead of a natural glycosidic bond, forodesine has a carbon–carbon bond which is not readily cleaved by purine nucleoside phosphorylase.[630]

Forodesine was shown to be a potent purine nucleoside phosphorylase inhibitor. Complete inhibition of the homotrimeric complex of the enzyme at a concentration as low as 72 pM has been reported, with the agent exhibiting a high equilibrium binding constant for and a very slow dissociation rate from the enzyme.[337,630] Inhibition occurs at a 1:1 molar ratio of inhibitor to purine nucleoside phosphorylase homotrimeric enzyme complex, with just one of the three substrate sites needing to be occupied by the agent to block enzyme activity.[34]

Forodesine hydrochloride **36** is administered by intravenous infusion. Like pentostatin **6** (see Section 6.1.5), the agent is active without metabolism, meaning it does not require phosphorylation. It blocks purine nucleoside phosphorylase, causing a high plasma concentration of deoxyguanosine, and it is not incorporated into DNA.[645] Deoxyguanosine is further converted to dGMP, dGDP, and dGTP by cellular kinases. High cellular levels of dGDP inhibit the ribonucleotide reductase complex and lead to imbalances of the nucleotide pool concentrations, resulting in p53-based apoptosis.[591] Because of this, forodesine works best in malignant cells containing high deoxyguanosine activity.[646]

Forodesine hydrochloride **36** has undergone clinical trials for the treatment of T-cell malignancies, including T-cell acute lymphocytic leukemia.[35,647] Additionally, the agent is being examined for treatment of chronic lymphocytic leukemia B cells, which may require

an additional nucleoside treatment for effectiveness.[35,648] Forodesine hydrochloride **36** did not inhibit the growth of colon cancer cell lines or normal human nonstimulated T cells, indicating some specificity of the drug toward normal versus malignant tissues.[33] In 2007 a phase IIb clinical trial of forodesine hydrochloride **36** in patients with leukemia/lymphoma was terminated due to manufacturing issues (ClinicalTrials.gov identifier: NCT00419081–terminated), indicating a need to improve the synthesis process in order to produce sufficient quantities of the agent for further clinical trials. In January 2013, Mundipharma began a Phase I/II clinical trial evaluating forodesine hydrochloride **36** in recurrent/refractory peripheral T cell lymphoma subjects in Japan (ClinicalTrials.gov identifier NCT01776411–active status–not recruiting patients).

6.3.4. Forodesine Synthesis.—The discovery synthesis of forodesine involved formation of the key iminoribitol intermediate **306** (see Scheme 57) followed by a 16-step linear construction of the base, producing only milligram quantities of the compound.[649] Therefore, a different route was designed, which could produce the required kilogram quantities of the agent needed for drug development. This new synthesis involved the coupling of imine **307** and brominated 9-deazahypoxanthine **313** (Scheme 57). One metric ton of **306** was prepared from D-ribose **213** by first bromine oxidation, protection, lactone epimerization,[650] and Fleet chemistry (five steps).[651] The same quantity of 9-deazahypoxanthine **310** was produced by condensing (ethoxymethylene)cyanoacetate **308** with diethyl aminomalonate followed by cyclization and acid-mediated decarboxylation.[652] Three more steps were required to produce the bromo hypoxanthine derivative **312**: chlorination, protection and methoxy displacement, and finally bromination. At this stage, the condensation of lithiated heterocycle **313** (generated *in-situ* from **312**) with imine **307** afforded the β-anomer of **314** exclusively, due to steric hindrance of the α-face by the isopropylidene protecting group. In order to avoid a hard-to-remove tarry byproduct, **314** was not converted directly to the product, but instead passed through a process of protection and sequential deprotection, eventually furnishing pure forodesine hydrochloride **36**.[653]

In order to avoid some of the cumbersome manipulations from the above-mentioned route, a different approach to forodesine hydrochloride **36** explored a novel route to prepare the aza-sugar core (Scheme 58).[654] Lactam **317**, which is prepared by esterification and reduction of L-pyroglutamic acid, was transformed into unsaturated bicycle **318** by treatment with benzaldehyde and catalytic acid followed by treatment with Meyer's reagent (methyl phenolsulfonate).[655] Osmium tetr-oxide-catalyzed dihydroxylation of compound **318** gave the desired diol stereochemistry, and subsequent acetal protection furnished the intermediate **319**. The coupling of lithiated 9-deazahypoxanthine to lactam **319** led to the ring-opened product 320. Treatment of the intermediate **320** with BBr_3 gave the cyclized product **321**, which was then reduced with $BH_3{\cdot}Me_2S$ and deprotected under acidic condition to give forodesine hydrochloride **36** as a 4:1 β:α mixture.

In 2016, a 50-g-scale synthesis of forodesine hydrochloride **36** was reported.[656] Differences from the original preparation (Scheme 57) include coupling of imine **307** with lithiated heterocycle **313** under milder condition, and the use of concentrated HCl instead of hydrogen for BOM removal in **315**.

7. ENZYME INHIBITOR AND CELL SURFACE RECEPTOR ANTAGONIST NUCLEOSIDE ANALOGS

7.1. Triciribine and Triciribine-5′-Monophosphate Biologies

In 1971, Schram and Townsend synthesized triciribine **30**, a tricyclic derivative of a purine nucleoside as a potential anticancer drug,[657] which has also been studied as an antiretroviral agent against HIV-1 and HIV-2.[658] Triciribine **30**, however, is poorly soluble, and this suboptimal property led to the development of the highly soluble prodrug triciribine-5′-monophosphate **33**.

Interestingly, triciribine **30** does not work by blocking host DNA or viral polymerases. It inhibits the phosphorylation of Akt1, Akt2, and Akt3,[659] and appears to influence the PI3K/Akt/mTOR cell survival pathway. The PI3K/Akt/mTOR pathway regulates a variety of cellular pathways, including cell proliferation and survival.[660,661] Akt is dysregulated in a variety of malignancies.[662] Initially, triciribine **30** was reported to inhibit *de novo* purine nucleotide and DNA synthesis in cell lines.[425,663] However, this would not explain the antiretroviral activity in HIV-1 and HIV-2 infected cell lines, because the reverse transcription step for proviral DNA synthesis of the viral life cycle was completed. Moreover, viral replication was no longer influenced by cellular dNTP concentrations or nucleoside reverse transcriptase inhibitors, which are chain terminators. Based on current data, the antiviral and anticancer activities of triciribine metabolite appear to be associated with Akt inhibition (Figure 17).[664] In addition to breast cancer, triciribine **30** may be effective when used as a combination therapy in bladder cancer patients prescreened for alterations in the Akt pathway.[665,666]

Triciribine-5′-monophosphate **33** is currently being evaluated in solid tumors, such as breast cancer.[662,667,668] A Phase I/II study is currently underway in patients with metastatic and locally advanced breast cancer that uses triciribine-5′-monophosphate monohydrate with paclitaxel (antimitotic taxane), which is followed by dose-dense doxorubicin (DNA intercalating agent) and the DNA alkylating-agent cyclophosphamide (ClinicalTrials.gov identifier: NCT01697293–recruiting patients). The H. Lee Moffitt Cancer Center and Research Institute recently began a Phase I/II clinical trial for the treatment of ovarian cancer with a combination of triciribine **30** and the DNA alkylating agent carboplatin (ClinicalTrials.gov identifier: NCT01690468–recruiting patients).

7.2. Triciribine and Triciribine-5′-Monophosphate Syntheses

Triciribine **30**[657] and triciribine-5′-monophosphate **33**[669] were originally prepared from the intermediate **322** by Townsend and Schram (Scheme 59). Aryl chloride displacement of **322** followed by tricycle formation led to triciribine **30**, and subsequent phosphorylation produced triciribine-5′-monophosphate **33**.

More recently Shen *et al.* developed a new approach to prepare triciribine **30** from commercially available intermediates in only 4 steps (Scheme 60).[659] Thus, reaction of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose with 6-chloro-7-iodo-7-deazapurine **324** under classical glycosylation conditions gave intermediate **325**, which was then selectively

cyanated by treatment with tributyltin cyanide in the presence of catalytic Pd(0) to give compound **326**. Aryl chloride displacement with methyl hydrazine followed by deprotection and ring closure with sodium methoxide in refluxing methanol afforded triciribine **30** in a 37% overall yield.

7.3. MLN4924 Biology

Whereas the function of most FDA-approved nucleoside analogs involves their incorporation into DNA or RNA after phosphorylation, MLN4924 (**44**; Pevonedistat; TAK-924) suppresses tumor development mainly through blocking the proteasomal degradation pathway. MLN4924 **44** is a structural analog of adenosine-5′-monophosphate. MLN4924 **44** tightly binds to NEDD8-activating enzyme (NAE), which is an ubiquitin-like protein involved in the ubiquitin-proteasome degradation system (Figure 18).[37] An initial high throughput screening of adenosine-5′-monophosphate analogs identified N^6-benzyl adenosine as an inhibitor of NAE, and further structural elaboration lead to MLN4924 **44**.[37]

Functionally, MLN4924 **44** was shown to selectively inhibit NAE, and did not inhibit transfer RNA synthetases or other ATP-consuming enzymes.[37] NAE is the enzyme that initiates the important pathway to maintain protein homeostasis, by forming a tight binding adduct with NEDD8.[670,671] This is a multistep enzymatic process in which Cullin-RING E3 ligases (CRLs) add the NEDD8 polypeptide onto target proteins.[672] MLN4924 44 was shown to block polypeptide addition to target proteins. Furthermore, in cancer cells MLN4924 **44** blocks the important homeostatic balance between protein synthesis and degradation, which is essential for cell survival, cell division, and regulating both innate and adaptive immune responses.[673–675] As a result, CRLs are deactivated and consequently multiple known substrates of CRLs accumulate inside the target cell (e.g., DEPTOR and HIF-1α), leading to cell cycle arrest and apoptosis of the cancer cells (Figure 17).[37,670,676]

Cells in the S phase are more sensitive to exposure to MLN4924 **44**, a unique feature of the agent.[37] A recent study using the A375 human melanoma-derived cell line indicated that MLN4924 **44** induced cell cycle arrest might largely be triggered by a p21-mediated intra-S phase checkpoint.[676] Parallel to this study, an *in vivo* study with diffuse large B cell lymphoma models indicated that MLN4924 **44** might also suppress tumor growth via its inhibition of the NF-κB pathway.[677] Studies in mice demonstrated good toleration for MLN4924 **44**, as well as potent antiproliferative activity against human tumor cell xenografts derived from human colorectal and lung carcinomas.[37] However, HLC116 cells, a human colorectal carcinoma cell line, exposed to MLN4924 **44** developed a point mutation at A171T in the UBS3 subunit of NAE, which greatly decreased sensitivity to the drug.[678] Another study attempted to address a similar issue and found that both HIF1-REDD1-TSC1-mTORC1 and DEPTOR might play important roles in MLN4924-induced autophagy, which is a survival strategy in cancer cells in response to chemotherapy-mediated DNA damage.[679] This study suggested that cells exposed to an autophagy inhibitor may have a marked improvement in MLN4924 **44** response.[679]

MLN4924 hydrochloride affords better solubility than the parent compound, MLN4924 **44**. However, clinical studies indicate that MLN4924 **44** pevonedistat is being administered.

Currently the safety and efficacy of MLN4924 **44** therapy are being assessed in several Phase I clinical studies involving solid tumors, melanoma, acute myeloid leukemia, refractory or relapsed/refractory multiply myeloma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, and cisplatin resistant ovarian cancers.[680,681] Current studies also include investigating fluconazole and itraconazole (antifungals often administered with cancer chemotherapeutics) effects on MLN4924 **44** safety and efficacy in solid tumors. Phase I trials using MLN4924 44 in combination therapy with docetaxel (antimitotic taxane), gemcitabine hydrochloride **9**, or a combination of carboplatin (DNA alkylating agent) and paclitaxel (antimitotic taxane) in solid tumors are underway (ClinicalTrials.gov identifiers: NCT02122770–recruiting patients, and NCT01862328–active status–not recruiting patients).

7.4. MLN4924 Synthesis

A preparation of MLN4924 **44** is summarized in Scheme 61.[682,683] Starting from diene **217**, ring closing metathesis led to the tertiary β-alcohol product **328**, which had the required stereochemistry that allowed oxidative rearrangement to cyclopentenone **329**.[682] Stereoselective catalytic hydrogenation, due to hydrogen incorporation from the convex face of the intermediate, followed by Luche reduction with $CeCl_3$, provided 330 as a single diastereomer. Regioselective cleavage of the acetonide group of **330** was followed by treatment with $SOCl_2$ and $RuCl_3/NaIO_4$ to furnish the cyclic sulfate **331**. Subsequent condensation with N^6-indanyl-7-deazaadenine in the presence of NaH afforded the nucleoside analogue **332**. Standard radical-mediated deoxygenation and O-TBDPS deprotection provided the intermediate **333**, which was then reacted with chlorosulfonamide and trifluoroacetic acid to furnish MLN4924 **44**.[683]

In 2007, Langston *et al.* published an alternative synthesis of MLN4924 **44** (Scheme 62).[684] The starting diol **334**,[685] formed in a four step sequence from cyclopentadiene and glycolic acid involving a hetero-Diels–Alder and oxidation/reduction reactions, was oxidized and treated with *p*-anisaldehyde to produce the cyclic epoxide **335**. Condensation with pyrrolopyrimidine **337** was followed by deoxygenation, deprotection, selective 5′-OH silylation, and 3′-OH acetylation. Finally, desilylation, sulfamate ester formation, and deacetylation led to MLN4924 **44**.

In 2015, a 100-g-scale process synthesis of MLN4924 hydrochloride **350** was reported (Scheme 63).[686] The key intermediate amino-diol **346** can be prepared from commercially available carboxylic acid **342** in four steps: bromolactonization, reductive ring opening, Boc deprotection, and hydrogenation. Subsequent coupling of **346** with acetaldehyde and (*S*)-(+)-1-aminoindane afforded diol **348**. Selective sulfamoylation of diol 348 was carried out using a Burgess-type reagent **349**, which was produced on a 100-kg-scale by reaction of chlorosulfonyl isocyanate with *tert*-butanol and subsequent treatment with DABCO. MLN4924 **44** was then separated from the disulfamoylated byproduct by stepwise hydrolysis with increasing concentration of HCl, requiring no chromatography. Finally, salt formation and crystallization from ethanol furnished MLN4924 hydrochloride **350**.

7.5. EPZ-004777 and EPZ-5676 Biologies

Histone methyl transferases (HMT) use *S*-adenosyl-L-methionine as a cofactor to methylate histone amino acid side chains, leading to post-translational gene modification. An important cellular HMT is DOT1-like histone H3K79 methyltransferase (DOT1L histone methyltransferase). It targets residue Lys79 of histone 3 (H3K79) for methylation, as illustrated in Figure 19.[687] The DOT1L histone methyltransferase is unique among HMTs for several reasons, making it an attractive therapeutic target.[688] The enzyme has recently been implicated as a therapeutic target for acute leukemias bearing rearrangements in the mixed lineage leukemia (MLL) gene.[689,690]

A translocation in the MLL gene occurs in 5–10% of acute leukemias and is especially common in childhood acute leukemias.[691,692] These cancers are particularly aggressive and have a relatively poor prognosis with a five-year survival of <40%.[693] In MLL-rearranged leukemia cells, DOT1L histone methyltransferase has been shown to physically bind to a complex of the rearranged MLL protein fused with sequences from genes such as AF4, AF9, AF10, and ENL. This interaction recruits DOT1L, leads to hypermethylation of H3K79 and aberrant expression of leukemogenic genes such as Hoxa9, Hoxa7, and Meis1.[559] Furthermore, preclinical models have shown that MLL-rearranged leukemias depend on abnormal DOT1L histone methyltransferase methylation of H3K79.[39]

Epizyme Inc. recently developed a potent and selective inhibitor of DOT1L histone methyltransferase. The compound, EPZ-004777 **351** (Figure 19) is a 7-deaza-5′-amino-alkylurea substituted analogue of adenosine. The agent inhibits *S*-adenosyl methionine in the enzyme-binding pocket. EPZ-004777 binding leads to a conformational change in DOT1L histone methyltransferase, which opens a hydrophobic pocket past the amino acid site and produces additional interaction surfaces.[694] Consequently, **351** selectively binds to DOT1L histone methyltransferase with high affinity. Furthermore, the agent was shown to selectively inhibit H3K79 methylation *in vitro* and inhibit the proliferation of MLL-rearranged cells. It also blocked MLL fusion target gene expression and led to prolonged survival in a mouse xenograft model of MLL leukemia.[695] However, the poor pharmacokinetic properties of EPZ-004777 **351** led to weak *in vivo* activity and precluded clinical development.

To overcome the suboptimal pharmacokinetic profile of **351**, Epizyme synthesized additional analogs. In 2013, the company reported promising preclinical data for EPZ-5676 **43**, which exhibited improved drug-like properties and potency.[696] Structural modifications were made to the 5′-side chain in order to decrease the number of hydrogen bonds and conformational freedom. EPZ-5676 **43** also occupies the *S*-adenosyl methionine binding pocket of DOT1L histone methyltransferase, and it was found to be a potent and selective inhibitor of the enzyme with a K_i of <0.08 nM, having >37,000-fold binding selectivity against 15 other human methyltransferases.[696]

EPZ-5676 **43** demonstrated selective inhibition of MLL-rearranged versus non-MLL-rearranged leukemia cells. In particular, an IC_{50} of 3.5 nM for the agent was demonstrated in the MV4–11 cell line following 14 days of exposure, with antiproliferative activity being most evident after 7 days.[696] This delayed onset could be explained by a progressive series mechanism involving a decrease of H3K79 methylation, lowered MLL-fusion target gene

expression, and inhibition of the expression of leukemogenic genes.[696] EPZ-5676 **43** produces a concentration-dependent decrease of both cellular methylated H3K79 levels as well as mRNA transcript levels of the MLL-fusion target genes HOXA9 and MEIS1. Continuous intravenous infusion of EPZ-5676 **43** (70 mg/kg) for 21 days produced a complete regression of tumors in a rat model of MLL-rearranged leukemia. Little or no tumor regrowth was detected 30 days after treatment cessation. Evaluation of subcutaneous administration of EPZ-5676 **43** has also been reported.[697]

However, EPZ-5676 **43** still exhibits suboptimal pharmacokinetics with a short plasma half-life and with fecal excretion being the main route of excretion.[698,699] A carbocyclic analogue of EPZ-5676 **43** was synthesized **352** in an attempt to produce increased metabolic stability, and this compound (**352**; see Figure 19) did indeed show increased stability in human plasma and liver microsomes while maintaining a selective DOT1L histone methyltransferase K_i of 1.1 nM.[559]

In September 2012, Epizyme began a phase I clinical trial of EPZ-5676 **43** for subjects with MLL-rearranged leukemia. Currently there are two ongoing Phase I clinical studies examining the agent for dose escalation and the first-in-human study for malignancies with rearrangement of the MLL gene (ClinicalTrials.gov identifiers: NCT02141828–competed in June 2016–no results posted, and NCT01684150–competed in February 2016–no results posted).

7.6. EPZ-5676 Synthesis

The synthesis of EPZ-5676 **43** is shown in Scheme 64.[700,701] Amine **353** is prepared from adenosine in four steps: 2′,3′-*O*-isopropylidine protection, installation of a 5′-*O* leaving group, 5′-azide displacement, and azide reduction. Reductive amination involving 5′-amino **353** and methyl 3-oxocyclobuganecarboxylate produced a *cis*/*trans* mixture (separated using chiral HPLC) furnishing intermediate **354**. Subsequent N-alkylation, combined reduction, and Wittig olefination, alkene reduction, and saponification furnished intermediate **357**. A two-step process was used to form the benzoimidazole ring of **359**, without isolation of the intermediate amide **358**. Finally, acid-mediated deprotection completed the synthesis of EPZ-5676 **43**.

In 2014, a 700-g-scale process of EPZ-5676 trihydrate was reported (Scheme 65).[702] Cyclobutanone **366** can be prepared from **360** on kilogram-scale in a six-step sequence: protection, [2+2] cyloaddition, dechlorination, deprotection, acyl substitution, and iron-catalyzed reduction followed by condensation. **353** was subsequently converted to isopropyl amine **367**, which was further converted to **368** as a mixture of diastereomers. Deprotection, followed by two crystallizations from MeCN/H_2O, afforded pure EPZ-5676 **43**.

7.7. CF102 Biology

In 2007, preclinical studies developed by Can-Fite Biopharma showed that CF102 (2-chloro-N6-(3-iodobenzyl)-adenosine-5′-N-methyl-uronamide) **42** had potent anticancer activity in hepatocellular carcinoma,[703] activity against hepatitis B virus, and anti-inflammatory activity (demonstrated in pre-clinical animal models of liver inflammation).[704]

CF102 **42** is as antagonist for the A3 adenosine receptor. Hepatocellular carcinoma cells treated with CF102 had Wnt and NF-κB signaling pathways deregulation, which promoted apoptosis.[703] Preclinical studies in rats with N1S1 hepatocellular carcinoma tumors, which highly express the A3 adenosine receptor, showed that CF102 **42** encouraged tumor apoptosis.[703] Furthermore, A3 adenosine receptor expression appears to be inflammation induced.[705,706] The A3 adenosine receptor is also highly expressed on PBMCs in hepatocellular carcinoma patients and patients with rheumatoid arthritis, psoriasis, dry eye syndrome, and glaucoma, in addition to hepatocellular carcinoma and hepatitis.[707,708]

Currently, a Phase I/II study for dose escalation using CF102 **42** was reported in patients with unresectable hepatocellular carcinoma.[709] Overall study results showed that CF102 **42** was well tolerated and had favorable pharmacokinetic characteristics. A Phase II study examining the safety and efficacy of CF102 **42** is being planned (ClinicalTrials.gov identifier: NCT02128958–recruiting patients).

7.8. CF102 Synthesis

Kim *et al.* reported the discovery synthesis of CF102 **42** in 1994 (Scheme 66).[710] Protecting group manipulation of 1-*O*-methyl-β-D-riboside **370** produced the intermediate **371**. Subsequent oxidation and esterification of the 5′-OH followed by methyl amide introduction, 2′,3′-*O*-benzoylation, and 1′-acetylation produced intermediate **374**. Condensation of intermediate **374** with silylated N^6-(3-iodobenzyl)-2-chloroadenine **376**, made by reacting 2,6-dichloropurine **375** with 3-iodobenzylamine, followed by debenzoylation produced CF102 **42** in milligram quantities in an overall yield of 1.7%.

Hou *et al.* recently reported an alternative preparation of CF102 **42** starting from the commercially available 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose **136β**, which is easily made from D-ribose **213** (Scheme 67).[711] The synthesis performed the glycosylation before constructing the 5′-methyl amide and furnished the target compound on a gram-scale in 27% overall yield.

8. ANTIVIRAL NUCLEOSIDE ANALOGS BEING EXPLORED FOR CANCER TREATMENT

8.1. Valacyclovir Hydrochloride and Valganciclovir Hydrochloride Biologies

Valacyclovir hydrochloride **17** and valganciclovir hydrochloride **35** are L-valyl ester prodrugs of acyclovir and ganciclovir, respectively. They were FDA-approved in 2009 and 2010, for the treatments of cytomegalovirus and herpes infections, respectively. After oral administration, the parent drug is released *in vivo*.[712] Importantly the monophosphorylated agents occur by the viral-specific thymidine kinases, making them less toxic to uninfected cells. The monophosphorylated agents are further converted to their active triphosphate forms to block viral DNA synthesis.[713] Since the viral thymidine kinase does not exist in human cells, gene therapy involving an adenoviral vector (disabled virus) designed to express the herpes simplex virus thymidine kinase gene in combination with valacyclovir hydrochloride **17** or valganciclovir hydrochloride **35** may lead to targeted cancer treatment

(see Section 5.1.1.6).[714] Furthermore, bystander killing of cancer cells has been proposed to be one mechanism of action for ganciclovir and acyclovir.[715,716]

Several clinical studies are evaluating valacyclovir hydrochloride **17**. A Phase I trial is underway using combination therapy with adenovirus expressing viral-specific thymidine kinase (AD-VTK) and radiation for pediatric brain tumors (ClinicalTrials.gov identifier: NCT00634231–active status–not recruiting patients). Another Phase I trial is using intrapleural AD-VTK therapy with valacyclovir hydrochloride **17** in patients with malignant pleural effusion (ClinicalTrials.gov identifier: NCT01997190–active status–not recruiting patients). A Phase I/II trial of the **17** in combination with HSV-TK and brachytherapy for recurrent prostate cancer is ongoing (ClinicalTrials.gov identifier: NCT01913106–recruiting patients), as well as a Phase III trial with AD-VTK and valacyclovir hydrochloride **17** combination therapy for localized prostate cancer (ClinicalTrials.gov identifier: NCT01436968–recruiting patients). Currently, valganciclovir hydrochloride **35** is being used in a Phase IV trial for chronic lymphocytic leukemia, but the action of the agent is to inhibit cytomegalovirus (CMV) replication in an attempt to stop chronic lymphocytic leukemia propagation (ClinicalTrials.gov identifier: NCT01552369–recruiting patients). The agent has also been examined as an add-on treatment for controlling CMV in patients being treated for the malignant brain tumor glioblastoma. Overall, results showed that the addition of valacyclovir hydrochloride **17** to the standard treatment improved patient survival,[717] which has encouraged a discourse on the possibility that CMV infection influences the development of brain tumors.[718,719]

8.2. Valacyclovir and Valganciclovir Syntheses

Valganciclovir hydrochloride **35** has been prepared almost exclusively from its parent compound ganciclovir **387**, following the chemistry described in Scheme 68.[720] Epichlorohydrin **383** was converted to dibenzyl-protected **384**, which was subsequently subjected to chloromethylation by treatment with paraformaldehyde and HCl. Exposure of the resulting intermediate to potassium acetate in acetone afforded **385**. Acid catalyzed condensation of **385** with diacetylguanine furnished a mixture of N^7:N^9 regioisomers, and the desired N^9 isomer was crystallized from toluene. Debenzylation followed by deacetylation completed the synthesis of ganciclovir **387**, on a 250-g-scale.

In 2004, a 100-g-scale process synthesis of triacetyl-protected ganciclovir **389** was reported that significantly increased the yield of the base condensation step.[721] The reaction coupled diacetylguanine **60** and protected glycerol (**388** in the presence of TsOH (Scheme 69). This step occurs under thermodynamic conditions, with the desired N^9 isomer being the thermodynamic product. Recycling of the undesired N^7 isomer was possible, allowing for increased yields of the desired N^9 isomer (overall 70%). Compound **389** can be deprotected to furnish ganciclovir **387**.

In 1998, Arzeno *et al.* reported a preparation of valganciclovir hydrochloride **35** from ganciclovir **387** (Scheme 70).[722] Treatment of **387** with trityl chloride and DMAP in DMF followed by coupling with Boc-L-valine furnished the intermediate **390**. Subsequent treatment with a TFA/TFE mixture, in order to remove the trityl groups, and hydrogenolysis

of the resulting Boc-protected intermediate with palladium on carbon in a methanol/HCl solution afforded kilograms of valganciclovir hydrochloride **35** with an HPLC purity of 98.4%.

Khanduri *et al.* reported a preparation of valganciclovir hydrochloride **35** directly from ganciclovir **387** (Scheme 71).[723] Treatment of ganciclovir **387** with Cbz-L-valine, DCC, and DMAP in DMSO led to a mixture of ganciclovir **387**, mono-L-valyl ester **391**, and bis-L-valyl ester **392**. Addition of water and ethyl acetate caused **387** and **391** to precipitate, while **392** was removed in the organic layer. Ganciclovir **387** was removed from the **387**/**391** mixture by treatment with TFA followed by extraction with dichloromethane. Mono-L-valyl ester **391** was then separated from the organic layer by recrystallization. Subsequent hydrogenolysis of **391** in acidic solution produced valganciclovir hydrochloride **35**, which was purified by recrystallization to give the target compound on a 60-g-scale in greater than 99% HPLC purity. Both ganciclovir **387** and bis-L-valyl ester **392** were recovered and recycled.

The discovery synthesis of valacyclovir hydrochloride **17** was reported in 1989 (Scheme 72). Acyclovir **393**, which is prepared in ways similar to those of ganciclovir **387**, was condensed directly with Cbz-L-valine.[724] Palladium-catalyzed deprotection of the intermediate followed by treatment with aqueous HCl produced **17**. An alternative preparation used Boc-L-valine instead of Cbz-L-valine, furnishing the compound on a 50-g-scale.[725]

An alternative route utilized azido-intermediate **395** to avoid protection–deprotection steps that increase risks of racemization (Scheme 72).[726] Sodium azide **394** was treated with sulfuryl chloride and imidazole followed by L-valine and copper sulfate, producing the intermediate **395**. Condensation of **395** with acyclovir followed by reduction with Rainey nickel furnished valacyclovir hydrochloride **17** in three steps.

8.3. Ribavirin Biology

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) **40** was first discovered as an effective antiviral agent against influenza A and B viruses in the 1970s using cell lines and and *in vivo* models.[727,728] It was FDA-approved 15 years ago for the combination treatment of hepatitis C virus.[729] The agent's antiviral activity is primarily attributed to ribavirin-5′-triphosphate, which can inhibit RNA polymerase.[730] Ribavirin-5′-monophosphate is also a potent inhibitor of inosine monophosphate dehydrogenase (IMPDH),[731] an enzyme involved in the rate-limiting step of purine *de novo* synthesis. It is an important factor in maintaining guanine nucleoside pools, and a virus is highly dependent on this process. IMPDH Type I is ubiquitously expressed in normal cells, whereas a high level of IMPDH Type II is also associated with many hematological cancers.[732] In addition, ribavirin **40** has a negative impact on eukaryotic translation initiation factor eIF4E activity, which is dysfunctional in 30% of cancers.[733–735] High levels of eIF4E have been correlated with clinical progression, increased angiogenesis, and poor prognosis in breast cancer,[736,737] and ribavirin **40** was shown to suppress growth in breast cell lines.[738]

Ribavirin **40** is currently being evaluated in a Phase I/II study for malignant solid tumors (ClinicalTrials.gov identifier: NCT01309490–unknown status–no updates for over 2 years)

and in a Phase I trial using combination therapy for head and neck cancer and squamous cell cancer (ClinicalTrials.gov identifier: NCT01721525–active status–not recruiting patients). Ribavirin **40** is also being examined in combination with an inhibitor of hedgehog proteins, which regulate cell growth, differentiation, and survival, with or without azacytidine **11** in acute myelogenous leukemia patients (ClinicalTrials.gov identifier: NCT02073838–recruiting patients). The pharmacodynamic effects of ribavirin **40** therapy are being investigated for patients with tonsil and/or base of tongue squamous cell carcinoma (ClinicalTrials.gov identifier: NCT01268579–active status–not recruiting patients). Ribavirin **40** is being evaluated in combination with decitabine **14** in high-risk acute myelogenous leukemia patients (ClinicalTrials.gov identifier: NCT02109744–recruiting patients). Furthermore, a Phase I/II study of ribavirin **40** given as monotherapy in solid tumor cancer patients is ongoing (ClinicalTrials.gov identifier: NCT01309490–unknown status–no updates for over 2 years).

8.4. Ribavirin Synthesis

Witkowski *et al.* reported the discovery synthesis of ribavirin **40** in 1972 (Scheme 73).[727,739] The compound was prepared by acid-catalyzed fusion of acylated sugar **136*β*** with 1,2,4-triazole-3-carboxylate **397**, which was prepared by reacting aminoguanidine bicarbonate with oxalic acid and subsequent esterification of the resulting 1,2,4-triazole-3-carboxylic acid. Treatment of methyl ester **398** with methanolic ammonia effected concomitant deprotection and amide formation, completing the synthesis.

In 2007, Banfi *et al.* reported a large-scale synthesis, which allowed for the preparation of ribavirin **40** on a 400-g-scale (Scheme 74).[740] Improvements over other preparations that allowed for scale up include the following: (1) a glycosylation involving direct coupling of triazole **397**, thereby avoiding the need for silylating reagents, (2) intermediates pure enough to be used directly in the following stages without difficult purifications, and (3) relatively mild reaction conditions.

Another recent preparation makes use of cheap nucleosides as starting materials.[741] Inosine, adenosine, and guanosine were cleaved to the tetra-acylated sugar **136*β*** and the corresponding acetylated purine base by treatment with acetic acid and TFA as catalyst. The glycosylation between **136*β*** and triazole **397** was catalyzed by TfOH, and after deprotection, ribavirin **40** was produced on a 30-g-scale.

Ribavirin **40** can also be synthesized enzymatically. It has been formed on gram-scale. A transglycosylation involving purine nucleoside phosphorylase, 7-methylguanosine and 1,2,4-triazole-3-carboxamide has been used to produce grams of the agent.[742] Furthermore, purine nucleoside phosphorylase has been used in conjunction with phosphopentomutase to couple ribose-5′-monophosphate **399** with the triazole **400**, forming ribavirin **40** (Scheme 75).[743]

8.5. Acadesine Biology

Acadesine (AICA-Ribonucleotide (AICAR); 5-amino-1-*β*-D-ribofuranosyl-imidazole-4-carboxamide, **41**) was previously under investigation for preventing ischemia-reperfusion injury in persons undergoing coronary artery bypass graft surgery.[744] The agent was found

to exert its cardio-protective effect via regulation of adenosine monophosphate activated protein kinase (AMPK) activity.[745] Acadesine-5′-monophosphate is a highly potent activator of AMPK, and it can bind to the allosteric site of the enzyme as an AMP mimic. In a recent study, this activation was found to exert anti-proliferative and apoptotic effects on acute lymphocytic leukemia cells by regulating different signaling pathways (Figure 17).[746,747] For this reason, cell-specific cytotoxicity of acadesine **41** might ascertain its future usage in anti-cancer therapy.

Preclinical trials with using acadesine **41** and rituximab showed synergistic anticancer activity in tissue culture and animal models.[748] Acadesine **41** therapy is currently being evaluated in a Phase I/II study for myelodysplastic syndromes and acute myelogenous leukemia (ClinicalTrials.gov identifier: NCT01813838–trial status suspended in 2015) and a Phase I/II open label dose escalation study for safety and tolerability in B cell chronic lymphocytic leukemia patients (ClinicalTrials.gov identifier: NCT00559624–completed in 2010). Clinical trial results indicated that acadesine **41** had a manageable toxicity profile and might be a valuable agent for the treatment of relapsed/refractory CLL patients.[749]

8.6. Acadesine Synthesis

Acadesine **41** is now produced industrially from the fermentation broth of glucose,[750–752] but Baddiley *et al.* reported the first synthesis of the agent in 1959 (Scheme 76).[753] The key intermediate **403** was synthesized from D-ribose **213** in a five-step sequence involving cyclic acetal formation and benzoylation, followed by anomeric bromination, acetylation, and chlorination.[754] In the presence of silver chloride, protected chlorosugar **403** was condensed with amide **405**, formed from 4-nitro-5-styrylglyoxaline **404** in three steps via a carboxylic acid and a methylester intermediate.[755] The resulting 1:1 mixture of regioisomers **406a** and **406b** was separated using descending paper chromatography. Hydrogenation of **406b** using Adams' catalyst afforded acadesine **41**.

Kohyama *et al.* reported a gram-scale synthesis of acadesine **41**, which features a hydrolysis of N-MEM protected inosine **408** by treatment with aqueous ammonia in methanol (Scheme 77).[756] The resulting deprotected triol was subjected to aqueous alkaline hydrolysis, furnishing the ring-opened product acadesine **41** in four steps from inosine.

9. CONCLUSIONS

Nucleoside analogs are used to treat a high percentage of clinical cancers, usually in combination with other agents. They interact with various biological targets, such as cellular kinases, ribonucleotide reductase, and cellular polymerases in order to produce cytotoxic effects. Overall, a majority of cancers treated with nucleoside analogs are haematological. Newer nucleoside analogs, however, such as RX-3117 **29**, triciribine-5′-monphosphate **33**, NUC-1031 **32**, and NUC-3373 **37** are exhibiting promising activity against solid tumors in patients, but further testing is needed. With the exceptions of 6-MP **1** and cytarabine **4**, nucleoside analogs usually do not produce cures as standalone agents.

As is the case with most classes of compounds explored in the clinic, attrition rates for nucleoside analogs remains high. Continuing efforts are directed toward improving the

selectivity and targeting of anticancer nucleoside analog therapies. Nucleoside analogs interact with various biological targets, such as cell membrane transporters for cellular uptake, kinases to promote their phosphorylation, as well as DNA and RNA polymerases in order to produce their cytotoxic effects. Therefore, the tendency to increase chemical modifications to the parent nucleoside analog in hopes to design a better derivative should be considered with caution. Major efforts to improve analog biological efficacy currently focus on developing new prodrug strategies and using antimetabolites in combination treatments.

Prodrug strategies have emerged as promising approaches in the search for new anti-cancer nucleoside analogs. Next generation nucleoside analogs have been developed in an attempt to overcome cellular uptake deficiencies (e.g., elacytarabine **51**, SGI-110 **22**, and sapacitabine **25**) as well as overcome sensitivity to cytidine deamination and adenosine deamination (e.g., FdCyd 38, MB 07133 **34**, fludarabine-5′-monophosphate **7**, clofarabine **12**). Compared to the parent compound, nucleoside analog prodrug candidates generally offer enhanced efficacy and less toxicity, with the most successful being capecitabine **10**.

Nucleoside analog prodrugs that have progressed to Phase III trials include elacytarabine **51**, SGI-110 **22**, and sapacitabine **25**. Elacytarabine **51** and CP-4126 **49** have stalled in development, with the former failing to achieve end point efficacy in a Phase III clinical trial involving elderly patients with acute myeloid leukemia,[490] indicating that 5′-elaidic acid ester conjugates are not successful clinical candidates. Elacytarabine **51** is still under clinical investigation, but questions remain about its clinical prospects as a monotherapy. The N^4-alkylester moiety was not a successful approach for LY2333727 **52**, but it may be for sapacitabine **25**, which is still being investigated in a Phase III clinical trial as a monotherapy. Furthermore, in June 2016, Cyclacel Pharmaceuticals announced positive results from a Phase I clinical trial involving sapacitabine **25** with seliciclib (CDK inhibitor) treatment for advanced solid tumors. SGI-110 **22** is also currently being evaluated in a Phase III clinical trial in patients with AML. In addition, in May 2016 BioCryst Pharmaceuticals submitted regulatory paperwork to obtain forodesine hydrochloride **36** approval in Japan.

The phosphoramidate prodrug strategy has been successful in HCV treatment.[757] Currently, ProTide analogs NUC-3373 **37** and NUC-1031 **32** are still being evaluated in Phase I clinical trials. Furthermore, the phosphoramidate prodrugs of many of the nucleoside analogs in this review (e.g., cladribine **8**, fludarabine-5′-monophosphate **7**, clofarabine **12**, thiarabine **47**, troxacitabine **48**, etc.) have been claimed in patents, but no interesting biological effects have been reported.[433,494] Beyond prodrugs, parent nucleoside analogs such as L-nucleosides seem to have an uncertain future, given the poor performance of troxacitabine **48**, which produced multiple terminated clinical trials.

There are several other prodrug strategies being evaluated in preclinical studies. SGI-110 **22** employs a duplex linked drug of decitabine(3′−5′)deoxyguanosine. Additional duplex drug linking of 5FdU(5′−5′)TAS-106[401] and 5-FdU(3′−5′)TAS-106[402] has been investigated *in vitro*. They may provide a means of delivering two nucleoside analogs at once to allow targeting of different cellular pathways. Preliminary data of the duplex drug linking approach seem promising, but further evidence is needed to show synergy and clinical efficacy. Other unique ways to modify nucleoside analogs being tested *in vitro* are

mesoporous silica nanoparticles,[361] 5′-mono-amino acid monoester nucleoside prodrugs,[362] and Hoechst nucleoside conjugates.[363]

A better understanding of cellular degradation pathways may greatly aid in maintaining high intracellular triphosphate forms of the anticancer nucleosides. SAMHD1 hydrolyzes dNTPs into 2′-deoxynucleoside and inorganic triphosphates.[49,50] The tight catalytic pocket of the enzyme excludes ribonucleoside-5′-triphosphates.[758] Clofarabine-5′-triphosphate was shown to be a substrate for SAMHD1 in a biochemical assay.[51] Therefore, we postulate that nucleoside analog triphosphates such as decitabine-5′-triphosphate, cladribine-5′-triphosphate, floxuri-dine-5′-triphosphate ara-C-5′-triphosphate, ara-A-5′-triphosphate, ara-G-5′-triphosphate, fludarabine-5′-triphosphate, cladribine-5′-triphosphate, and clofarabine-5′-triphosphate may be sensitive to dNTP hydrolysis activity by SAMHD1 both *in vitro* and *in vivo*. Cotreatment of these nucleoside analogs with an effective inhibitor of SAMHD1, which still has not been developed, may increase the intracellular antimetabolite concentrations. Moreover, decreasing SAMHD1 protein levels have been shown to promote dNTP imbalance both *in vitro* and *in vivo*,[759,760] and inhibition of the dNTP hydrolytic activity of the enzyme might lead to higher mutation rates in cancer cells. An alternative approach has been suggested to increase SAMHD1 expression in cancer cells. Methylation of the promoter of SAMHD1 has been shown to decrease mRNA and protein levels in certain cancers,[53,761,762] and gene dosage of SAMHD1 has been shown to influence cellular dNTP levels in a mouse model.[763] The current concept is to increase the SAMHD1 protein level within cells in order to decrease cellular dNTPs, which, in turn, might inhibit cell proliferation.[764,765]

Continued investment in basic synthetic organic chemistry provides the ability to access novel nucleoside analogs on both discovery and production scales. For example, the Jamison group's syntheses of capecitabine **10** and doxifluridine **23** (see section 5.1.2.2) using continuous flow chemistry show great potential for the synthesis of nucleoside, nucleotide, and base analogs. Flow chemistry is widely used in academia and currently is attracting great interest in the pharmaceutical industry (e.g., establishment of the MIT-Novartis Center for Continuous Manufacture).[766] This burgeoning technology offers great potential for both microscale synthetic drug discovery efforts and macroscale production of active pharmaceutical agents. On the laboratory scale, the microreactor allows for rigorously controlled reaction conditions, leading to better heat and mass transfer, while also requiring much smaller reaction volumes.[767] This allows for much greater efficiency in comparison to batch systems—a reduction in reaction times (minutes versus hours), and a significant drop in waste (particularly solvent) production. On the macroscale, the significantly reduced reaction time has the potential to produce larger quantities of drug per unit time, in comparison to batch. Furthermore, the continuous flow process joins well with other enabling technologies (microwave irradiation, electrochemistry, 3D-printing, etc.), potentially leading to fully automated processes.[768] Although questions remain about anomeric selectivity in glycosylations involving 2′-deoxysugars as well as phosphoramidate prodrug synthesis, application of this technology to nucleoside analog drug discovery and drug production seems promising.

The recent FDA approval of TAS-102 (**15** + **16**) illustrates that possibilities still exist for anticancer nucleoside analogs. The wealth of information available for these analogs produced from decades of research offers a strong foundation for future pursuits, including prodrug and combination strategies. In addition, further development of novel nucleoside analog chemical entities as well as continued refinement of biological applications of current nucleoside analogs will continue to contribute positively to the fight against cancer.

ACKNOWLEDGMENTS

We would like to thank Megan Browning for her initial contributions to this manuscript and Judy Mathew for her critical evaluation. This work was supported in part by NIH Grant 5P30-AI-50409 (CFAR).

Biographies

Jadd Shelton received his B.S. in Biology (Chemistry minor) from Brigham Young University—Idaho in 2008, where he worked in the lab of Jason Hunt, studying mink glycogen metabolism as a result of hormonal treatment. Also as an undergraduate, and NIH-funded Idaho INBRE fellow in the laboratory of Ron Strohmeyer at Northwest Nazarene University, he researched pharmacologic manipulation of microglial cell inflammatory gene expression related to Alzheimer's disease. In 2012, he received his Ph.D. in Chemistry from Brigham Young University, working in the lab of Matt Peterson, where his research focused on the synthesis of anticancer nucleoside analogs. From 2012 to 2015, Jadd worked as a postdoctoral researcher in the laboratory of Raymond F. Schinazi at Emory University, synthesizing and studying nucleoside analogs for antiviral applications. He currently works as an R&D chemist for Autoliv Inc. His research interests include the design and synthesis of energetic materials for pyrotechnic formulations.

Xiao Lu received his B.S. degree in Pharmaceutical Sciences in 2005 and his M.S. degree in Chemical Biology in 2008 from Peking University. He then earned his Ph.D. degree in Pharmaceutical and Biological Sciences from The University of Georgia in 2012. After finishing his doctoral studies, he joined Prof. Schinazi's Laboratory of Biochemical Pharmacology (LOBP) as a postdoctoral fellow at Emory University, working on medicinal chemistry for viral infectious diseases. He is currently a postdoctoral researcher at The National Center for Advancing Translational Sciences (NCATS), where he is contributing to developing drugs that block malaria transmission.

Joseph A. Hollenbaugh received his doctorate from the Department of Cellular and Molecular Biology at University of Vermont in 2006. He was trained as a cellular immunologist under the mentorship Richard Dutton (Trudeau Institute in Saranac Lake, NY) and examined CD8+ T cell responses to EG7OVA tumor in C57BL/6 mice. He did postdoctoral training in immunology and virology in the laboratories of David Topham and Baek Kim (University of Rochester). He is currently an Instructor in the Pediatrics Department, in the Laboratory of Biochemical Pharmacology at Emory University School of Medicine. His research interests include viral biology, cancer biology, and nucleoside biosynthesis regulation at the cellular level. His recent focus has been on the newly identified cellular protein SAMHD1, which degrades nucleoside triphosphates, in order to

understand their biological implications in diseases and to develop a nucleoside inhibitor for potential therapeutic applications.

Jong Hyun Cho was born in Jangyumyeon, Gimhae-si, South Korea, in 1967. In 2002, he received his Ph.D. in Organic Chemistry from Seoul National University, working on biologically active peptide mimetics under the direction of B. M. Kim. He then joined David C. K. Chu's group at the Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, where he studied nucleoside chemistry and medicinal chemistry from 2003 to 2006. He is currently working as an instructor research fellow for Raymond F. Schinazi at Emory University. His research interests include synthetic approaches to nucleosides and peptidomimetics with antiviral activities against DNA and RNA viruses.

Franck Amblard was born in Châteauroux, France. He studied chemistry at the University of Orléans (France), where he received his Ph.D. in 2004 under the guidance of Luigi A. Agrofoglio, working on the synthesis of new nucleoside analogs using metathesis and palladium-catalyzed reactions. In 2005, he moved to the USA to join Raymond F. Schinazi's research group at Emory University (Atlanta, GA) and worked, as a postdoctoral fellow, on new nucleosides and nucleotides prodrugs. He is now Assistant Professor at the Department of Pediatrics, Emory University School of Medicine. His main research interests include the study of nucleosides analogs and small molecules as potential antiviral agents.

Raymond F. Schinazi is the Frances Winship Walters Professor of Pediatrics, Director of the Laboratory of Biochemical Pharmacology at Emory University, and Adjunct Professor at Georgia State University. He also serves as Director of the HIV Cure Scientific Working Group within the NIH-sponsored Emory University Center for AIDS Research. Dr. Schinazi is a world leader in the area of nucleoside chemistry and biology, having published more than 500 papers and having 100 US patents issued. He is the founder of several biotechnology companies focusing on antiviral drug discovery and development, including Pharmasset, Inc., Triangle Pharmaceuticals, Idenix Pharmaceuticals, and Cocrystal Pharmaceuticals, Inc. He is best known for his innovative and pioneering work on stavudine, lamivudine, emtricitabine, telbivudine, and sofosbuvir, all of which have been approved by the United States Food and Drug Administration and are widely used clinically. More than 94% of HIV-infected individuals take at least one of the antiretroviral agents he invented as a fixed dose combination.

REFERENCES

(1). Siegel R; Jemal A Cancer facts & figures 2014; American Cancer Society: 2014; pp 1–68.

(2). Rasool S; Kadla SA; Rasool V; Ganai BA A comparative overview of general risk factors associated with the incidence of colorectal cancer. Tumor Biol 2013, 34 (5), 2469–2476.

(3). Saeki N; Ono H; Sakamoto H; Yoshida T Genetic factors related to gastric cancer susceptibility identified using a genome-wide association study. Cancer Sci 2013, 104 (1), 1–8. [PubMed: 23057512]

(4). Zimet GD; Buffler P Prevention of human papillomavirus-related diseases: Impediments to progress. Prev. Med 2013, 57 (5), 407–408. [PubMed: 23954187]

(5). Yanik EL; Tamburro K; Eron JJ; Damania B; Napravnik S; Dittmer DP Recent cancer incidence trends in an observational clinical cohort of HIV-infected patients in the US, 2000 to 2011. Infect. Agents Cancer 2013, 8 (1), 18.

(6). PhRMA's Communications & Public Affairs Department. Medicines in development: Cancer 2014 report; Pharmaceutical Research and Manufacturers of America: 2014; pp 1–104.

(7). Siegel RL; Miller KD; Jemal A Cancer statistics, 2016. Ca-Cancer J. Clin 2016, 66 (1), 7–30. [PubMed: 26742998]

(8). Fortin S; Berube G Advances in the development of hybrid anticancer drugs. Expert Opin. Drug Discovery 2013, 8 (8), 1029–1047.

(9). Povirk LF; Goldberg IH A role of oxidative DNA sugar damage in mutagenesis by neocarzinostatin and bleomycin. Biochimie 1987, 69 (8), 815–823. [PubMed: 2447954]

(10). Chen T; Sun Y; Ji P; Kopetz S; Zhang W Topoisomerase IIα in chromosome instability and personalized cancer therapy. Oncogene 2015, 34 (31), 4019–4031. [PubMed: 25328138]

(11). Casaluce F; Sgambato A; Maione P; Ciardiello F; Gridelli C Emerging mitotic inhibitors for non-small cell carcinoma. Expert Opin. Emerging Drugs 2013, 18 (1), 97–107.

(12). Deep G; Agarwal R New combination therapies with cell-cycle agents. Curr. Opin. Investig. Drugs 2008, 9 (6), 591–604.

(13). Paulsen O; Aass N; Kaasa S; Dale O Do corticosteroids provide analgesic effects in cancer patients? A systematic literature review. J. Pain Symptom Manage 2013, 46 (1), 96–105. [PubMed: 23149084]

(14). Antonioli L; Yegutkin GG; Pacher P; Blandizzi C; Hasko G Anti-CD73 in cancer immunotherapy: awakening new opportunities. Trends Cancer 2016, 2 (2), 95–109. [PubMed: 27014745]

(15). Voena C; Chiarle R Advances in cancer immunology and cancer immunotherapy. Discovery Med 2016, 21 (114), 125–133.

(16). Carvajal RD; Yang J; Asmar R Clinical utility of nivolumab in the treatment of advanced melanoma. Ther. Clin. Risk Manage 2016, 12, 313–325.

(17). Parker WB Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer. Chem. Rev 2009, 109 (7), 2880–2893. [PubMed: 19476376]

(18). Zhang J; Visser F; King KM; Baldwin SA; Young JD; Cass CE The role of nucleoside transporters in cancer chemotherapy with nucleoside drugs. Cancer Metastasis Rev 2007, 26 (1), 85–110. [PubMed: 17345146]

(19). Pastor-Anglada M; Casado FJ In Deoxynucleoside Analogs in Cancer Therapy; Humana Press: Totowa, NJ, 2006; pp 1–28.

(20). Gourdeau H; Clarke ML; Ouellet F; Mowles D; Selner M; Richard A; Lee N; Mackey JR; Young JD; Jolivet J; et al. Mechanisms of uptake and resistance to troxacitabine, a novel deoxycytidine nucleoside analogue, in human leukemic and solid tumor cell lines. Cancer Res 2001, 61 (19), 7217–7224. [PubMed: 11585758]

(21). Van Rompay AR; Johansson M; Karlsson A Phosphorylation of nucleosides and nucleoside analogs by mammalian nucleoside monophosphate kinases. Pharmacol. Ther 2000, 87 (2–3), 189–198. [PubMed: 11008000]

(22). Smal C; Vertommen D; Bertrand L; Ntamashimikiro S; Rider MH; Van Den Neste E; Bontemps F Identification of in vivo phosphorylation sites on human 2′-deoxycytidine kinase. Role of Ser-74 in the control of enzyme activity. J. Biol. Chem 2006, 281 (8), 4887–4893. [PubMed: 16361699]

(23). Arner ES; Eriksson S Mammalian deoxyribonucleoside kinases. Pharmacol. Ther 1995, 67 (2), 155–186. [PubMed: 7494863]

(24). Saada-Reisch A Deoxyribonucleoside kinases in mitochondrial DNA depletion. Nucleosides, Nucleotides Nucleic Acids 2004, 23 (8–9), 1205–1215. [PubMed: 15571232]

(25). Knecht W; Petersen GE; Munch-Petersen B; Piskur J Deoxyribonucleoside kinases belonging to the thymidine kinase 2 (TK2)-like group vary significantly in substrate specificity, kinetics and feed-back regulation. J. Mol. Biol 2002, 315 (4), 529–540. [PubMed: 11812127]

(26). Matsuda A; Sasaki T Antitumor activity of sugar-modified cytosine nucleosides. Cancer Sci 2004, 95 (2), 105–111. [PubMed: 14965358]

(27). Achanta G; Pelicano H; Feng L; Plunkett W; Huang P Interaction of p53 and DNA-PK in response to nucleoside analogues: potential role as a sensor complex for DNA damage. Cancer Res 2001, 61 (24), 8723–8729. [PubMed: 11751391]

(28). Jackson SP; Bartek J The DNA-damage response in human biology and disease. Nature 2009, 461 (7267), 1071–1078. [PubMed: 19847258]

(29). Melvin WT; Milne HB; Slater AA; Allen HJ; Keir HM Incorporation of 6-thioguanosine and 4-thiouridine into RNA. Application to isolation of newly synthesised RNA by affinity chromatography. Eur. J. Biochem 1978, 92 (2), 373–379. [PubMed: 570106]

(30). Sahasranaman S; Howard D; Roy S Clinical pharmacology and pharmacogenetics of thiopurines. Eur. J. Clin. Pharmacol 2008, 64 (8), 753–767. [PubMed: 18506437]

(31). Perez MA; Cerqueira NM; Fernandes PA; Ramos MJ Ribonucleotide reductase: a mechanistic portrait of substrate analogues inhibitors. Curr. Med. Chem 2010, 17 (26), 2854–2872. [PubMed: 20858168]

(32). Hofer A; Crona M; Logan DT; Sjoberg BM DNA building blocks: keeping control of manufacture. Crit. Rev. Biochem. Mol. Biol 2012, 47 (1), 50–63. [PubMed: 22050358]

(33). Kicska GA; Long L; Horig H; Fairchild C; Tyler PC; Furneaux RH; Schramm VL; Kaufman HL Immucillin H, a powerful transition-state analog inhibitor of purine nucleoside phosphorylase, selectively inhibits human T lymphocytes. Proc. Natl. Acad. Sci. U. S. A 2001, 98 (8), 4593–4598. [PubMed: 11287638]

(34). Miles RW; Tyler PC; Furneaux RH; Bagdassarian CK; Schramm VL One-third-the-sites transition-state inhibitors for purine nucleoside phosphorylase. Biochemistry 1998, 37 (24), 8615–8621. [PubMed: 9628722]

(35). Robak P; Robak T Older and new purine nucleoside analogs for patients with acute leukemias. Cancer Treat. Rev 2013, 39 (8), 851–861. [PubMed: 23566572]

(36). Heidelberger C; Chaudhuri NK; Danneberg P; Mooren D; Griesbach L; Duschinsky R; Schnitzer RJ; Pleven E; Scheiner J Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. Nature 1957, 179 (4561), 663–666. [PubMed: 13418758]

(37). Soucy TA; Smith PG; Milhollen MA; Berger AJ; Gavin JM; Adhikari S; Brownell JE; Burke KE; Cardin DP; Critchley S; et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature 2009, 458 (7239), 732–736. [PubMed: 19360080]

(38). Tatum D; Li S Evidence that the histone methyltransferase Dot1 mediates global genomic repair by methylating histone H3 on lysine 79. J. Biol. Chem 2011, 286 (20), 17530–17535. [PubMed: 21460225]

(39). Bernt KM; Zhu N; Sinha AU; Vempati S; Faber J; Krivtsov AV; Feng Z; Punt N; Daigle A; Bullinger L; et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. Cancer Cell 2011, 20 (1), 66–78. [PubMed: 21741597]

(40). Hunsucker SA; Mitchell BS; Spychala J The 5′-nucleotidases as regulators of nucleotide and drug metabolism. Pharmacol. Ther 2005, 107 (1), 1–30. [PubMed: 15963349]

(41). Eriksson S Is the expression of deoxynucleoside kinases and 5′-nucleotidases in animal tissues related to the biological effects of nucleoside analogs? Curr. Med. Chem 2013, 20 (34), 4241–4248. [PubMed: 23992317]

(42). Buschmann J; Moritz B; Jeske M; Lilie H; Schierhorn A; Wahle E Identification of Drosophila and human 7-methyl GMP-specific nucleotidases. J. Biol. Chem 2013, 288 (4), 2441–2451. [PubMed: 23223233]

(43). Bianchi V; Spychala J Mammalian 5′-nucleotidases. J. Biol. Chem 2003, 278 (47), 46195–46198. [PubMed: 12947102]

(44). Chernogorova P; Zeiser R Ectonucleotidases in solid organ and allogeneic hematopoietic cell transplantation. J. Biomed. Biotechnol 2012, 2012, 1–17. [PubMed: 21836813]

(45). Reichard P Interactions between deoxyribonucleotide and DNA synthesis. Annu. Rev. Biochem 1988, 57, 349–374. [PubMed: 3052277]

(46). Stagg J; Smyth MJ Extracellular adenosine triphosphate and adenosine in cancer. Oncogene 2010, 29 (39), 5346–5358. [PubMed: 20661219]

(47). Gaudreau PO; Allard B; Turcotte M; Stagg J CD73-adenosine reduces immune responses and survival in ovarian cancer patients. Oncoimmunology 2016, 5 (5), e1127496. [PubMed: 27467942]

(48). Stella J; Bavaresco L; Braganhol E; Rockenbach L; Farias PF; Wink MR; Azambuja AA; Barrios CH; Morrone FB; Oliveira Battastini AM Differential ectonucleotidase expression in human bladder cancer cell lines. Urol Oncol 2010, 28 (3), 260–267. [PubMed: 19372055]

(49). Powell RD; Holland PJ; Hollis T; Perrino FW Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. J. Biol. Chem 2011, 286 (51), 43596–43600. [PubMed: 22069334]

(50). Goldstone DC; Ennis-Adeniran V; Hedden JJ; Groom HC; Rice GI; Christodoulou E; Walker PA; Kelly G; Haire LF; Yap MW; et al. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature 2011, 480 (7377), 379–382. [PubMed: 22056990]

(51). Arnold LH; Kunzelmann S; Webb MR; Taylor IA A continuous enzyme-coupled assay for triphosphohydrolase activity of HIV-1 restriction factor SAMHD1. Antimicrob. Agents Chemother 2015, 59 (1), 186–192. [PubMed: 25331707]

(52). Rossi D SAMHD1: a new gene for CLL. Blood 2014, 123 (7), 951–952. [PubMed: 24526775]

(53). Wang JL; Lu FZ; Shen XY; Wu Y; Zhao LT SAMHD1 is down regulated in lung cancer by methylation and inhibits tumor cell proliferation. Biochem. Biophys. Res. Commun 2014, 455 (3–4), 229–233. [PubMed: 25449277]

(54). Holohan C; Van Schaeybroeck S; Longley DB; Johnston PG Cancer drug resistance: An evolving paradigm. Nat. Rev. Cancer 2013, 13 (10), 714–726. [PubMed: 24060863]

(55). Wiley JS; Jones SP; Sawyer WH; Paterson AR Cytosine arabinoside influx and nucleoside transport sites in acute leukemia. J. Clin. Invest 1982, 69 (2), 479–489. [PubMed: 6948829]

(56). Jamieson GP; Brocklebank AM; Snook MB; Sawyer WH; Buolamwini JK; Paterson AR; Wiley JS Flow cytometric quantitation of nucleoside transporter sites on human leukemic cells. Cytometry 1993, 14 (1), 32–38. [PubMed: 8432200]

(57). Phua LC; Mal M; Koh PK; Cheah PY; Chan EC; Ho HK Investigating the role of nucleoside transporters in the resistance of colorectal cancer to 5-fluorouracil therapy. Cancer Chemother. Pharmacol 2013, 71 (3), 817–823. [PubMed: 23271323]

(58). Hu X; Chen W; Xu J Downregulation of human equilibrative nucleoside transporter 1 by RNAi enhances 5-fluorouracil response in pancreatic cancer. Hepatogastroenterology 2010, 57 (104), 1567–1572. [PubMed: 21443122]

(59). Mohelnikova-Duchonova B; Melichar B Human equilibrative nucleoside transporter 1 (hENT1): do we really have a new predictive biomarker of chemotherapy outcome in pancreatic cancer patients? Pancreatology 2013, 13 (6), 558–563. [PubMed: 24280569]

(60). Saiki Y; Yoshino Y; Fujimura H; Manabe T; Kudo Y; Shimada M; Mano N; Nakano T; Lee Y; Shimizu S; et al. DCK is frequently inactivated in acquired gemcitabine-resistant human cancer cells. Biochem. Biophys. Res. Commun 2012, 421 (1), 98–104. [PubMed: 22490663]

(61). Flasshove M; Strumberg D; Ayscue L; Mitchell BS; Tirier C; Heit W; Seeber S; Schutte J Structural analysis of the 2′-deoxycytidine kinase gene in patients with acute myeloid leukemia and resistance to cytosine arabinoside. Leukemia 1994, 8 (5), 780–785. [PubMed: 7514246]

(62). Veuger MJ; Honders MW; Willemze R; Barge RM 2′-deoxycytidine kinase expression and activity in patients with resistant versus sensitive acute myeloid leukemia. Eur. J. Haematol 2002, 69 (3), 171–178. [PubMed: 12406011]

(63). Ferry KV; Hamilton TC; Johnson SW Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells: role of ERCC1-XPF. Biochem. Pharmacol 2000, 60 (9), 1305–1313. [PubMed: 11008124]

(64). Nambaru PK; Hubner T; Kock K; Mews S; Grube M; Payen L; Guitton J; Sendler M; Jedlitschky G; Rimmbach C; et al. Drug efflux transporter multidrug resistance-associated protein 5 affects sensitivity of pancreatic cancer cell lines to the nucleoside anticancer drug 5-fluorouracil. Drug Metab. Dispos 2011, 39 (1), 132–139. [PubMed: 20930123]

(65). Fukuda Y; Schuetz JD ABC transporters and their role in nucleoside and nucleotide drug resistance. Biochem. Pharmacol 2012, 83 (8), 1073–1083. [PubMed: 22285911]

(66). Gonzalez-Haba E; Garcia MI; Cortejoso L; Lopez-Lillo C; Barrueco N; Garcia-Alfonso P; Alvarez S; Jimenez JL; Martin ML; Munoz-Fernandez MA; et al. ABCB1 gene polymorphisms are associated with adverse reactions in fluoropyrimidine-treated colorectal cancer patients. Pharmacogenomics 2010, 11 (12), 1715–1723. [PubMed: 21142915]

(67). Steuart CD; Burke PJ Cytidine deaminase and the development of resistance to arabinosyl cytosine. Nature: New biology 1971, 233 (38), 109–110. [PubMed: 5285962]

(68). Abraham A; Varatharajan S; Abbas S; Zhang W; Shaji RV; Ahmed R; George B; Srivastava A; Chandy M; Mathews V; et al. Cytidine deaminase genetic variants influence RNA expression and cytarabine cytotoxicity in acute myeloid leukemia. Pharmacogenomics 2012, 13 (3), 269–282. [PubMed: 22304580]

(69). Damaraju VL; Damaraju S; Young JD; Baldwin SA; Mackey J; Sawyer MB; Cass CE Nucleoside anticancer drugs: the role of nucleoside transporters in resistance to cancer chemotherapy. Oncogene 2003, 22 (47), 7524–7536. [PubMed: 14576856]

(70). Vorbrüggen H; Ruh-Pohlenz C Organic Reactions; American Chemical Society: 1999; pp 1–630.

(71). Elion GB The purine path to chemotherapy. Science 1989, 244 (4900), 41–47. [PubMed: 2649979]

(72). Levinsen M; Rotevatn EO; Rosthoj S; Nersting J; Abrahamsson J; Appell ML; Bergan S; Bechensteen AG; Harila-Saari A; Heyman M; et al. Pharmacogenetically based dosing of thiopurines in childhood acute lymphoblastic leukemia: Influence on cure rates and risk of second cancer. Pediatr. Blood Cancer 2014, 61 (5), 797–802. [PubMed: 24395436]

(73). Vora A; Mitchell CD; Lennard L; Eden TO; Kinsey SE; Lilleyman J; Richards SM; Medical Research C; National Cancer Research Network Childhood Leukaemia Working, P.. Toxicity and efficacy of 6-thioguanine versus 6-mercaptopurine in childhood lymphoblastic leukaemia: a randomised trial. Lancet 2006, 368 (9544), 1339–1348. [PubMed: 17046466]

(74). Lemann M Treatment of chronic inflammatory bowel diseases. Bull. Acad. Natl. Med 2007, 191 (6), 1125–1141. [PubMed: 18402168]

(75). Jabin D; Kumar S; Gow PJ Outcome of patients on azathioprine: a need for a better pre-treatment assessment and dosing guideline. N. Z. Med. J 2010, 123 (1324), 67–73.

(76). Chaparro M; Ordas I; Cabre E; Garcia-Sanchez V; Bastida G; Penalva M; Gomollon F; Garcia-Planella E; Merino O; Gutierrez A; et al. Safety of thiopurine therapy in inflammatory bowel disease: Long-term follow-up study of 3931 patients. Inflamm. Bowel Dis 2013, 19 (7), 1404–1410. [PubMed: 23665964]

(77). Kudo M; Saito Y; Sasaki T; Akasaki H; Yamaguchi Y; Uehara M; Fujikawa K; Ishikawa M; Hirasawa N; Hiratsuka M Genetic variations in the HGPRT, ITPA, IMPDH1, IMPDH2, and GMPS genes in Japanese individuals. Drug Metab. Pharmacokinet 2009, 24 (6), 557–564. [PubMed: 20045992]

(78). Petit E; Langouet S; Akhdar H; Nicolas-Nicolaz C; Guillouzo A; Morel F Differential toxic effects of azathioprine, 6-mercaptopurine and 6-thioguanine on human hepatocytes. Toxicol. In Vitro 2008, 22 (3), 632–642. [PubMed: 18222062]

(79). Aarbakke J; Janka-Schaub G; Elion GB Thiopurine biology and pharmacology. Trends Pharmacol. Sci 1997, 18 (1), 3–7. [PubMed: 9114722]

(80). Nelson JA; Carpenter JW; Rose LM; Adamson DJ Mechanisms of action of 6-thioguanine, 6-mercaptopurine, and 8-azaguanine. Cancer Res 1975, 35 (10), 2872–2878. [PubMed: 1157053]

(81). Karran P Thiopurines, DNA damage, DNA repair and therapy-related cancer. Br. Med. Bull 2006, 79–80, 153–170.

(82). Chrzanowska M; Kuehn M; Januszkiewicz-Lewandowska D; Kurzawski M; Drozdzik M Thiopurine S-methyltransferase pheno-type-genotype correlation in children with acute lymphoblastic leukemia. Acta. Polym. Pharm 2012, 69 (3), 405–410.

(83). de Boer NK; van Bodegraven AA; Jharap B; de Graaf P; Mulder CJ Drug insight: Pharmacology and toxicity of thiopurine therapy in patients with IBD. Nat. Clin. Pract. Gastroenterol. Hepatol 2007, 4 (12), 686–694. [PubMed: 18043678]

(84). Karim H; Ghalali A; Lafolie P; Vitols S; Fotoohi AK Differential role of thiopurine methyltransferase in the cytotoxic effects of 6-mercaptopurine and 6-thioguanine on human leukemia cells. Biochem. Biophys. Res. Commun 2013, 437 (2), 280–286. [PubMed: 23811272]

(85). Chen ZS; Lee K; Kruh GD Transport of cyclic nucleotides and estradiol 17-β-D-glucuronide by multidrug resistance protein 4. Resistance to 6-mercaptopurine and 6-thioguanine. J. Biol. Chem 2001, 276 (36), 33747–33754. [PubMed: 11447229]

(86). Wielinga PR; Reid G; Challa EE; van der Heijden I; van Deemter L; de Haas M; Mol C; Kuil AJ; Groeneveld E; Schuetz JD; et al. Thiopurine metabolism and identification of the thiopurine metabolites transported by MRP4 and MRP5 overexpressed in human embryonic kidney cells. Mol. Pharm 2002, 62 (6), 1321–1331.

(87). Peng XX; Chen ZS; Tiwari AK; Damaraju VL; Fu L; Cass CE; Ashby CR Jr.; Kruh GD; Shi Z Up-regulation of P-glycoprotein confers acquired resistance to 6-mercaptopurine in human chronic myeloid leukemia cells. Oncol. Lett 2011, 2 (3), 549–556. [PubMed: 22866119]

(88). J Pharmacol Exp Ther; Baskin F; Rosenberg RN Decreased 6-mercaptopurine retention by two resistant variants of mouse neuroblastoma with normal hypoxanthine-guanine-phospho-ribosyltransferase activities. J. Pharmacol. Exp. Ther 1975, 193 (1), 293–300. [PubMed: 1169317]

(89). Chouchana L; Fernandez-Ramos AA; Dumont F; Marchetti C; Ceballos-Picot I; Beaune P; Gurwitz D; Loriot MA Molecular insight into thiopurine resistance: transcriptomic signature in lymphoblastoid cell lines. Genome Med 2015, 7 (1), 37. [PubMed: 26015807]

(90). Tzoneva G; Perez-Garcia A; Carpenter Z; Khiabanian H; Tosello V; Allegretta M; Paietta E; Racevskis J; Rowe JM; Tallman MS; et al. Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. Nat. Med 2013, 19 (3), 368–371. [PubMed: 23377281]

(91). Meyer JA; Wang J; Hogan LE; Yang JJ; Dandekar S; Patel JP; Tang Z; Zumbo P; Li S; Zavadil J; et al. Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. Nat. Genet 2013, 45 (3), 290–294. [PubMed: 23377183]

(92). Elion GB; Burgi E; Hitchings GH Studies on condensed pyrimidine systems. IX. The synthesis of some 6-substituted purines. J. Am. Chem. Soc 1952, 74 (2), 411–414.

(93). Albert A; Brown DJ; Cheeseman G 103. Pteridine studies. Part I. Pteridine, and 2- and 4-amino- and 2- and 4-hydroxy-pteridines. J. Chem. Soc 1951, 474–485.

(94). Lin J; Zhang C; Deng B CN Patent 101817822, 2010.

(95). Bergman J; Pettersson B; Hasimbegovic V; Svensson PH Thionations using a P4S10-pyridine complex in solvents such as acetonitrile and dimethyl sulfone. J. Org. Chem 2011, 76 (6), 1546–1553. [PubMed: 21341727]

(96). Elion GB; Hitchings GH The synthesis of 6-thioguanine. J. Am. Chem. Soc 1955, 77 (6), 1676.

(97). Ma Q CN Patent 1690058, 2005.

(98). Kulikowski T; Grynkiewicz G PL Patent 162708, 1990.

(99). Kramata P; Downey KM; Paborsky LR Incorporation and excision of 9-(2-phosphonylmethoxyethyl)guanine (PMEG) by DNA polymerase delta and epsilon in vitro. J. Biol. Chem 1998, 273 (34), 21966–21971. [PubMed: 9705337]

(100). Romeo R; Carnovale C; Rescifina A; Chiacchio MA In Chemical synthesis of nucleoside analogues; Wiley: Hoboken, NJ, 2013; pp 163–208.

(101). Hatse S; De Clercq E; Balzarini J Role of antimetabolites of purine and pyrimidine nucleotide metabolism in tumor cell differentiation. Biochem. Pharmacol 1999, 58 (4), 539–555. [PubMed: 10413291]

(102). Kramata P; Downey KM 9-(2-phosphonylmethoxyethyl) derivatives of purine nucleotide analogs: A comparison of their metabolism and interaction with cellular DNA synthesis. Mol. Pharm 1999, 56 (6), 1262–1270.

(103). Reiser H; Wang J; Chong L; Watkins WJ; Ray AS; Shibata R; Birkus G; Cihlar T; Wu S; Li B; et al. GS-9219–a novel acyclic nucleotide analogue with potent antineoplastic activity in dogs with spontaneous non-Hodgkin's lymphoma. Clin. Cancer Res 2008, 14 (9), 2824–2832. [PubMed: 18451250]

(104). Pradere U; Garnier-Amblard EC; Coats SJ; Amblard F; Schinazi RF Synthesis of nucleoside phosphate and phosphonate prodrugs. Chem. Rev 2014, 114 (18), 9154–9218. [PubMed: 25144792]

(105). Schinkmanova M; Votruba I; Holy A N6-methyl-AMP aminohydrolase activates N6-substituted purine acyclic nucleoside phosphonates. Biochem. Pharmacol 2006, 71 (9), 1370–1376. [PubMed: 16513094]

(106). Frey CR; Andrei G; Votruba I; Cannizzaro C; Han B; Fung W; Hung M; Liu X; Geleziunas R; Fiten P; et al. Mutations in adenosine deaminase-like (ADAL) protein confer resistance to the antiproliferative agents N6-cyclopropyl-PMEDAP and GS-9219. Anticancer Res 2013, 33 (5), 1899–1912. [PubMed: 23645737]

(107). Mertlikova-Kaiserova H; Rumlova M; Tloustova E; Prochazkova E; Holy A; Votruba I Point mutations in human guanylate kinase account for acquired resistance to anticancer nucleotide analogue PMEG. Biochem. Pharmacol 2011, 82 (2), 131–138. [PubMed: 21515241]

(108). Thamm DH; Vail DM; Kurzman ID; Babusis D; Ray AS; Sousa-Powers N; Tumas DB GS-9219/VDC-1101 - a prodrug of the acyclic nucleotide PMEG has antitumor activity inspontaneous canine multiple myeloma. BMC Vet. Res 2014, 10 (30), 1–7. [PubMed: 24383544]

(109). Lawrence JA; Huelsmeyer MK; Thamm DH; Tumas DB; Birkus G; Kurzman I; Vail DM Novel acyclic nucleotide analogues GS-343074 and GS-424044 demonstrate antiproliferative and pro-apoptotic activity in canine neoplastic cell lines. Vet. Comp. Oncol 2015, 13 (3), 246–254. [PubMed: 23672437]

(110). Morges MA; Burton JH; Saba CF; Vail DM; Burgess KE; Thamm DH Phase II evaluation of VDC-1101 in canine cutaneous T-cell lymphoma. J. Vet. Intern. Med 2014, 28 (5), 1569–1574. [PubMed: 25274443]

(111). Cheng X; Cook GP; Desai MC; Doerffler E; He G-X; Kim CU; Lee WA; Rohloff JC; Wang J; Yang Z-Y WO Patent 2005066189, 2005.

(112). Jansa P; Baszczynski O; Dracinsky M; Votruba I; Zidek Z; Bahador G; Stepan G; Cihlar T; Mackman R; Holy A; Janeba Z A novel and efficient one-pot synthesis of symmetrical diamide (bisamidate) prodrugs of acyclic nucleoside phosphonates and evaluation of their biological activities. Eur. J. Med. Chem 2011, 46 (9), 3748–3754. [PubMed: 21664011]

(113). Horejsi K; Andrei G; De Clercq E; Snoeck R; Pohl R; Holy A Tricyclic etheno analogs of PMEG and PMEDAP: Synthesis and biological activity. Bioorg. Med. Chem 2006, 14 (23), 8057–8065. [PubMed: 16904327]

(114). Rutman RJ; Cantarow A; Paschkis KE Studies in 2-acetylaminofluorene carcinogenesis. III. The utilization of uracil-2-C14 by preneoplastic rat liver and rat hepatoma. Cancer Res 1954, 14 (2), 119–123. [PubMed: 13126946]

(115). Callery P; Gannett P Cancer and Cancer Chemotherapy; Lippincott Williams & Wilkins: Philadelphia, PA, 2002; pp 924–951.

(116). Heidelberger C; Griesbach L; Cruz O; Schnitzer RJ; Grunberg E Fluorinated pyrimidines. VI. Effects of 5-fluorouridine and 5-fluoro-2′-deoxyuridine on transplanted tumors. Exp. Biol. Med 1958, 97 (2), 470–475.

(117). Bosch L; Harbers E; Heidelberger C Studies on fluorinated pyrimidines. V. Effects on nucleic acid metabolism in vitro. Cancer Res 1958, 18 (3), 335–343.

(118). Chaudhuri NK; Montag BJ; Heidelberger C Studies on fluorinated pyrimidines III. The metabolism of 5-fluorouracil-2-C14 and 5-fluoroorotic-2-C14 acid in vivo. Cancer Res 1958, 18 (3), 318–328. [PubMed: 13523598]

(119). Longley DB; Harkin DP; Johnston PG 5-fluorouracil: Mechanisms of action and clinical strategies. Nat. Rev. Cancer 2003, 3 (5), 330–338. [PubMed: 12724731]

(120). Guo Y; Shi M; Shen X; Yang C; Yang L; Zhang J Capecitabine plus irinotecan versus 5-FU/leucovorin plus irinotecan in the treatment of colorectal cancer: A meta-analysis. Clin. Colorectal Cancer 2014, 13 (2), 110–118. [PubMed: 24461997]

(121). Du Z; Wang Y; Zhou Y; Wen F; Li Q First-line irinotecan combined with 5-fluorouracil and leucovorin for high-grade metastatic gastrointestinal neuroendocrine carcinoma. Tumori 2013, 99 (1), 57–60. [PubMed: 23549001]

(122). Diasio RB; Harris BE Clinical pharmacology of 5-fluorouracil. Clin. Pharmacokinet 1989, 16 (4), 215–237. [PubMed: 2656050]

(123). Wohlhueter RM; McIvor RS; Plagemann PG Facilitated transport of uracil and 5-fluorouracil, and permeation of orotic acid into cultured mammalian cells. J. Cell. Physiol 1980, 104 (3), 309–319. [PubMed: 7419607]

(124). Grem JL 5-Fluorouracil: forty-plus and still ticking. A review of its preclinical and clinical development. Invest. New Drugs 2000, 18 (4), 299–313. [PubMed: 11081567]

(125). Ghoshal K; Jacob ST An alternative molecular mechanism of action of 5-fluorouracil, a potent anticancer drug. Biochem. Pharmacol 1997, 53 (11), 1569–1575. [PubMed: 9264308]

(126). Santi DV; Hardy LW Catalytic mechanism and inhibition of tRNA (uracil-5-)methyltransferase: evidence for covalent catalysis. Biochemistry 1987, 26 (26), 8599–8606. [PubMed: 3327525]

(127). Huehls AM; Huntoon CJ; Joshi PM; Baehr CA; Wagner JM; Wang X; Lee MY; Karnitz LM Genomically incorporated 5-fluorouracil that escapes UNG-initiated base excision repair blocks DNA replication and activates homologous recombination. Mol. Pharmacol 2016, 89 (1), 53–62. [PubMed: 26494862]

(128). Berger SH; Pittman DL; Wyatt MD Uracil in DNA: consequences for carcinogenesis and chemotherapy. Biochem. Pharmacol 2008, 76 (6), 697–706. [PubMed: 18599024]

(129). Guillet M; Van Der Kemp PA; Boiteux S dUTPase activity is critical to maintain genetic stability in Saccharomyces cerevisiae. Nucleic Acids Res 2006, 34 (7), 2056–2066. [PubMed: 16617146]

(130). Nilsen H; Rosewell I; Robins P; Skjelbred CF; Andersen S; Slupphaug G; Daly G; Krokan HE; Lindahl T; Barnes DE Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication. Mol. Cell 2000, 5 (6), 1059–1065. [PubMed: 10912000]

(131). Pratt S; Shepard RL; Kandasamy RA; Johnston PA; Perry W 3rd; Dantzig AH The multidrug resistance protein 5 (ABCC5) confers resistance to 5-fluorouracil and transports its monophosphorylated metabolites. Mol. Cancer Ther 2005, 4 (5), 855–863. [PubMed: 15897250]

(132). Guo Y; Kotova E; Chen ZS; Lee K; Hopper-Borge E; Belinsky MG; Kruh GD MRP8, ATP-binding cassette C11 (ABCC11), is a cyclic nucleotide efflux pump and a resistance factor for fluoropyrimidines 2′,3′-dideoxycytidine and 9′-(2′-phosphonylmethoxyethyl)adenine. J. Biol. Chem 2003, 278 (32), 29509–29514. [PubMed: 12764137]

(133). Wang W; Zhao L; Wei X; Wang L; Liu S; Yang Y; Wang F; Sun G; Zhang J; Ma Y; et al. MicroRNA-320a promotes 5-FU resistance in human pancreatic cancer cells. Sci. Rep 2016, 6 (27641), 1–11. [PubMed: 28442746]

(134). Wei X; Wang W; Wang L; Zhang Y; Zhang X; Chen M; Wang F; Yu J; Ma Y; Sun G MicroRNA-21 induces 5-fluorouracil resistance in human pancreatic cancer cells by regulating PTEN and PDCD4. Cancer Med 2016, 5 (4), 693–702. [PubMed: 26864640]

(135). Xie X; Liu H; Wang Y; Zhou Y; Yu H; Li G; Ruan Z; Li F; Wang X; Zhang J Nicotinamide N-methyltransferase enhances resistance to 5-fluorouracil in colorectal cancer cells through inhibition of the ASK1-p38 MAPK pathway. Oncotarget 2016, 45837–45848. [PubMed: 27323852]

(136). Zhang X; Chen Y; Hao L; Hou A; Chen X; Li Y; Wang R; Luo P; Ruan Z; Ou J; et al. Macrophages induce resistance to 5-fluorouracil chemotherapy in colorectal cancer through the release of putrescine. Cancer Lett 2016, 381 (2), 305–313. [PubMed: 27514455]

(137). Zhao T; Xu Z; Gu D; Wu P; Huo X; Wei X; Tang Y; Gong W; He ML; Chen J The effects of genomic polymorphisms in one-carbon metabolism pathways on survival of gastric cancer patients received fluorouracil-based adjuvant therapy. Sci. Rep 2016, 6, 1–11. [PubMed: 28442746]

(138). Mader RM; Muller M; Steger GG Resistance to 5-fluorouracil. Gen. Pharmacol 1998, 31 (5), 661–666. [PubMed: 9809460]

(139). Schoffski P The modulated oral fluoropyrimidine prodrug S-1, and its use in gastrointestinal cancer and other solid tumors. Anti-Cancer Drugs 2004, 15 (2), 85–106. [PubMed: 15075664]

(140). Kono A; Hara Y; Sugata S; Karube Y; Matsushima Y; Ishitsuka H Activation of 5′-deoxy-5-fluorouridine by thymidine phosphorylase in human tumors. Chem. Pharm. Bull 1983, 31 (1), 175–178.

(141). Nishioka M; Miyamoto H; Kurita N; Higashijima J; Yoshikawa K; Miyatani T; Shimada M Pyrimidine nucleoside phosphorylase and dihydropyrimidine dihydrogenase activities as

predictive factors for the efficacy of doxifluridine together with mitomycin C as adjuvant chemotherapy in primary colorectal cancer. Hepatogastroenterology 2007, 54 (76), 1089–1093. [PubMed: 17629045]

(142). Osanai T; Ichikawa W; Takagi Y; Uetake H; Nihei Z; Sugihara K Expression of pyrimidine nucleoside phosphorylase (PyNPase) in colorectal cancer. Jnp. J. Clin. Onco 2001, 31 (10), 500–505.

(143). Noguchi T; Moriyama H; Wada S; Takeno S; Kimura Y; Uchida Y; Gabbert HE High level concentration of pyrimidine nucleoside phosphorylase in esophageal squamous cell carcinoma but no correlation with clinicopathological parameters. Dis. Esophagus 2003, 16 (4), 307–311. [PubMed: 14641294]

(144). Mori K; Hasegawa M; Nishida M; Toma H; Fukuda M; Kubota T; Nagasue N; Yamana H; Hirakawa YSCK; Ikeda T; et al. Expression levels of thymidine phosphorylase and dihydropyrimidine dehydrogenase in various human tumor tissues. Int. J. Oncol 2000, 17 (1), 33–38. [PubMed: 10853015]

(145). Ogata Y; Sasatomi T; Mori S; Matono K; Ishibashi N; Akagi Y; Fukushima T; Murakami H; Ushijima M; Shirouzu K Significance of thymidine phosphorylase in metronomic chemotherapy using CPT-11 and doxifluridine for advanced colorectal carcinoma. Anticancer Res 2007, 27 (4C), 2605–2611. [PubMed: 17695422]

(146). Lamont EB; Schilsky RL The oral fluoropyrimidines in cancer chemotherapy. Clin. Cancer Res 1999, 5 (9), 2289–2296. [PubMed: 10499595]

(147). Alberto P; Mermillod B; Germano G; Kaplan S; Weber W; Joss R; Spati B; Martz G; Cavalli F A randomized comparison of doxifluridine and fluorouracil in colorectal carcinoma. Eur. J. Cancer Clin. Oncol 1988, 24 (3), 559–563. [PubMed: 2968263]

(148). Kang YK; Yook JH; Chang HM; Ryu MH; Yoo C; Zang DY; Lee JL; Kim TW; Yang DH; Jang SJ; et al. Enhanced efficacy of postoperative adjuvant chemotherapy in advanced gastric cancer: results from a phase 3 randomized trial (AMC0101). Cancer Chemother. Pharmacol 2014, 73 (1), 139–149. [PubMed: 24162381]

(149). Herr RJ In Modern Drug Synthesis; Wiley: Hoboken, NJ, 2010; pp 57–71.

(150). Shimma N; Umeda I; Arasaki M; Murasaki C; Masubuchi K; Kohchi Y; Miwa M; Ura M; Sawada N; Tahara H; Kuruma I; Horii I; Ishitsuka H The design and synthesis of a new tumor-selective fluoropyrimidine carbamate, capecitabine. Bioorg. Med. Chem 2000, 8 (7), 1697–1706. [PubMed: 10976516]

(151). Walko CM; Lindley C Capecitabine: A review. Clin. Ther 2005, 27 (1), 23–44. [PubMed: 15763604]

(152). Ishitsuka H In Discovery and preclinical pharmacology of capecitabine; Humana Press: Totowa, NJ, 2003; pp 249–259.

(153). Ishikawa T; Utoh M; Sawada N; Nishida M; Fukase Y; Sekiguchi F; Ishitsuka H Tumor selective delivery of 5-fluorouracil by capecitabine, a new oral fluoropyrimidine carbamate, in human cancer xenografts. Biochem. Pharmacol 1998, 55 (7), 1091–1097. [PubMed: 9605432]

(154). Richardson MA Complementary and alternative therapy use in gynecologic oncology: implications for clinical practice. Gynecol. Oncol 2002, 84 (3), 360–362. [PubMed: 11855869]

(155). Kosuri KV; Wu X; Wang L; Villalona-Calero MA; Otterson GA An epigenetic mechanism for capecitabine resistance in mesothelioma. Biochem. Biophys. Res. Commun 2010, 391 (3), 1465–1470. [PubMed: 20035722]

(156). McGuigan C; Murziani P; Slusarczyk M; Gonczy B; Vande Voorde J; Liekens S; Balzarini J Phosphoramidate ProTides of the anticancer agent FUDR successfully deliver the preformed bioactive monophosphate in cells and confer advantage over the parent nucleoside. J. Med. Chem 2011, 54 (20), 7247–7258. [PubMed: 21892829]

(157). Vande Voorde J; Liekens S; McGuigan C; Murziani PG; Slusarczyk M; Balzarini J The cytostatic activity of NUC-3073, a phosphoramidate prodrug of 5-fluoro-2′-deoxyuridine, is independent of activation by thymidine kinase and insensitive to degradation by phosphorolytic enzymes. Biochem. Pharmacol 2011, 82 (5), 441–452. [PubMed: 21658373]

(158). Tsume Y; Hilfinger JM; Amidon GL Enhanced cancer cell growth inhibition by dipeptide prodrugs of floxuridine: increased transporter affinity and metabolic stability. Mol. Pharmaceutics 2008, 5 (5), 717–727.

(159). DiMasi JA; Paquette C The economics of follow-on drug research and development: trends in entry rates and the timing of development. PharmacoEconomics 2004, 22 (Suppl 2), 1–14.

(160). Homsi J; Garrett CR Hepatic arterial infusion of chemotherapy for hepatic metastases from colorectal cancer. Cancer Control 2006, 13 (1), 42–47. [PubMed: 16508625]

(161). Landowski CP; Song X; Lorenzi PL; Hilfinger JM; Amidon GL Floxuridine amino acid ester prodrugs: enhancing Caco-2 permeability and resistance to glycosidic bond metabolism. Pharm. Res 2005, 22 (9), 1510–1518. [PubMed: 16132363]

(162). Power DG; Kemeny NE The role of floxuridine in metastatic liver disease. Mol. Cancer Ther 2009, 8 (5), 1015–1025. [PubMed: 19383854]

(163). Konstantinidis IT; Groot Koerkamp B; Do RK; Gonen M; Fong Y; Allen PJ; D'Angelica MI; Kingham TP; DeMatteo RP; Klimstra DS; et al. Unresectable intrahepatic cholangiocarcinoma: Systemic plus hepatic arterial infusion chemotherapy is associated with longer survival in comparison with systemic chemotherapy alone. Cancer 2016, 122 (5), 758–765. [PubMed: 26695839]

(164). Li C; Gu Y; Zhao M; Yuan Y; Wang F; Wang Z; Li W; Luo H; Chen C; Chen G; et al. Phase I trial of hepatic arterial infusion (HAI) of floxuridine with modified oxaliplatin, 5-fluorouracil and leucovorin (m-FOLFOX6) in Chinese patients with unresectable liver metastases from colorectal cancer. Cancer Chemother. Pharmacol 2014, 74 (5), 1079–1087. [PubMed: 25217393]

(165). Ko YJ; Karanicolas PJ Hepatic arterial infusion pump chemotherapy for colorectal liver metastases: An old technology in a new era. Curr. Oncol 2014, 21 (1), e116–121. [PubMed: 24523608]

(166). Murakami Y; Kazuno H; Emura T; Tsujimoto H; Suzuki N; Fukushima M Different mechanisms of acquired resistance to fluorinated pyrimidines in human colorectal cancer cells. Int. J. Oncol 2000, 17 (2), 277–283. [PubMed: 10891536]

(167). Libra M; Navolanic PM; Talamini R; Cecchin E; Sartor F; Tumolo S; Masier S; Travali S; Boiocchi M; Toffoli G Thymidylate synthetase mRNA levels are increased in liver metastases of colorectal cancer patients resistant to fluoropyrimidine-based chemotherapy. BMC Cancer 2004, 4 (11), 1–6. [PubMed: 14725718]

(168). Beumer JH; Eiseman JL; Gilbert JA; Holleran JL; Yellow-Duke AE; Clausen DM; D'Argenio DZ; Ames MM; Hershberger PA; Parise RA; et al. Plasma pharmacokinetics and oral bioavailability of the 3,4,5,6-tetrahydrouridine (THU) prodrug, triacetyl-THU (taTHU), in mice. Cancer Chemother. Pharmacol 2011, 67 (2), 421–430. [PubMed: 20443002]

(169). Ebrahem Q; Mahfouz RZ; Ng KP; Saunthararajah Y High cytidine deaminase expression in the liver provides sanctuary for cancer cells from decitabine treatment effects. Oncotarget 2012, 3 (10), 1137–1145. [PubMed: 23087155]

(170). Funamizu N; Lacy CR; Fujita K; Furukawa K; Misawa T; Yanaga K; Manome Y Tetrahydrouridine inhibits cell proliferation through cell cycle regulation regardless of cytidine deaminase expression levels. PLoS One 2012, 7 (5), e37424. [PubMed: 22616006]

(171). Neil GL; Moxley TE; Manak RC Enhancement by tetrahydrouridine of 1-β-D-arabinofuranosylcytosine (cytarabine) oral activity in L1210 leukemic mice. Cancer Res 1970, 30 (8), 2166–2172. [PubMed: 4990003]

(172). Kelly CJ; Gaudio L; Yesair DW; Schoenemann PT; Wodinsky I Pharmacokinetic considerations in evaluating the effects of tetrahydrouridine on 5-azacytidine chemotherapy in L1210 leukemic mice. Cancer Treat. Rep 1978, 62 (7), 1025–1032. [PubMed: 80268]

(173). Laliberte J; Marquez VE; Momparler RL Potent inhibitors for the deamination of cytosine arabinoside and 5-aza-2′-deoxycytidine by human cytidine deaminase. Cancer Chemother. Pharmacol 1992, 30 (1), 7–11. [PubMed: 1375134]

(174). Beumer JH; Eiseman JL; Parise RA; Florian JA Jr.; Joseph E; D'Argenio DZ; Parker RS; Kay B; Covey JM; Egorin MJ Plasma pharmacokinetics and oral bioavailability of 3,4,5,6-tetrahydrouridine, a cytidine deaminase inhibitor, in mice. Cancer Chemother. Pharmacol 2008, 62 (3), 457–464. [PubMed: 18008070]

(175). Sugiyama E; Kaniwa N; Kim SR; Kikura-Hanajiri R; Hasegawa R; Maekawa K; Saito Y; Ozawa S; Sawada J; Kamatani N; et al. Pharmacokinetics of gemcitabine in Japanese cancer patients: the impact of a cytidine deaminase polymorphism. J. Clin. Oncol 2006, 25 (1), 32–42.

(176). Yonemori K; Ueno H; Okusaka T; Yamamoto N; Ikeda M; Saijo N; Yoshida T; Ishii H; Furuse J; Sugiyama E; et al. Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. Clin. Cancer Res 2005, 11 (7), 2620–2624. [PubMed: 15814642]

(177). Micozzi D; Carpi FM; Pucciarelli S; Polzonetti V; Polidori P; Vilar S; Williams B; Costanzi S; Vincenzetti S Human cytidine deaminase: a biochemical characterization of its naturally occurring variants. Int. J. Biol. Macromol 2014, 63, 64–74. [PubMed: 24183806]

(178). Beumer JH; Eiseman JL; Parise RA; Joseph E; Holleran JL; Covey JM; Egorin MJ Pharmacokinetics, metabolism, and oral bioavailability of the DNA methyltransferase inhibitor 5-fluoro-2′-deoxycytidine in mice. Clin. Cancer Res 2006, 12 (24), 7483–7491. [PubMed: 17138702]

(179). Kaysen J; Spriggs D; Kufe D Incorporation of 5-fluorodeoxycytidine and metabolites into nucleic acids of human MCF-7 breast carcinoma cells. Cancer Res 1986, 46 (9), 4534–4538. [PubMed: 2425957]

(180). Beumer JH; Joseph E; Egorin MJ; Covey JM; Eiseman JL Quantitative determination of zebularine (NSC 309132), a DNA methyltransferase inhibitor, and three metabolites in murine plasma by high-performance liquid chromatography coupled with on-line radioactivity detection. J. Chromatogr. B: Anal. Technol. Biomed. Life Sci 2006, 831 (1–2), 147–155.

(181). Morfouace M; Nimmervoll B; Boulos N; Patel YT; Shelat A; Freeman BB 3rd; Robinson GW; Wright K; Gajjar A; Stewart CF; et al. Preclinical studies of 5-fluoro-2′-deoxycytidine and tetrahydrouridine in pediatric brain tumors. J. Neuro-Oncol 2016, 126 (2), 225–234.

(182). Holleran JL; Eiseman JL; Parise RA; Kummar S; Beumer JH LC-MS/MS assay for the quantitation of FdCyd and its metabolites FdUrd and FU in human plasma. J. Pharm. Biomed. Anal 2016, 129, 359–366. [PubMed: 27454087]

(183). Newman EM; Morgan RJ; Kummar S; Beumer JH; Blanchard MS; Ruel C; El-Khoueiry AB; Carroll MI; Hou JM; Li C; et al. A phase I, pharmacokinetic, and pharmacodynamic evaluation of the DNA methyltransferase inhibitor 5-fluoro-2′-deoxycytidine, administered with tetrahydrouridine. Cancer Chemother. Pharmacol 2015, 75 (3), 537–546. [PubMed: 25567350]

(184). Holleran JL; Beumer JH; McCormick DL; Johnson WD; Newman EM; Doroshow JH; Kummar S; Covey JM; Davis M; Eiseman JL Oral and intravenous pharmacokinetics of 5-fluoro-2′-deoxycytidine and THU in cynomolgus monkeys and humans. Cancer Chemother. Pharmacol 2015, 76 (4), 803–811. [PubMed: 26321472]

(185). Ferraris D; Duvall B; Delahanty G; Mistry B; Alt J; Rojas C; Rowbottom C; Sanders K; Schuck E; Huang KC; et al. Design, synthesis, and pharmacological evaluation of fluorinated tetrahydrouridine derivatives as inhibitors of cytidine deaminase. J. Med. Chem 2014, 57 (6), 2582–2588. [PubMed: 24520856]

(186). Sakamoto J; Oba K; Matsui T; Kobayashi M Efficacy of oral anticancer agents for colorectal cancer. Dis. Colon Rectum 2006, 49 (10 Suppl), S82–91. [PubMed: 17106820]

(187). Hsu CH; Shen YC; Lin ZZ; Chen PJ; Shao YY; Ding YH; Hsu C; Cheng AL Phase II study of combining sorafenib with metronomic tegafur/uracil for advanced hepatocellular carcinoma. J. Hepatol 2010, 53 (1), 126–131. [PubMed: 20416968]

(188). Osugi H; Takada N; Takemura M; Kaseno S; Lee S; Ueno M; Tanaka Y; Fukuhara K; Fujiwara Y; Kinoshita H Oral fluoropyrimidine anticancer drug TS-1 for gastric cancer patients with peritoneal dissemination. Oncol. Rep 2002, 9 (4), 811–815. [PubMed: 12066214]

(189). Rich TA; Shepard RC; Mosley ST Four decades of continuing innovation with fluorouracil: current and future approaches to fluorouracil chemoradiation therapy. J. Clin. Oncol 2004, 22 (11), 2214–2232. [PubMed: 15169811]

(190). Utsugi T New challenges and inspired answers for anticancer drug discovery and development. Jpn. J. Clin. Oncol 2013, 43 (10), 945–953. [PubMed: 24014883]

(191). Yamada T; Okamura S; Okazaki T; Ushiroyama T; Yanagawa Y; Ueki M; Sugimoto O; Yamazaki H; Sugino M; Masui Y Leukoencephalopathy following treatment with carmofur: a

case report and review of the Japanese literature. Asia-Oceania J. Obstet. Gynaecol 1989, 15 (2), 161–168. [PubMed: 2667512]

(192). Mizutani T [Leukoencephalopathy caused by antineoplastic drugs]. Brain Nerve 2008, 60 (2), 137–141. [PubMed: 18306661]

(193). Baehring JM; Fulbright RK Delayed leukoencephalopathy with stroke-like presentation in chemotherapy recipients. J. Neurol., Neurosurg. Psychiatry 2008, 79 (5), 535–539. [PubMed: 17682013]

(194). Yamamoto M; Arii S; Sugahara K; Tobe T Adjuvant oral chemotherapy to prevent recurrence after curative resection for hepatocellular carcinoma. Br. J. Surg 1996, 83 (3), 336–340. [PubMed: 8665186]

(195). Lee CH; Wu CL; Shiau AL Hypoxia-induced cytosine deaminase gene expression for cancer therapy. Hum. Gene Ther 2007, 18 (1), 27–38. [PubMed: 17184154]

(196). Matuskova M; Baranovicova L; Kozovska Z; Durinikova E; Pastorakova A; Hunakova L; Waczulikova I; Nencka R; Kucerova L Intrinsic properties of tumour cells have a key impact on the bystander effect mediated by genetically engineered mesenchymal stromal cells. J. Gene Med 2012, 14 (12), 776–787. [PubMed: 23150190]

(197). Wheeler HL; Merriam HF Über einige kondensationsprodukte des pseudothioharnstoffes: Synthese von uracil, thymin und ähnlichen verbindungen. Am. Chem. J 1903, 29, 378.

(198). Dornow A; Boberg F; Schurer L Various condensations with chloroformylacetic acid ethyl ester. VIII. Synthesis of nitrogenous heterocycles. Arch. Pharm 1953, 286 (10), 494–501.

(199). Duschinsky R; Edward Pleven E; Heidelberger C The synthesis of 5-fluoropyrimides. J. Am. Chem. Soc 1957, 79 (16), 4559–4560.

(200). Robins MJ; MacCoss M; Naik SR; Ramani G Nucleic acid related compounds. 21. Direct fluorination of uracil and cytosine bases and nucleosides using trifluoromethyl hypofluorite. Mechanism, stereochemistry, and synthetic applications. J. Am. Chem. Soc 1976, 98 (23), 7381–7389. [PubMed: 977870]

(201). Yamazaki A; Morisawa H; Oda Y; Uchida K US Patent 4186266, 1980.

(202). Baasner B; Klauke E A new route to the synthesis of 5-fluorouracil. J. Fluorine Chem 1989, 45 (3), 417–430.

(203). Kamiya T; Ishiduka I; Nakajima H US Patent 5453497, 1995.

(204). Robins MJ; Naik SR A direct synthesis of 5-fluorocytosine and its nucleosides using trifluoromethyl hypofluorite. J. Chem. Soc., Chem. Commun 1972, No. 1, 18–19.

(205). Brinkman HR; Kalaritis P; Morrissey JF US Patent 5476932, 1995.

(206). Gore VG; Patkar LN; Bhalerao R; Hublikar MG; Pokharkar KS WO Patent 2011104540, 2011.

(207). Cook AF Fluorinated Pyrimidine nucleosides. 1. Synthesis of a nitrogen analogue of the antitumor agent 2,2′-anhydro-1-beta-D-arabinofuranosyl-5-fluorocytosine hydrochloride. J. Med. Chem 1977, 20 (3), 344–348. [PubMed: 66315]

(208). Cook AF; Holman MJ; Kramer MJ; Trown PW Fluorinated pyrimidine nucleosides. 3. Synthesis and antitumor activity of a series of 5′-deoxy-5-fluoropyrimidine nucleosides. J. Med. Chem 1979, 22 (11), 1330–1335. [PubMed: 533880]

(209). Shen B; Jamison TF Rapid continuous synthesis of 5′-deoxyribonucleosides in flow via Bronsted acid catalyzed glycosylation. Org. Lett 2012, 14 (13), 3348–3351. [PubMed: 22694250]

(210). Duschinsky R; Pleven E; Malbica J; Heidelberger C Synthesis of 5-fluorouracil nucleosides. 132nd Meeting Am. Chem. Soc., New York, 1957; p 19c.

(211). Fox JJ; Wempen I; Duschinsky R Nucleosides of 5-fluorocytosine. 4th Intl. Cong. of Biochem., Vienna, 1958; p 6.

(212). Hoffer M; Duschinsky R; Fox JJ; Yung N Simple syntheses of pyrimidine-2′-deoxy-ribonucleosides. J. Am. Chem. Soc 1959, 81 (15), 4112–4113.

(213). Hanze AR Nucleic acids. IV. The catalytic reduction of pyrimidine nucleosides (human liver deaminase inhibitors). J. Am. Chem. Soc 1967, 89 (25), 6720–6725. [PubMed: 6063658]

(214). Aoyama H Stereoselective synthesis of anomers of 5-substituted 2′-deoxyuridines. Bull. Chem. Soc. Jpn 1987, 60 (6), 2073–2077.

(215). Wang ZX; Duan W; Wiebe LI; Balzarini J; De Clercq E; Knaus EE Synthesis of 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-substituted-benzene thymidine mimics, some related alpha-anomers, and their evaluation as antiviral and anticancer agents. Nucleosides, Nucleotides Nucleic Acids 2001, 20 (1–2), 11–40. [PubMed: 11303557]

(216). Kotick MP; Szantay C; Bardos TJ Synthesis of 5-S-substituted 2′-deoxyuridines. Study of the factors influencing stereoselectivity of the silyl modification of the Hilbert-Johnson reaction. J. Org. Chem 1969, 34 (12), 3806–3813. [PubMed: 5357523]

(217). Yasumoto M; Marunaka T; Hashimoto S; Harima K; Suzue T JP Patent 76146482, 1976.

(218). Miyashita O; Masumura K; Shimadzu H; Hashimoto N Studies on fluorinated pyrimidines. I. A new method of synthesizing 5-fluorouracil and its derivatives. Chem. Pharm. Bull 1981, 29 (11), 3181–3190.

(219). Ozaki S; Ike Y; Mizuno H; Ishikawa K; Mori H The synthesis of 1-carbamoyl-5-fluorouracils. Bull. Chem. Soc. Jpn 1977, 50 (9), 2406–2412.

(220). Ozaki S Synthesis and antitumor activity of 5-fluorouracil derivatives. Med. Res. Rev 1996, 16 (1), 51–86. [PubMed: 8788214]

(221). Xiong J; Zhou S; Huang Z; Chao G; Wang L; Xu J CN Patent 102229571, 2011.

(222). Kolder CR; den Hertog HJ Synthesis and reactivity of 5-chloro-2,4-dihydroxypyridine. Rec. Trav. Chim 1953, 72, 285–295.

(223). Yano S; Ohno T; Ogawa K Convenient and practical synthesis of 5-chloro-4-hydroxy-2(1H)-pyridinone. Heterocycles 1993, 36, 145–148.

(224). Mittelbach M; Kastner G; Junek H Synthesen mit Nitrilen, 71. Mitt. Zur Synthese von 4-Hydroxynicotinsaure aus Butadiendicarbonitrilen. Arch. Pharm 1985, 318 (6), 481–486.

(225). Xu Y; Mao D; Zhang F CN Patent 1915976, 2007.

(226). Poje M; Sokolić-Maravić L The mechanism for the conversion of uric acid into allantoin and dehydro-allantoin: A new look at an old problem. Tetrahedron 1986, 42 (2), 747–751.

(227). Sugi M; Igi M EP Patent 0957096, 1999.

(228). Temmink OH; Emura T; de Bruin M; Fukushima M; Peters GJ Therapeutic potential of the dual-targeted TAS-102 formulation in the treatment of gastrointestinal malignancies. Cancer Sci 2007, 98 (6), 779–789. [PubMed: 17441963]

(229). Heidelberger C; Anderson SW Fluorinated pyrimidines. XXI. The tumor-inhibitory activity of 5-trifluoromethyl-2′-deoxyuridine. Cancer Res 1964, 24, 1979–1985. [PubMed: 14247510]

(230). Heidelberger C; Boohar J; Kampschroer B Fluorinated pyrimidines. XXIV. In vivo metabolism of 5-trifluoromethyluracil-2-C-14 and 5-trifluoromethyl-2′-deoxyuridine-2-C-14. Cancer Res 1965, 25, 377–381. [PubMed: 14281103]

(231). Heidelberger C; Parsons DG; Remy DC Syntheses of 5-trifluoromethyluracil and 5-trifluoromethyl-2′-deoxyuridine. J. Med. Chem 1964, 7, 1–5. [PubMed: 14186018]

(232). Dexter DL; Wolberg WH; Ansfield FJ; Helson L; Heidelberger C The clinical pharmacology of 5-trifluoromethyl-2′-deoxyuridine. Cancer Res 1972, 32 (2), 247–253. [PubMed: 4333494]

(233). Doi T; Ohtsu A; Yoshino T; Boku N; Onozawa Y; Fukutomi A; Hironaka S; Koizumi W; Sasaki T Phase I study of TAS-102 treatment in Japanese patients with advanced solid tumours. Br. J. Cancer 2012, 107 (3), 429–434. [PubMed: 22735906]

(234). Emura T; Nakagawa F; Fujioka A; Ohshimo H; Yokogawa T; Okabe H; Kitazato K An optimal dosing schedule for a novel combination antimetabolite, TAS-102, based on its intracellular metabolism and its incorporation into DNA. Int. J. Mol. Med 2004, 13 (2), 249–255. [PubMed: 14719131]

(235). Reyes P; Heidelberger C Fluorinated pyrimidines. XXVI. Mammalian thymidylate synthetase: its mechanism of action and inhibition by fluorinated nucleotides. Mol. Pharm 1965, 1 (1), 14–30.

(236). Eckstein JW; Foster PG; Finer-Moore J; Wataya Y; Santi DV Mechanism-based inhibition of thymidylate synthase by 5-(trifluoromethyl)-2′-deoxyuridine 5′-monophosphate. Biochemistry 1994, 33 (50), 15086–15094. [PubMed: 7999767]

(237). Peters GJ; Backus HH; Freemantle S; van Triest B; Codacci-Pisanelli G; van der Wilt CL; Smid K; Lunec J; Calvert AH; Marsh S; et al. Induction of thymidylate synthase as a 5-fluorouracil resistance mechanism. Biochim. Biophys. Acta, Mol. Basis Dis 2002, 1587 (2–3), 194–205.

(238). Emura T; Suzuki N; Yamaguchi M; Ohshimo H; Fukushima M A novel combination antimetabolite, TAS-102, exhibits antitumor activity in FU-resistant human cancer cells through a mechanism involving FTD incorporation in *DNA*. Int. J. Oncol 2004, 25 (3), 571–578. [PubMed: 15289858]

(239). Suzuki N; Emura T; Fukushima M Mode of action of trifluorothymidine (TFT) against DNA replication and repair enzymes. Int. J. Oncol 2011, 39 (1), 263–270. [PubMed: 21491084]

(240). Suzuki N; Nakagawa F; Nukatsuka M; Fukushima M Trifluorothymidine exhibits potent antitumor activity via the induction of DNA double-strand breaks. Exp. Ther. Med 2011, 2 (3), 393–397. [PubMed: 22977515]

(241). Markley JC; Chirakul P; Sologub D; Sigurdsson ST Incorporation of 2′-deoxy-5-(trifluoromethyl)uridine and 5-cyano-2′-deoxyuridine into DNA. Bioorg. Med. Chem. Lett 2001, 11 (18), 2453–2455. [PubMed: 11549445]

(242). Emura T; Suzuki N; Fujioka A; Ohshimo H; Fukushima M Potentiation of the antitumor activity of alpha, alpha, alpha-trifluorothymidine by the co-administration of an inhibitor of thymidine phosphorylase at a suitable molar ratio in vivo. Int. J. Oncol 2005, 27 (2), 449–455. [PubMed: 16010427]

(243). Fukushima M; Suzuki N; Emura T; Yano S; Kazuno H; Tada Y; Yamada Y; Asao T Structure and activity of specific inhibitors of thymidine phosphorylase to potentiate the function of antitumor 2′-deoxyribonucleosides. Biochem. Pharmacol 2000, 59 (10), 1227–1236. [PubMed: 10736423]

(244). Ursem C; Van Loon K; Venook A Adjuvant Therapy Trials. Cancer J 2016, 22 (3), 196–198. [PubMed: 27341598]

(245). De Clercq E Antiviral drugs in current clinical use. J. Clin. Virol 2004, 30 (2), 115–133. [PubMed: 15125867]

(246). De Clercq E Selective anti-herpesvirus agents. Antiviral Chem. Chemo 2013, 23 (3), 93–101.

(247). Komatsu H; Umetani H Synthesis of trifluorothymidine: Green tlycosylation condition using neither chloroform nor transition metals. Org. Process Res. Dev 2002, 6, 847–850.

(248). Hubbard AJ; Jones AS; Walker RT An investigation by 1H NMR spectroscopy into the factors determining the β:α ratio of the product in 2′-deoxynucleoside synthesis. Nucleic Acids Res 1984, 12 (17), 6827–6837. [PubMed: 6483621]

(249). Yano S; Kazuno H; Sato T; Suzuki N; Emura T; Wierzba K; Yamashita J; Tada Y; Yamada Y; Fukushima M; Asao T Synthesis and evaluation of 6-methylene-bridged uracil derivatives. Part 2: Optimization of inhibitors of human thymidine phosphorylase and their selectivity with uridine phosphorylase. Bioorg. Med. Chem 2004, 12, 3443–3450. [PubMed: 15186830]

(250). Moriconi EJ; Cevasco AA Synthesis and reactions of cyclic amidines. J. Org. Chem 1968, 33 (5), 2109–2111.

(251). Sun L; Li J; Bera H; Dolzhenko AV; Chiu GN; Chui WK Fragment-based approach to the design of 5-chlorouracil-linked-pyrazolo[1,5-a][1,3,5]triazines as thymidine phosphorylase inhibitors. Eur. J. Med. Chem 2013, 70, 400–410. [PubMed: 24177367]

(252). Yano S; Kazuno H; Suzuki N; Emura T; Wierzba K; Yamashita J; Tada Y; Yamada Y; Fukushima M; Asao T Synthesis and evaluation of 6-methylene-bridged uracil derivatives. Part 1: Discovery of novel orally active inhibitors of human thymidine phosphorylase. Bioorg. Med. Chem 2004, 12, 3431–3441. [PubMed: 15186829]

(253). Sorm F; Piskala A; Cihak A; Vesely J 5-azacytidine, a new, highly effective cancerostatic. Experientia 1964, 20 (4), 202–203.

(254). Sorm F; Vesely J Effect of 5-aza-2′-deoxycytidine against leukemic and hemopoietic tissues in AKR mice. Neoplasma 1968, 15 (4), 339–343. [PubMed: 5684460]

(255). Hubeek I; Stam RW; Peters GJ; Broekhuizen R; Meijerink JP; van Wering ER; Gibson BE; Creutzig U; Zwaan CM; Cloos J; et al. The human equilibrative nucleoside transporter 1 mediates in vitro cytarabine sensitivity in childhood acute myeloid leukaemia. Br. J. Cancer 2005, 93 (12), 1388–1394. [PubMed: 16333246]

(256). Bouchard J; Momparler RL Incorporation of 5-Aza-2′-deoxycytidine-5′-triphosphate into DNA. Interactions with mammalian DNA polymerase alpha and DNA methylase. Mol. Pharm 1983, 24 (1), 109–114.

(257). Singh V; Sharma P; Capalash N DNA methyltransferase-1 inhibitors as epigenetic therapy for cancer. Curr. Cancer Drug Targets 2013, 13 (4), 379–399. [PubMed: 23517596]

(258). Hamm CA; Costa FF Epigenomes as therapeutic targets. Pharmacol. Ther 2015, 151, 72–86. [PubMed: 25797698]

(259). Yoo J; Choi S; Medina-Franco JL Molecular modeling studies of the novel inhibitors of DNA methyltransferases SGI-1027 and CBC12: implications for the mechanism of inhibition of DNMTs. PLoS One 2013, 8 (4), e62152. [PubMed: 23637988]

(260). Galm O; Herman JG; Baylin SB The fundamental role of epigenetics in hematopoietic malignancies. Blood Rev 2006, 20 (1), 1–13. [PubMed: 16426940]

(261). Baylin SB DNA methylation and gene silencing in cancer. Nat. Clin. Pract. Oncol 2005, 2 (Suppl 1), S4–11. [PubMed: 16341240]

(262). Legendre CR; Demeure MJ; Whitsett TG; Gooden GC; Bussey KJ; Jung S; Waibhav T; Kim S; Salhia B Pathway implications of aberrant global methylation in adrenocortical cancer. PLoS One 2016, 11 (3), e0150629. [PubMed: 26963385]

(263). Wong KY; Chim CS DNA methylation of tumor suppressor protein-coding and non-coding genes in multiple myeloma. Epigenomics 2015, 7 (6), 985–1001. [PubMed: 26417914]

(264). Qin T; Si J; Raynal NJ; Wang X; Gharibyan V; Ahmed S; Hu X; Jin C; Lu Y; Shu J; et al. Epigenetic synergy between decitabine and platinum derivatives. Clin. Epigenet 2015, 7 (97), 1–14.

(265). Al-Salihi M; Yu M; Burnett DM; Alexander A; Samlowski WE; Fitzpatrick FA The depletion of DNA methyltransferase-1 and the epigenetic effects of 5-aza-2′deoxycytidine (decitabine) are differentially regulated by cell cycle progression. Epigenetics 2011, 6 (8), 1021–1028. [PubMed: 21725200]

(266). Stresemann C; Lyko F Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. Int. J. Cancer 2008, 123 (1), 8–13. [PubMed: 18425818]

(267). Takahashi A; Imai Y; Yamakoshi K; Kuninaka S; Ohtani N; Yoshimoto S; Hori S; Tachibana M; Anderton E; Takeuchi T; et al. DNA damage signaling triggers degradation of histone methyltransferases through APC/C(Cdh1) in senescent cells. Mol. Cell 2012, 45 (1), 123–131. [PubMed: 22178396]

(268). Ghoshal K; Datta J; Majumder S; Bai S; Kutay H; Motiwala T; Jacob ST 5-Aza-deoxycytidine induces selective degradation of DNA methyltransferase 1 by a proteasomal pathway that requires the KEN box, bromo-adjacent homology domain, and nuclear localization signal. Mol. Cell Biol 2005, 25 (11), 4727–4741. [PubMed: 15899874]

(269). Rogstad DK; Herring JL; Theruvathu JA; Burdzy A; Perry CC; Neidigh JW; Sowers LC Chemical decomposition of 5-aza-2′-deoxycytidine (Decitabine): kinetic analyses and identification of products by NMR, HPLC, and mass spectrometry. Chem. Res. Toxicol 2009, 22 (6), 1194–1204. [PubMed: 19480391]

(270). Gnyszka A; Jastrzebski Z; Flis S DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. Anticancer Res 2013, 33 (8), 2989–2996. [PubMed: 23898051]

(271). Qin T; Castoro R; El Ahdab S; Jelinek J; Wang X; Si J; Shu J; He R; Zhang N; Chung W; et al. Mechanisms of resistance to decitabine in the myelodysplastic syndrome. PLoS One 2011, 6 (8), e23372. [PubMed: 21858090]

(272). Saunthararajah Y; Hillery CA; Lavelle D; Molokie R; Dorn L; Bressler L; Gavazova S; Chen YH; Hoffman R; DeSimone J Effects of 5-aza-2′-deoxycytidine on fetal hemoglobin levels, red cell adhesion, and hematopoietic differentiation in patients with sickle cell disease. Blood 2003, 102 (12), 3865–3870. [PubMed: 12907443]

(273). Jones PA; Taylor SM Cellular differentiation, cytidine analogs and DNA methylation. Cell 1980, 20 (1), 85–93. [PubMed: 6156004]

(274). Jansen RS; Rosing H; Wijermans PW; Keizer RJ; Schellens JH; Beijnen JH Decitabine triphosphate levels in peripheral blood mononuclear cells from patients receiving prolonged low-

dose decitabine administration: a pilot study. Cancer Chemother. Pharmacol 2012, 69 (6), 1457–1466. [PubMed: 22382880]

(275). Lavelle D; Chin J; Vaitkus K; Redkar S; Phiasivongsa P; Tang C; Will R; Hankewych M; Roxas B; Singh M; et al. Oral decitabine reactivates expression of the methylated gamma-globin gene in Papio anubis. Am. J. Hematol 2007, 82 (11), 981–985. [PubMed: 17696208]

(276). Lavelle D; Vaitkus K; Ling Y; Ruiz MA; Mahfouz R; Ng KP; Negrotto S; Smith N; Terse P; Engelke KJ; et al. Effects of tetrahydrouridine on pharmacokinetics and pharmacodynamics of oral decitabine. Blood 2012, 119 (5), 1240–1247. [PubMed: 22160381]

(277). Plagemann PG; Behrens M; Abraham D Metabolism and cytotoxicity of 5-azacytidine in cultured Novikoff rat hepatoma and P388 mouse leukemia cells and their enhancement by preincubation with pyrazofurin. Cancer Res 1978, 38 (8), 2458–2466. [PubMed: 78761]

(278). Meininger V Clinical trials in ALS: what did we learn from recent trials in humans? Neurodegener. Dis 2005, 2 (3–4), 208–214. [PubMed: 16909027]

(279). Lee TT; Momparier RL Inhibition of uridine-cytidine kinase by 5-azacytidine 5′-triphosphate. Med. Pediatr. Oncol 1976, 2 (3), 265–270. [PubMed: 62274]

(280). Garcia-Manero G Myelodysplastic syndromes: 2014 update on diagnosis, risk-stratification, and management. Am. J. Hematol 2014, 89 (1), 97–108. [PubMed: 24464505]

(281). Raj K; Mufti GJ Azacytidine (Vidaza(R)) in the treatment of myelodysplastic syndromes. Ther. Clin. Risk. Manag 2006, 2 (4), 377–388. [PubMed: 18360650]

(282). Van Camp JA Decitabine (Dacogen): A DNA methyltransferace inhibitor for cancer; Wiley: Hoboken, NJ, 2010; pp 47–55.

(283). Fanciullino R; Mercier C; Serdjebi C; Venton G; Colle J; Fina F; Ouafik L; Lacarelle B; Ciccolini J; Costello R Yin and yang of cytidine deaminase roles in clinical response to azacitidine in the elderly: a pharmacogenetics tale. Pharmacogenomics 2015, 16 (17), 1907–1912. [PubMed: 26556583]

(284). Fanciullino R; Mercier C; Serdjebi C; Berda Y; Fina F; Ouafik L; Lacarelle B; Ciccolini J; Costello R Lethal toxicity after administration of azacytidine: implication of the cytidine deaminase-deficiency syndrome. Pharmacogenet. Genomics 2015, 25 (6), 317–321. [PubMed: 25850965]

(285). Serdjebi C; Milano G; Ciccolini J Role of cytidine deaminase in toxicity and efficacy of nucleosidic analogs. Expert Opin. Drug Metab. Toxicol 2015, 11 (5), 665–672. [PubMed: 25495470]

(286). Brueckner B; Rius M; Markelova MR; Fichtner I; Hals PA; Sandvold ML; Lyko F Delivery of 5-azacytidine to human cancer cells by elaidic acid esterification increases therapeutic drug efficacy. Mol. Cancer Ther 2010, 9 (5), 1256–1264. [PubMed: 20442313]

(287). Hummel-Eisenbeiss J; Hascher A; Hals PA; Sandvold ML; Muller-Tidow C; Lyko F; Rius M The role of human equilibrative nucleoside transporter 1 on the cellular transport of the DNA methyltransferase inhibitors 5-azacytidine and CP-4200 in human leukemia cells. Mol. Pharmacol 2013, 84 (3), 438–450. [PubMed: 23814180]

(288). Chabner BA; Drake JC; Johns DG Deamination of 5-azacytidine by a human leukemia cell cytidine deaminase. Biochem. Pharmacol 1973, 22 (21), 2763–2765. [PubMed: 4128550]

(289). Scholl J; Joshi-Hangal R; Inloes R; Shi C; Tavema P; Choy GS; Redkar S; Azab M SGI-110, a novel second generation potent DNA methylation inhibitor, in development for the treatment of MDS and AML. Preclinical safety, pharmacokinetics, and DNA methylation results of a low volume subcutaneous (SC) formulation. Blood 2010, 116, 1872.

(290). Tellez CS; Grimes MJ; Picchi MA; Liu Y; March TH; Reed MD; Oganesian A; Taverna P; Belinsky SA SGI-110 and entinostat therapy reduces lung tumor burden and reprograms the epigenome. Int. J. Cancer 2014, 135 (9), 2223–2231. [PubMed: 24668305]

(291). Coral S; Parisi G; Nicolay HJ; Colizzi F; Danielli R; Fratta E; Covre A; Taverna P; Sigalotti L; Maio M Immunomodulatory activity of SGI-110, a 5-aza-2′-deoxycytidine-containing demethylating dinucleotide. Cancer Immunol. Immunother 2013, 62 (3), 605–614. [PubMed: 23138873]

(292). Srivastava P; Paluch BE; Matsuzaki J; James SR; Collamat-Lai G; Karbach J; Nemeth MJ; Taverna P; Karpf AR; Griffiths EA Immunomodulatory action of SGI-110, a hypomethylating

agent, in acute myeloid leukemia cells and xenografts. Leuk. Res 2014, 38 (11), 1332–1341. [PubMed: 25260825]

(293). Fang F; Munck J; Tang J; Taverna P; Wang Y; Miller DF; Pilrose J; Choy G; Azab M; Pawelczak KS; et al. The novel, small-molecule DNA methylation inhibitor SGI-110 as an ovarian cancer chemosensitizer. Clin. Cancer Res 2014, 20 (24), 6504–6516. [PubMed: 25316809]

(294). Covre A; Parisi G; Nicolay HJ; Fonsatti E; Fratta E; Sigalotti L; Taverna P; Coral S; Maio M In vivo immunomodulatory activity of SGI-110, a second generation hypomethylating agent, in hematologic malignancies. AACR 104th Annual Meeting, Washington, DC, 2013.

(295). Píškala A; Šorm F Nucleic acids components and their analogues. LI. Synthesis of 1-glycosyl derivatives of 5-azauracil and 5-azacytosine. Collect. Czech. Chem. Commun 1964, 29 (9), 2060–2076.

(296). Ionescu D; Blumbergs P US Patent 7038038, 2006.

(297). Vujjini SK; Varanasi G; Arevelli S; Kandala SC; Tirumalaraju SR; Bandichhor R; Kagga M; Cherukupally P An improved and scalable process for the synthesis of 5-azacytidine: An antineoplastic drug. Org. Process Res. Dev 2013, 17, 303–306.

(298). Pliml J;Šorm F Synthesis of a 2-deoxy-D-ribofuranosyl-5-azacytosine. Collect. Czech. Chem. Commun 1964, 29 (10), 2576–2578.

(299). Winkley MW; Robins RK Direct glycosylation of 1,3,5-triazinones. A new approach to the synthesis of the nucleoside antibiotic 5-azacytidine (4-amino-1-beta-D-ribofuranosyl-1,3,5-triazin-2-one) and related derivatives. J. Org. Chem 1970, 35 (2), 491–495. [PubMed: 5412138]

(300). Lin KT; Momparler RL; Rivard GE High-performance liquid chromatographic analysis of chemical stability of 5-aza-2′-deoxycytidine. J. Pharm. Sci 1981, 70 (11), 1228–1232. [PubMed: 6170748]

(301). Henschke JP; Zhang X; Yu J; Hu K; Mei L US Patent 8586729, 2013.

(302). Kolla NK; Neelam UK; Baddam SR; Gangula S EP Patent 2424845, 2012.

(303). Redkar S. Scale up and development of a process for a low volume subcutaneous formulation of SGI-110, a potent hypomethylating agent. AACR Annual Meeting 2012; Chicago, IL. 2012.

(304). Hertel LW; Boder GB; Kroin JS; Rinzel SM; Poore GA; Todd GC; Grindey GB Evaluation of the antitumor activity of gemcitabine (2′,2′-difluoro-2′-deoxycytidine). Cancer Res 1990, 50 (14), 4417–4422. [PubMed: 2364394]

(305). Abratt RP; Bezwoda WR; Falkson G; Goedhals L; Hacking D; Rugg TA Efficacy and safety profile of gemcitabine in non-small-cell lung cancer: a phase II study. J. Clin. Oncol 1994, 12 (8), 1535–1540. [PubMed: 8040664]

(306). Heinemann V Gemcitabine: progress in the treatment of pancreatic cancer. Oncology 2001, 60 (1), 8–18. [PubMed: 11150902]

(307). Ansari D; Tingstedt B; Andersson R Pancreatic cancer - cost for overtreatment with gemcitabine. Acta Oncol 2013, 52 (6), 1146–1151. [PubMed: 23244671]

(308). Ritzel MW; Ng AM; Yao SY; Graham K; Loewen SK; Smith KM; Hyde RJ; Karpinski E; Cass CE; Baldwin SA; et al. Recent molecular advances in studies of the concentrative Na +-dependent nucleoside transporter (CNT) family: identification and characterization of novel human and mouse proteins (hCNT3 and mCNT3) broadly selective for purine and pyrimidine nucleosides (system cib). Mol. Membr. Biol 2001, 18 (1), 65–72. [PubMed: 11396613]

(309). Garcia-Manteiga J; Molina-Arcas M; Casado FJ; Mazo A; Pastor-Anglada M Nucleoside transporter profiles in human pancreatic cancer cells: role of hCNT1 in 2′,2′-difluorodeoxycytidine- induced cytotoxicity. Clin. Cancer Res 2003, 9 (13), 5000–5008. [PubMed: 14581375]

(310). Perez-Torras S; Garcia-Manteiga J; Mercade E; Casado FJ; Carbo N; Pastor-Anglada M; Mazo A Adenoviral-mediated overexpression of human equilibrative nucleoside transporter 1 (hENT1) enhances gemcitabine response in human pancreatic cancer. Biochem. Pharmacol 2008, 76 (3), 322–329. [PubMed: 18589402]

(311). Bergman AM; Peters GJ Gemcitabine; Humana Press: Totowa, NJ, 2006; pp 225–251.

(312). Huang P; Chubb S; Hertel LW; Grindey GB; Plunkett W Action of 2′,2′-difluorodeoxycytidine on DNA synthesis. Cancer Res 1991, 51 (22), 6110–6117. [PubMed: 1718594]

(313). Mini E; Nobili S; Caciagli B; Landini I; Mazzei T Cellular pharmacology of gemcitabine. Ann. Oncol 2006, 17 (Suppl 5), v7–12. [PubMed: 16807468]

(314). Wickremsinhe ER; Lutzke BS; Jones BR; Schultz GA; Freeman AB; Pratt SE; Bones AM; Ackermann BL Quantification of gemcitabine incorporation into human DNA by LC/MS/MS as a surrogate measure for target engagement. Anal. Chem 2010, 82 (15), 6576–6583. [PubMed: 20698580]

(315). Ruiz van Haperen VW; Veerman G; Vermorken JB; Peters GJ 2′,2′-Difluoro-deoxycytidine (gemcitabine) incorporation into RNA and DNA of tumour cell lines. Biochem. Pharmacol 1993, 46 (4), 762–766. [PubMed: 8363650]

(316). Modrak DE; Cardillo TM; Newsome GA; Goldenberg DM; Gold DV Synergistic interaction between sphingomyelin and gemcitabine potentiates ceramide-mediated apoptosis in pancreatic cancer. Cancer Res 2004, 64 (22), 8405–8410. [PubMed: 15548711]

(317). Heinemann V; Xu YZ; Chubb S; Sen A; Hertel LW; Grindey GB; Plunkett W Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2′,2′-difluorodeoxycytidine. Mol. Pharm 1990, 38 (4), 567–572.

(318). Muggia F; Diaz I; Peters GJ Nucleoside and nucleobase analogs in cancer treatment: not only sapacitabine, but also gemcitabine. Expert Opin. Invest. Drugs 2012, 21 (4), 403–408.

(319). Plunkett W; Huang P; Searcy CE; Gandhi V Gemcitabine: preclinical pharmacology and mechanisms of action. Semin. Oncol 1996, 23 (5 Suppl 10), 3–15.

(320). Cerqueira NM; Fernandes PA; Ramos MJ Understanding ribonucleotide reductase inactivation by gemcitabine. Chem. - Eur. J 2007, 13 (30), 8507–8515. [PubMed: 17636467]

(321). Morgan MA; Parsels LA; Maybaum J; Lawrence TS Improving gemcitabine-mediated radiosensitization using molecularly targeted therapy: a review. Clin. Cancer Res 2008, 14 (21), 6744–6750. [PubMed: 18980967]

(322). Gilbert JA; Salavaggione OE; Ji Y; Pelleymounter LL; Eckloff BW; Wieben ED; Ames MM; Weinshilboum RM Gemcitabine pharmacogenomics: cytidine deaminase and deoxycytidylate deaminase gene resequencing and functional genomics. Clin. Cancer Res 2006, 12 (6), 1794–1803. [PubMed: 16551864]

(323). Hodge LS; Taub ME; Tracy TS The deaminated metabolite of gemcitabine, 2′,2′-difluorodeoxyuridine, modulates the rate of gemcitabine transport and intracellular phosphorylation via 2′-deoxycytidine kinase. Drug Metab. Dispos 2011, 39 (11), 2013–2016. [PubMed: 21832002]

(324). Adema AD; Losekoot N; Smid K; Kathmann I; Myhren F; Sandvold ML; Peters GJ Induction of resistance to the lipophilic cytarabine prodrug elacytarabine (CP-4055) in CEM leukemic cells. Nucleosides, Nucleotides Nucleic Acids 2010, 29 (4–6), 394–399. [PubMed: 20544525]

(325). Aksoy P; Zhu MJ; Kalari KR; Moon I; Pelleymounter LL; Eckloff BW; Wieben ED; Yee VC; Weinshilboum RM; Wang L Cytosolic 5′-nucleotidase III (NT5C3): Gene sequence variation and functional genomics. Pharmacogenet. Genomics 2009, 19 (8), 567–576. [PubMed: 19623099]

(326). Hagmann W; Jesnowski R; Lohr JM Interdependence of gemcitabine treatment, transporter expression, and resistance in human pancreatic carcinoma cells. Neoplasia 2010, 12 (9), 740–747. [PubMed: 20824050]

(327). Pan MR; Hsu MC; Chen LT; Hung WC G9a orchestrates PCL3 and KDM7A to promote histone H3K27 methylation. Sci. Rep 2015, 5, 18709. [PubMed: 26688070]

(328). Garcia-Cano J; Roche O; Cimas FJ; Pascual-Serra R; Ortega-Muelas M; Fernandez-Aroca DM; Sanchez-Prieto R p38MAPK and chemotherapy: We always need to hear both sides of the story. Front. Cell Dev. Biol 2016, 4 (69), 1–8. [PubMed: 26858948]

(329). Wu Q; Wang X; Liu J; Zheng J; Liu Y; Li Y; Su F; Ou W; Wang R Nutlin-3 reverses the epithelial-mesenchymal transition in gemcitabine-resistant hepatocellular carcinoma cells. Oncol. Rep 2016, 36 (3), 1325–1332. [PubMed: 27430152]

(330). Hayes GM; Carrigan PE; Beck AM; Miller LJ Targeting the RNA splicing machinery as a novel treatment strategy for pancreatic carcinoma. Cancer Res 2006, 66 (7), 3819–3827. [PubMed: 16585209]

(331). Serdjebi C; Gagniere J; Desrame J; Fein F; Guimbaud R; Francois E; Andre T; Seitz JF; Monterymard C; Arsene D; et al. FFCD-1004 clinical trial: Impact of cytidine deaminase activity

on clinical outcome in gemcitabine-monotherapy treated patients. PLoS One 2015, 10 (8), e0135907. [PubMed: 26308942]

(332). Serdjebi C; Seitz JF; Ciccolini J; Duluc M; Norguet E; Fina F; Lacarelle B; Ouafik L; Dahan L Rapid deaminator status is associated with poor clinical outcome in pancreatic cancer patients treated with a gemcitabine-based regimen. Pharmacogenomics 2013, 14 (9), 1047–1051. [PubMed: 23837479]

(333). Bepler G; Kusmartseva I; Sharma S; Gautam A; Cantor A; Sharma A; Simon G RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non-small-cell lung cancer. J. Clin. Oncol 2006, 24 (29), 4731–4737. [PubMed: 16966686]

(334). Rosell R; Danenberg KD; Alberola V; Bepler G; Sanchez JJ; Camps C; Provencio M; Isla D; Taron M; Diz P; et al. Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. Clin. Cancer Res 2004, 10 (4), 1318–1325. [PubMed: 14977831]

(335). Duxbury MS; Ito H; Benoit E; Waseem T; Ashley SW; Whang EE RNA interference demonstrates a novel role for integrin-linked kinase as a determinant of pancreatic adenocarcinoma cell gemcitabine chemoresistance. Clin. Cancer Res 2005, 11 (9), 3433–3438. [PubMed: 15867245]

(336). Noble S; Goa KL Gemcitabine. A review of its pharmacology and clinical potential in non-small cell lung cancer and pancreatic cancer. Drugs 1997, 54 (3), 447–472. [PubMed: 9279506]

(337). Barton-Burke M Gemcitabine: a pharmacologic and clinical overview. Cancer Nursing 1999, 22 (2), 176–183. [PubMed: 10217035]

(338). Toschi L; Finocchiaro G; Bartolini S; Gioia V; Cappuzzo F Role of gemcitabine in cancer therapy. Future Oncol 2005, 1 (1), 7–17. [PubMed: 16555971]

(339). Sun C; Ansari D; Andersson R; Wu DQ Does gemcitabine-based combination therapy improve the prognosis of unresectable pancreatic cancer? World J. Gastroenterol 2012, 18 (35), 4944–4958. [PubMed: 23002368]

(340). Ciliberto D; Botta C; Correale P; Rossi M; Caraglia M; Tassone P; Tagliaferri P Role of gemcitabine-based combination therapy in the management of advanced pancreatic cancer: a meta-analysis of randomised trials. Eur. J. Cancer 2013, 49 (3), 593–603. [PubMed: 22989511]

(341). Qi WX; Tang LN; He AN; Shen Z; Lin F; Yao Y Doublet versus single cytotoxic agent as first-line treatment for elderly patients with advanced non-small-cell lung cancer: a systematic review and meta-analysis. Lung 2012, 190 (5), 477–485. [PubMed: 22711060]

(342). Bai B; Huang HQ; Cai QQ; Wang XX; Cai QC; Lin ZX; Gao Y; Xia Y; Bu Q; Guo Y Promising long-term outcome of gemcitabine, vinorelbine, liposomal doxorubicin (GVD) in 14-day schedule as salvage regimen for patients with previously heavily treated Hodgkin's lymphoma and aggressive non-Hodgkin's lymphoma. Med. Oncol 2013, 30 (1), 350. [PubMed: 23329307]

(343). Chan SL; Chan ST; Chan EH; He ZX Systemic treatment for inoperable pancreatic adenocarcinoma: review and update. Aizheng 2014, 33 (6), 267–276.

(344). Wagner AD; Buechner-Steudel P; Moehler M; Schmalenberg H; Behrens R; Fahlke J; Wein A; Behl S; Kuss O; Kleber G; et al. Gemcitabine, oxaliplatin and 5-FU in advanced bile duct and gallbladder carcinoma: two parallel, multicentre phase-II trials. Br. J. Cancer 2009, 101 (11), 1846–1852. [PubMed: 19904267]

(345). Aurilio G; Macarulla T; Ramos JF; Fazio N; Nole F; Iglesias C Successful treatment with GEMOX in patient with metastatic pancreatic adenosquamous carcinoma. Tumori 2011, 97 (2), 239–242. [PubMed: 21617724]

(346). Fang P; Hu JH; Cheng ZG; Liu ZF; Wang JL; Jiao SC Efficacy and safety of bevacizumab for the treatment of advanced hepatocellular carcinoma: a systematic review of phase II trials. PLoS One 2012, 7 (12), e49717. [PubMed: 23284624]

(347). Prince SK; Forgeson G Bleeding from gastrointestinal tract recurrence of non-seminomatous germ cell tumour testis, showing temporary response to gemcitabine and oxaliplatin chemotherapy. N. Z. Med. J 2013, 126 (1385), 76–80. [PubMed: 24217594]

(348). Vici P; Sergi D; Pizzuti L; Mariani L; Arena MG; Barba M; Maugeri-Sacca M; Vincenzoni C; Vizza E; Corrado G; et al. Gemcitabine-oxaliplatin (GEMOX) as salvage treatment in pretreated

epithelial ovarian cancer patients. J. Exp. Clin. Cancer Res 2013, 32 (1), 49. [PubMed: 23927758]

(349). Jordheim LP; Durantel D; Zoulim F; Dumontet C Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. Nat. Rev. Drug Discovery 2013, 12 (6), 447–464. [PubMed: 23722347]

(350). Bender DM; Bao J; Dantzig AH; Diseroad WD; Law KL; Magnus NA; Peterson JA; Perkins EJ; Pu YJ; Reutzel-Edens SM; Remick DM; Starling JJ; Stephenson GA; Vaid RK; Zhang D; McCarthy JR Synthesis, crystallization, and biological evaluation of an orally active prodrug of gemcitabine. J. Med. Chem 2009, 52 (22), 6958–6961. [PubMed: 19860433]

(351). Pratt SE; Durland-Busbice S; Shepard RL; Heinz-Taheny K; Iversen PW; Dantzig AH Human carboxylesterase-2 hydrolyzes the prodrug of gemcitabine (LY2334737) and confers prodrug sensitivity to cancer cells. Clin. Cancer Res 2013, 19 (5), 1159–1168. [PubMed: 23325581]

(352). Pratt SE; Durland-Busbice S; Shepard RL; Donoho GP; Starling JJ; Wickremsinhe ER; Perkins EJ; Dantzig AH Efficacy of low-dose oral metronomic dosing of the prodrug of gemcitabine, LY2334737, in human tumor xenografts. Mol. Cancer Ther 2013, 12 (4), 481–490. [PubMed: 23371859]

(353). Koolen SL; Witteveen PO; Jansen RS; Langenberg MH; Kronemeijer RH; Nol A; Garcia-Ribas I; Callies S; Benhadji KA; Slapak CA; et al. Phase I study of oral gemcitabine prodrug (LY2334737) alone and in combination with erlotinib in patients with advanced solid tumors. Clin. Cancer Res 2011, 17 (18), 6071–6082. [PubMed: 21753156]

(354). Williams R Discontinued in 2013: Oncology drugs. Expert Opin. Invest. Drugs 2015, 24, 95–110.

(355). Infante JR; Benhadji KA; Dy GK; Fetterly G; Ma WW; Bendell J; Callies S; Adjei AA Phase 1b study of the oral gemcitabine 'pro-drug' LY2334737 in combination with capecitabine in patients with advanced solid tumors. Invest. New Drugs 2015, 33 (2), 432–439. [PubMed: 25640850]

(356). Bergman AM; Adema AD; Balzarini J; Bruheim S; Fichtner I; Noordhuis P; Fodstad O; Myhren F; Sandvold ML; Hendriks HR; et al. Antiproliferative activity, mechanism of action and oral antitumor activity of CP-4126, a fatty acid derivative of gemcitabine, in in vitro and in vivo tumor models. Invest. New Drugs 2011, 29 (3), 456–466. [PubMed: 20066470]

(357). Stuurman FE; Voest EE; Awada A; Witteveen PO; Bergeland T; Hals PA; Rasch W; Schellens JH; Hendlisz A Phase I study of oral CP-4126, a gemcitabine derivative, in patients with advanced solid tumors. Invest. New Drugs 2013, 31 (4), 959–966. [PubMed: 23345000]

(358). Poplin E; Wasan H; Rolfe L; Raponi M; Ikdahl T; Bondarenko I; Davidenko I; Bondar V; Garin A; Boeck S; et al. Randomized, multicenter, phase II study of CO-101 versus gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma: including a prospective evaluation of the role of hENT1 in gemcitabine or CO-101 sensitivity. J. Clin. Oncol 2013, 31 (35), 4453–4461. [PubMed: 24220555]

(359). Stuurman FE; Lolkema MP; Huitema AD; Soetekouw PM; Rosing H; Rolfe L; Kaur P; Beijnen JH; van Tinteren H; Voest EE; et al. A phase 1 comparative pharmacokinetic and cardiac safety study of two intravenous formulations of CO-101 in patients with advanced solid tumors. J. Clin. Pharmacol 2013, 53 (8), 878–883. [PubMed: 23775853]

(360). Venugopal B; Awada A; Evans TR; Dueland S; Hendlisz A; Rasch W; Hernes K; Hagen S; Aamdal S A first-in-human phase I and pharmacokinetic study of CP-4126 (CO-101), a nucleoside analogue, in patients with advanced solid tumours. Cancer Chemother. Pharmacol 2015, 76 (4), 785–792. [PubMed: 26289594]

(361). Malfanti A; Miletto I; Bottinelli E; Zonari D; Blandino G; Berlier G; Arpicco S Delivery of gemcitabine prodrugs employing mesoporous silica nanoparticles. Molecules 2016, 21 (4), 522. [PubMed: 27110750]

(362). Tsume Y; Incecayir T; Song X; Hilfinger JM; Amidon GL The development of orally administrable gemcitabine prodrugs with D-enantiomer amino acids: enhanced membrane permeability and enzymatic stability. Eur. J. Pharm. Biopharm 2014, 86 (3), 514–523. [PubMed: 24361461]

(363). Dasari M; Acharya AP; Kim D; Lee S; Rhea J; Molinaro R; Murthy N H-gemcitabine: a new gemcitabine prodrug for treating cancer. Bioconjugate Chem 2013, 24 (1), 4–8.

(364). Wi LI US Patent 20140134160, 2014.

(365). Mehellou Y; Balzarini J; McGuigan C Aryloxy phosphoramidate triesters: a technology for delivering monophosphorylated nucleosides and sugars into cells. ChemMedChem 2009, 4 (11), 1779–1791. [PubMed: 19760699]

(366). Saif MW; Lee Y; Kim R Harnessing gemcitabine metabolism: a step towards personalized medicine for pancreatic cancer. Ther. Adv. Med. Oncol 2012, 4 (6), 341–346. [PubMed: 23118809]

(367). McGuigan C; Habib N; Wasan H; Gabra H; Jiao L; Slusarczyk M; Chabot J; Saif MW A phosphoramidate ProTide (NUC-1031) and acquired and intrinsic resistance to gemcitabine. 2011 ASCO Annual Meeting, Chicago, IL, 2011.

(368). Slusarczyk M; Lopez MH; Balzarini J; Mason M; Jiang WG; Blagden S; Thompson E; Ghazaly E; McGuigan C Application of ProTide technology to gemcitabine: a successful approach to overcome the key cancer resistance mechanisms leads to a new agent (NUC-1031) in clinical development. J. Med. Chem 2014, 57 (4), 1531–1542. [PubMed: 24471998]

(369). Ghazaly EA; Simon J; Gribben JG; Mohammad T; Oluwadunni E; Stavraka C; Hopkins T; Gabra H; harpreet W; Habib NAet al. proGem1: Phase 1 first-in-human study of the novel nucleotide analogue NUC-1031 in adult patients with advanced solid tumors. ASCO Annual Meeting, Chicago, IL, 2013.

(370). Hertel LW US Patent 4808614, 1989.

(371). Chang Y-K; Lee J; Park G-S; Lee M; Park CH; Kim HK; Lee G; Lee B-Y; Baek JY; Kim KS An efficient large-scale synthesis of gemcitabine employing a crystalline 2,2-difluoro-α-ribofuranosyl bromide. Tetrahedron 2010, 66, 5687–5691.

(372). Jiang X; Li J; Zhang R; Zhu Y; Shen J An improved preparation process for gemcitabine. Org. Process Res. Dev 2008, 12, 888–891.

(373). Myhren F; Borretzen B; Dalen A; Sandvold M EP Patent 0986570, 2004.

(374). McGuigan C US Patent 20060142238, 2005.

(375). Dammalapati VLNR; Kotala MB; Madhabaram S WO Patent 2016012781, 2016.

(376). Matsuda A; Nakajima Y; Azuma A; Tanaka M; Sasaki T Nucleosides and nucleotides. 100. 2′-C-cyano-2′-deoxy-1-β-D-arabinofuranosyl-cytosine (CNDAC): design of a potential mechanism-based DNA-strand-breaking antineoplastic nucleoside. J. Med. Chem 1991, 34 (9), 2917–2919. [PubMed: 1895307]

(377). Tener GM 2-Cyanoethyl phosphate and its use in the synthesis of phosphate esters. J. Am. Chem. Soc 1961, 83, 159–168.

(378). Liu XJ; Nowak B; Wang YQ; Plunkett W Sapacitabine, the prodrug of CNDAC, is a nucleoside analog with a unique action mechanism of inducing DNA strand breaks. Aizheng 2012, 31 (8), 373–380.

(379). Hanaoka K; Suzuki M; Kobayashi T; Tanzawa F; Tanaka K; Shibayama T; Miura S; Ikeda T; Iwabuchi H; Nakagawa A; et al. Antitumor activity and novel DNA-self-strand-breaking mechanism of CNDAC (1-(2-C-cyano-2-deoxy-β-D-arabino-pentofuranosyl) cyto-sine) and its N4-palmitoyl derivative (CS-682). Int. J. Cancer 1999, 82 (2), 226–236. [PubMed: 10389757]

(380). Yoshida T; Endo Y; Obata T; Kosugi Y; Sakamoto K; Sasaki T Influence of cytidine deaminase on antitumor activity of 2′-deoxycytidine analogs in vitro and in vivo. Drug Metab. Dispos 2010, 38 (10), 1814–1819. [PubMed: 20587622]

(381). Serova M; Galmarini CM; Ghoul A; Benhadji K; Green SR; Chiao J; Faivre S; Cvitkovic E; Le Tourneau C; Calvo F; et al. Antiproliferative effects of sapacitabine (CYC682), a novel 2′-deoxycytidine-derivative, in human cancer cells. Br. J. Cancer 2007, 97 (5), 628–636. [PubMed: 17637678]

(382). Azuma A; Huang P; Matsuda A; Plunkett W 2′-C-cyano-2′-deoxy-1-β-D-arabino-pentofuranosylcytosine: a novel anticancer nucleoside analog that causes both DNA strand breaks and G(2) arrest. Mol. Pharm 2001, 59 (4), 725–731.

(383). Liu X; Guo Y; Li Y; Jiang Y; Chubb S; Azuma A; Huang P; Matsuda A; Hittelman W; Plunkett W Molecular basis for G2 arrest induced by 2′-C-cyano-2′-deoxy-1-β-D-arabino-pentofuranosylcytosine and consequences of checkpoint abrogation. Cancer Res 2005, 65 (15), 6874–6881. [PubMed: 16061671]

(384). Wang Y; Liu X; Matsuda A; Plunkett W Repair of 2′-C-cyano-2′-deoxy-1-β-D-arabino-pentofuranosylcytosine-induced DNA single-strand breaks by transcription-coupled nucleotide excision repair. Cancer Res 2008, 68 (10), 3881–3889. [PubMed: 18483273]

(385). Liu X; Wang Y; Benaissa S; Matsuda A; Kantarjian H; Estrov Z; Plunkett W Homologous recombination as a resistance mechanism to replication-induced double-strand breaks caused by the antileukemia agent CNDAC. Blood 2010, 116 (10), 1737–1746. [PubMed: 20479284]

(386). Arnaudeau C; Lundin C; Helleday T DNA double-strand breaks associated with replication forks are predominantly repaired by homologous recombination involving an exchange mechanism in mammalian cells. J. Mol. Biol 2001, 307 (5), 1235–1245. [PubMed: 11292338]

(387). Liu X; Kantarjian H; Plunkett W Sapacitabine for cancer. Expert Opin. Invest. Drugs 2012, 21 (4), 541–555.

(388). Kantarjian H; Faderl S; Garcia-Manero G; Luger S; Venugopal P; Maness L; Wetzler M; Coutre S; Stock W; Claxton D; et al. Oral sapacitabine for the treatment of acute myeloid leukaemia in elderly patients: a randomised phase 2 study. Lancet Oncol 2012, 13 (11), 1096–1104. [PubMed: 23075701]

(389). Hansske F; Madej D; Robins MJ 2′ and 3′-ketonucleosides and their arabino and xylo reduction products: Convenient access via selective protection and oxidation of ribonucleosides. Tetrahedron 1984, 40 (1), 125–135.

(390). Kaneko M; Hotoda H; Shibata T; Kobayashi T; Mitsuhashi Y; Matsuda A; Sasaki T EP Patent 0536936, 1996.

(391). Westwood R; Wood G; Meades CK GB Patent 2459779, 2009.

(392). Wood GJ; Westwood R WO Patent 2009136162, 2009.

(393). Hattori H; Nozawa E; Iino T; Yoshimura Y; Shuto S; Shimamoto Y; Nomura M; Fukushima M; Tanaka M; Sasaki T; et al. Nucleosides and nucleotides. 175. Structural requirements of the sugar moiety for the antitumor activities of new nucleoside antimetabolites, 1-(3-C-ethynyl-β-D-ribo-pentofuranosyl)cytosine and -uracil. J. Med. Chem 1998, 41 (15), 2892–2902. [PubMed: 9667977]

(394). Hammond-Thelin LA; Thomas MB; Iwasaki M; Abbruzzese JL; Lassere Y; Meyers CA; Hoff P; de Bono J; Norris J; Matsushita H; et al. Phase I and pharmacokinetic study of 3′-C-ethynylcytidine (TAS-106), an inhibitor of RNA polymerase I, II and III,in patients with advanced solid malignancies. Invest. New Drugs 2012, 30 (1), 316–326. [PubMed: 20839029]

(395). Meike S; Yamamori T; Yasui H; Eitaki M; Matsuda A; Morimatsu M; Fukushima M; Yamasaki Y; Inanami O A nucleoside anticancer drug, 1-(3-C-ethynyl-β-D-ribo-pentofuranosyl)cytosine (TAS106), sensitizes cells to radiation by suppressing BRCA2 expression. Mol. Cancer 2011, 10, 92. [PubMed: 21798026]

(396). Ahmed NK Multiple forms and inhibitors of uridine-cytidine kinase in neoplastic cells. Int. J. Biochem 1982, 14 (4), 259–262. [PubMed: 6279452]

(397). Shimamoto Y; Koizumi K; Okabe H; Kazuno H; Murakami Y; Nakagawa F; Matsuda A; Sasaki T; Fukushima M Sensitivity of human cancer cells to the new anticancer ribo-nucleoside TAS-106 is correlated with expression of uridine-cytidine kinase 2. Jpn. J. Cancer Res 2002, 93 (7), 825–833. [PubMed: 12149149]

(398). Kanda H; Takatori S; Matsuda A; Sasaki T; Tanaka M; Fukushima M; Wataya Y Cytotoxic mechanisms of new antitumor nucleoside analogues, 3′-ethynylcytidine (ECyd) and 3′-ethynyluridine (EUrd). Nucleic Acids Symp. Ser 1997, 37, 137–138.

(399). Hattori H; Tanaka M; Fukushima M; Sasaki T; Matsuda A Nucleosides and nucleotides. 158. 1-(3-C-ethynyl-β-D-ribo-pentofuranosyl)-cytosine, 1-(3-C-ethynyl-β-D-ribo-pentofuranosyl)uracil, and their nucleobase analogues as new potential multifunctional antitumor nucleosides with a broad spectrum of activity. J. Med. Chem 1996, 39 (25), 5005–5011. [PubMed: 8960561]

(400). Takatori S; Kanda H; Takenaka K; Wataya Y; Matsuda A; Fukushima M; Shimamoto Y; Tanaka M; Sasaki T Antitumor mechanisms and metabolism of the novel antitumor nucleoside analogues, 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)cytosine and 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)uracil. Cancer Chemother. Pharmacol 1999, 44 (2), 97–104. [PubMed: 10412942]

(401). Weinreich J; Schott S; Konigsrainer I; Zieker D; Konigsrainer A; Schott H Cytostatic activity of the duplex drug linking 2′-deoxy-5-fluorouridine (5FdU) with 3′-C-ethynylcytidine (ECyd) against gastric adenocarcinoma cell lines. Invest. New Drugs 2011, 29 (6), 1294–1302. [PubMed: 20596746]

(402). Eicher C; Dewerth A; Ellerkamp V; Fuchs J; Schott S; Armeanu-Ebinger S Effect of duplex drugs linking 2′-deoxy-5-fluorouridine (5-FdU) with 3′-C-ethynylcytidine (ECyd) on hepatoblastoma cell lines. Pediatr. Surg. Int 2013, 29 (2), 121–127. [PubMed: 23187893]

(403). Tsao A; Hui EP; Juergens R; Marur S; Huat TE; Cher GB; Hong R-L; Hong WK; Chan AT-C Phase II study of TAS-106 in patients with platinum-failure recurrent or metastatic head and neck cancer and nasopharyngeal cancer. Cancer Med 2013, 2 (3), 351–359. [PubMed: 23930212]

(404). Naing A; Fu S; Zinner RG; Wheler JJ; Hong DS; Arakawa K; Falchook GS; Kurzrock R Phase I dose-escalating study of TAS-106 in combination with carboplatin in patients with solid tumors. Invest. New Drugs 2014, 32 (1), 154–159. [PubMed: 23609829]

(405). Yoshimura Y; Sano T; Matsuda A; Ueda T Nucleosides and nucleotides. LXXVII: Synthesis of 6,3′-methanocytidine, 6,3′-methanouridine, and Their 2′-deoxyribonucleosides. Chem. Pharm. Bull. (Tokyo) 1988, 36, 162–167.

(406). Nomura M; Sato T; Washinosu M; Tanaka M; Asao T; Shuto S; Matsuda A Nucleosides and nucleotides. Part 212: Practical large-scale synthesis of 1-(3-C-ethynyl-β-D-ribo-pentofuranosyl)-cytosine (ECyd), a potent antitumor nucleoside. Isobutyryloxy group as an efficient anomeric leaving group in the Vorbrüggen glycosylation reaction. Tetrahedron 2002, 58, 1279–1288.

(407). Bitonti AJ; Bush TL; Lewis MT; Sunkara PS Response of human colon and prostate tumor xenografts to (E)-2′-deoxy-2′-(fluoromethylene) cytidine, an inhibitor of ribonucleotide reductase. Anticancer Res 1995, 15 (4), 1179–1182. [PubMed: 7653997]

(408). Avendano C; Menendez JA Antimetabolites; Elsevier: Oxford, UK, 2008; pp 9–52.

(409). Stubbe J; van der Donk WA Ribonucleotide reductases: radical enzymes with suicidal tendencies. Chem. Biol 1995, 2 (12), 793–801. [PubMed: 8807812]

(410). Seley KL Tezacitabine Hoechst Marion Roussel. Curr. Opin. Investig. Drugs 2000, 1 (1), 135–140.

(411). Takahashi T; Nakashima A; Kanazawa J; Yamaguchi K; Akinaga S; Tamaoki T; Okabe M Metabolism and ribonucleotide reductase inhibition of (E)-2′-deoxy-2′-(fluoromethylene)cytidine, MDL 101,731, in human cervical carcinoma HeLa S3 cells. Cancer Chemother. Pharmacol 1998, 41 (4), 268–274. [PubMed: 9488595]

(412). Rodriguez GI; Jones RE; Orenberg EK; Stoltz ML; Brooks DJ Phase I clinical trials of tezacitabine [(E)-2′-deoxy-2′-(fluoromethylene)cytidine] in patients with refractory solid tumors. Clin. Cancer Res 2002, 8 (9), 2828–2834. [PubMed: 12231523]

(413). Piepmeier JM; Rabidou N; Schold SC Jr.; Bitonti AJ; Prakash NJ; Bush TL In vitro and in vivo inhibition of glioblastoma and neuroblastoma with MDL101731, a novel ribonucleoside diphosphate reductase inhibitor. Cancer Res 1996, 56 (2), 359–361. [PubMed: 8542592]

(414). Bitonti AJ; Dumont JA; Bush TL; Cashman EA; Cross-Doersen DE; Wright PS; Matthews DP; McCarthy JR; Kaplan DA Regression of human breast tumor xenografts in response to (E)-2′-deoxy-2′-(fluoromethylene)cytidine, an inhibitor of ribonucleoside diphosphate reductase. Cancer Res 1994, 54 (6), 1485–1490. [PubMed: 8137252]

(415). Coucke PA; Cottin E; Decosterd LA Simultaneous alteration of de novo and salvage pathway to the deoxynucleoside triphosphate pool by (E)-2′-deoxy-(fluoromethylene)cytidine (FMdC) and zidovudine (AZT) results in increased radiosensitivity in vitro. Acta Oncol 2007, 46 (5), 612–620. [PubMed: 17562437]

(416). Woessner RD; Loudy DE; Wallace CD; Montgomery LR; Cross-Doersen DE; Bush TL; Lewis MT; Prakash N; Bitonti AJ; Wright PS Decreased vascular endothelial growth factor expression associated with tumor regression induced by (E)-2′-deoxy-2′-(fluoromethylene)cytidine (MDL 101,731). Oncol. Res 1997, 9 (10), 543–552. [PubMed: 9507532]

(417). Bendell JC; Eder JP; Clark JW; Fidias P; Lynch TJ; Seiden MV; Ryan DP Phase I dose-escalation study of tezacitabine in combination with 5-fluorouracil in patients with advanced solid tumors. Cancer 2005, 103 (9), 1925–1931. [PubMed: 15772958]

(418). McCarthy JR; Matthews DP; Stemerick DM; Huber EW; Bey P; Lippert BJ; Snyder RD; Sunkara PS Stereospecific method to (E) and (Z) terminal fluoroolefins and its application to the synthesis of 2′-deoxy-2′-fluoromethylenenucleosides as potential inhibitors of ribonucleoside diphosphate reductase. J. Am. Chem. Soc 1991, 113, 7439–7440.

(419). Matthews DP; Persichetti RA; Sabol JS; Stewart KT; McCarthy JR Improved synthesis of (E)-2′-deoxy-2′-(fluoromethylene)cytidine - a potent inhibitor of ribonucleotide diphosphate reductase. Nucleosides Nucleotides 1993, 12, 115–123.

(420). Chou KM; Kukhanova M; Cheng YC A novel action of human apurinic/apyrimidinic endonuclease: excision of L-configuration deoxyribonucleoside analogs from the 3′ termini of DNA. J. Biol. Chem 2000, 275 (40), 31009–31015. [PubMed: 10906132]

(421). Quintas-Cardama A; Cortes J Evaluation of the L-stereo-isomeric nucleoside analog troxacitabine for the treatment of acute myeloid leukemia. Expert Opin. Invest. Drugs 2007, 16 (4), 547–557.

(422). Griffith DA; Jarvis SM Nucleoside and nucleobase transport systems of mammalian cells. Biochim. Biophys. Acta, Rev. Biomembr 1996, 1286 (3), 153–181.

(423). Lin CC; Beeram M; Rowinsky EK; Takimoto CH; Ng CM; Geyer CE Jr.; Denis LJ; De Bono JS; Hao D; Tolcher AW; et al. Phase I and pharmacokinetic study of cisplatin and troxacitabine administered intravenously every 28 days in patients with advanced solid malignancies. Cancer Chemother. Pharmacol 2009, 65 (1), 167–175. [PubMed: 19449006]

(424). Gourdeau H; Jolivet J [Troxacitabine]. Bull. Cancer 2004, 91 (3), 213–218. [PubMed: 15171046]

(425). Moore EC; Hurlbert RB; Boss GR; Massia SP Inhibition of two enzymes in de novo purine nucleotide synthesis by triciribine phosphate (TCN-P). Biochem. Pharmacol 1989, 38 (22), 4045–4051. [PubMed: 2480792]

(426). Krishnan P; Fu Q; Lam W; Liou JY; Dutschman G; Cheng YC Phosphorylation of pyrimidine deoxynucleoside analog diphosphates: selective phosphorylation of L-nucleoside analog diphosphates by 3-phosphoglycerate kinase. J. Biol. Chem 2002, 277 (7), 5453–5459. [PubMed: 11741981]

(427). Dent SF; Arnold A; Stewart DJ; Gertler S; Ayoub J; Batist G; Goss G; Nevile A; Soulieres D; Jolivet J; et al. Phase II study of troxacitabine (BCH-4556) in patients with advanced non-small-cell lung cancer. Lung 2005, 183 (4), 265–272. [PubMed: 16211462]

(428). Kukhanova M; Liu SH; Mozzherin D; Lin TS; Chu CK; Cheng YC L- and D-enantiomers of 2′,3′-dideoxycytidine 5′-triphosphate analogs as substrates for human DNA polymerases. Implications for the mechanism of toxicity. J. Biol. Chem 1995, 270 (39), 23055–23059. [PubMed: 7559445]

(429). Adema AD; Zuurbier L; Floor K; Hubeek I; Kaspers GJ; Albertoni F; Peters GJ Cellular resistance against troxacitabine in human cell lines and pediatric patient acute myeloid leukemia blast cells. Nucleosides, Nucleotides Nucleic Acids 2006, 25 (9–11), 981–986. [PubMed: 17065050]

(430). Giles FJ; Garcia-Manero G; Cortes JE; Baker SD; Miller CB; O'Brien SM; Thomas DA; Andreeff M; Bivins C; Jolivet J; et al. Phase II study of troxacitabine, a novel dioxolane nucleoside analog, in patients with refractory leukemia. J. Clin. Oncol 2002, 20 (3), 656–664. [PubMed: 11821445]

(431). Lapointe R; Letourneau R; Steward W; Hawkins RE; Batist G; Vincent M; Whittom R; Eatock M; Jolivet J; Moore M Phase II study of troxacitabine in chemotherapy-naive patients with advanced cancer of the pancreas: gastrointestinal tumors. Ann. Oncol 2005, 16 (2), 289–293. [PubMed: 15668286]

(432). Townsley CA; Chi K; Ernst DS; Belanger K; Tannock I; Bjarnason GA; Stewart D; Goel R; Ruether JD; Siu LL; et al. Phase II study of troxacitabine (BCH-4556) in patients with advanced and/or metastatic renal cell carcinoma: a trial of the National Cancer Institute of Canada-Clinical Trials Group. J. Clin. Oncol 2003, 21 (8), 1524–1529. [PubMed: 12697876]

(433). Adema AD; Radi M; Daft J; Narayanasamy J; Hoebe EK; Alexander LE; Chu CK; Peters GJ Troxacitabine prodrugs for pancreatic cancer. Nucleosides, Nucleotides Nucleic Acids 2007, 26 (8–9), 1073–1077. [PubMed: 18058539]

(434). Jimeno A; Messersmith WA; Lee CK; Ma WW; Laheru D; Donehower RC; Baker SD; Hidalgo M Phase I study of troxacitabine administered by continuous infusion in subjects with advanced solid malignancies. Ann. Oncol 2008, 19 (2), 374–379. [PubMed: 18245131]

(435). Roboz GJ; Giles FJ; Ritchie EK; Allen-Bard S; Curcio TJ; Wilkes MA; Park SL; Kantarjian HM; Faderl S; Ravandi F; et al. Phase I/II study of continuous-infusion troxacitabine in refractory acute myeloid leukemia. J. Clin. Oncol 2006, 25 (1), 10–15. [PubMed: 17146106]

(436). Vose JM; Panwalkar A; Belanger R; Coiffier B; Baccarani M; Gregory SA; Facon T; Fanin R; Caballero D; Ben-Yehuda D; et al. A phase II multicenter study of troxacitabine in relapsed or refractory lymphoproliferative neoplasms or multiple myeloma. Leuk. Lymphoma 2007, 48 (1), 39–45. [PubMed: 17325846]

(437). Belleau BR; Evans CA; Tse HLA; Jin H; Dixit DM; Mansour TS Oxidative degradation of L-ascorbic acid acetals to 2′,3′-dideoxy-3′-oxaribofuranosides. Synthesis of enantiomerically pure 2′,3′-dideoxy-3′-oxacytidine stereoisomers as potential antiviral agents. Tetrahedron Lett 1992, 33 (46), 6949–6952.

(438). Kim HO; Schinazi RF; Shanmuganathan K; Jeong LS; Beach JW; Nampalli S; Cannon DL; Chu CK L-β-(2S,4S)- and L-α-(2S,4R)-dioxolanyl nucleosides as potential anti-HIV agents: asymmetric synthesis and structure-activity relationships. J. Med. Chem 1993, 36 (5), 519–528. [PubMed: 8496934]

(439). Whistler RL; Doner LW; Nayak UG 4-thio-D-arabinofuranosylpyrimidine nucleosides. J. Org. Chem 1971, 36 (1), 108–110. [PubMed: 5543043]

(440). Ototani N; Whistler RL Preparation and antitumor activity of 4′-thio analogs of 2,2′-anhydro-1-β-D-arabinofuranosylcytosine. J. Med. Chem 1974, 17, 535–537. [PubMed: 4830257]

(441). Secrist JA 3rd; Tiwari KN; Shortnacy-Fowler AT; Messini L; Riordan JM; Montgomery JA; Meyers SC; Ealick SE Synthesis and biological activity of certain 4′-thio-D-arabinofuranosylpurine nucleosides. J. Med. Chem 1998, 41 (20), 3865–3871. [PubMed: 9748361]

(442). Roy AM; Tiwari KN; Parker WB; Secrist JA 3rd; Li R; Qu Z Antiangiogenic activity of 4′-thio-β-D-arabinofuranosylcytosine. Mol. Cancer Ther 2006, 5 (9), 2218–2224. [PubMed: 16985055]

(443). Tiwari KN; Shortnacy-Fowler AT; Cappellacci L; Parker WB; Waud WR; Montgomery JA; Secrist JA 3rd Synthesis of 4′-thio-β-D-arabinofuranosylcytosine (4′-thio-ara-C) and comparison of its anticancer activity with that of ara-C. Nucleosides, Nucleotides Nucleic Acids 2000, 19 (1–2), 329–340. [PubMed: 10772718]

(444). Parker WB; Shaddix SC; Rose LM; Waud WR; Shewach DS; Tiwari KN; Secrist JA 3rd Metabolism of 4′-thio-β-D-arabinofuranosylcytosine in CEM cells. Biochem. Pharmacol 2000, 60 (12), 1925–1932. [PubMed: 11108809]

(445). Someya H; Shaddix SC; Tiwari KN; Secrist JA 3rd; Parker WB Phosphorylation of 4′-thio-β-D-arabinofuranosylcytosine and its analogs by human 2′-deoxycytidine kinase. J. Pharmacol. Exp. Ther 2003, 304 (3), 1314–1322. [PubMed: 12604712]

(446). Someya H; Waud WR; Parker WB Long intracellular retention of 4′-thio-arabinofuranosylcytosine 5′-triphosphate as a critical factor for the anti-solid tumor activity of 4′-thio-arabinofuranosylcytosine. Cancer Chemother. Pharmacol 2006, 57 (6), 772–780. [PubMed: 16180016]

(447). Robak T New nucleoside analogs for patients with hematological malignancies. Expert Opin. Invest. Drugs 2011, 20 (3), 343–359.

(448). Chen YW; Chou KM DNA lesion bypass polymerases and 4′-thio-β-D-arabinofuranosylcytosine (T-araC). Int. J. Biochem. Mol. Biol 2011, 2 (4), 340–346. [PubMed: 22187668]

(449). Parker WB; Waud WR; Secrist JA 3rd Thiarabine, 1-(4-thio-β-D-arabinofuranosyl)cytosine. A deoxycytidine analog with excellent anticancer activity. Curr. Med. Chem 2015, 22 (34), 3881–3896. [PubMed: 26597061]

(450). Thottassery JV; Westbrook L; Someya H; Parker WB c-Abl-independent p73 stabilization during gemcitabine- or 4′-thio-β-D-arabinofuranosylcytosine-induced apoptosis in wild-type and p53-null colorectal cancer cells. Mol. Cancer Ther 2006, 5 (2), 400–410. [PubMed: 16505115]

(451). Waud WR; Gilbert KS; Secrist JA 3rd Lack of in vivo cross-resistance with 4′-thio-ara-C against drug-resistant murine P388 and L1210 leukemias. Cancer Chemother. Pharmacol 2011, 68 (2), 399–403. [PubMed: 21069340]

(452). Waud WR; Gilbert KS; Secrist JA 3rd Preclinical combination therapy of thiarabine plus various clinical anticancer agents. Nucleosides, Nucleotides Nucleic Acids 2012, 31 (8), 630–646. [PubMed: 22908953]

(453). Faderl S; Gandhi V; O'Brien S; Bonate P; Cortes J; Estey E; Beran M; Wierda W; Garcia-Manero G; Ferrajoli A; et al. Results of a phase 1–2 study of clofarabine in combination with cytarabine (araC) in relapsed and refractory acute leukemias. Blood 2005, 105 (3), 940–947. [PubMed: 15486072]

(454). Lee CP; de Jonge MJ; O'Donnell AE; Schothorst KL; Hanwell J; Chick JB; Brooimans RA; Adams LM; Drolet DW; de Bono JS; et al. A phase I study of a new nucleoside analogue, OSI-7836, using two administration schedules in patients with advanced solid malignancies. Clin. Cancer Res 2006, 12 (9), 2841–2848. [PubMed: 16675579]

(455). Goss G; Siu LL; Gauthier I; Chen EX; Oza AM; Goel R; Maroun J; Powers J; Walsh W; Maclean M; et al. A phase I, first in man study of OSI-7836 in patients with advanced refractory solid tumors: IND.147, a study of the Investigational New Drug Program of the National Cancer Institute of Canada Clinical Trials Group. Cancer Chemother. Pharmacol 2006, 58 (5), 703–710. [PubMed: 16528532]

(456). Nayak UG; Whistler RL Synthesis of 5-thio-D-glucose. J. Org. Chem 1969, 34 (1), 97–100.

(457). Meyer AS; Reichstein T l-Idomethylose. Desoxyzucker. 8. Mitteilung. Helv. Chim. Acta 1946, 29 (1), 139–152.

(458). Whistler RL; Nayak UG; Perkins AW Anomeric methyl 4-thio-D-arabinofuranosides. J. Org. Chem 1970, 35 (2), 519–521.

(459). Ness RK; Fletcher HG Jr. The anomeric 2,3,5-tri-O-benzoyl-D-arabinosyl bromides and other D-arabinofuranose derivatives. J. Am. Chem. Soc 1958, 80 (8), 2007–2010.

(460). Jeong LS; Zhao LX; Choi WJ; Pal S; Park YH; Lee SK; Chun MW; Lee YB; Ahn CH; Moon HR Synthesis and antitumor activity of fluorocyclopentenyl-pyrimidines. Nucleosides, Nucleotides Nucleic Acids 2007, 26 (6–7), 713–716. [PubMed: 18066886]

(461). Zhao LX; Yun M; Kim HO; Lee JA; Choi WJ; Lee KM; Lee SK; Lee YB; Ahn CH; Jeong LS Design, synthesis, and anticancer activity of fluorocyclopentenyl-pyrimidines. Nucleic Acids Symp. Ser. (Oxf) 2005, 49, 107–108.

(462). Peters GJ; Smid K; Vecchi L; Kathmann I; Sarkisjan D; Honeywell RJ; Losekoot N; Ohne O; Orbach A; Blaugrund E; et al. Metabolism, mechanism of action and sensitivity profile of fluorocyclopentenylcytosine (RX-3117; TV-1360). Invest. New Drugs 2013, 31 (6), 1444–1457. [PubMed: 24048768]

(463). Choi WJ; Chung HJ; Chandra G; Alexander V; Zhao LX; Lee HW; Nayak A; Majik MS; Kim HO; Kim JH; Lee YB; Ahn CH; Lee SK; Jeong LS Fluorocyclopentenyl-cytosine with broad spectrum and potent antitumor activity. J. Med. Chem 2012, 55 (9), 4521–4525. [PubMed: 22524616]

(464). Yang MY; Lee YB; Ahn CH; Kaye J; Fine T; Kashi R; Ohne O; Smid K; Peters GJ; Kim DJ A novel cytidine analog, RX-3117, shows potent efficacy in xenograft models, even in tumors that are resistant to gemcitabine. Anticancer Res 2014, 34 (12), 6951–6959. [PubMed: 25503121]

(465). Ahn CH; Choi WJ; Lee YB; Jeong LS; Lee SK US Patent 20050222185, 2005.

(466). Cohen SS Sponges, cancer chemotherapy, and cellular aging. Perspect. Biol. Med 1963, 6 (2), 215–227. [PubMed: 4924849]

(467). Sundaram M; Yao SY; Ingram JC; Berry ZA; Abidi F; Cass CE; Baldwin SA; Young JD Topology of a human equilibrative, nitrobenzylthioinosine (NBMPR)-sensitive nucleoside transporter (hENT1) implicated in the cellular uptake of adenosine and anti-cancer drugs. J. Biol. Chem 2001, 276 (48), 45270–45275. [PubMed: 11584005]

(468). Clarke ML; Mackey JR; Baldwin SA; Young JD; Cass CE The role of membrane transporters in cellular resistance to anticancer nucleoside drugs. Cancer Treat. Res 2002, 112, 27–47. [PubMed: 12481710]

(469). Liliemark JO; Plunkett W Regulation of 1-β-D-arabinofuranosylcytosine 5′-triphosphate accumulation in human leukemia cells by deoxycytidine 5′-triphosphate. Cancer Res 1986, 46 (3), 1079–1083. [PubMed: 3484676]

(470). Cohen SS The mechanisms of lethal action of arabinosyl cytosine (araC) and arabinosyl adenine (araA). Cancer 1977, 40 (1 Suppl), 509–518. [PubMed: 328134]

(471). Guchelaar HJ; Vermes I; Koopmans RP; Reutelingsperger CP; Haanen C Apoptosis- and necrosis-inducing potential of cladribine, cytarabine, cisplatin, and 5-fluorouracil in vitro: a quantitative pharmacodynamic model. Cancer Chemother. Pharmacol 1998, 42 (1), 77–83. [PubMed: 9619762]

(472). Ross DD; Cuddy DP; Cohen N; Hensley DR Mechanistic implications of alterations in HL-60 cell nascent DNA after exposure to 1-β-D-arabinofuranosylcytosine. Cancer Chemother. Pharmacol 1992, 31 (1), 61–70. [PubMed: 1458561]

(473). Drake JC; Hande KR; Fuller RW; Chabner BA Cytidine and deoxycytidylate deaminase inhibition by uridine analogs. Biochem. Pharmacol 1980, 29 (5), 807–811. [PubMed: 20227960]

(474). Gandhi V; Plunkett W Modulation of arabinosylnucleoside metabolism by arabinosylnucleotides in human leukemia cells. Cancer Res 1988, 48 (2), 329–334. [PubMed: 3335008]

(475). Cai J; Damaraju VL; Groulx N; Mowles D; Peng Y; Robins MJ; Cass CE; Gros P Two distinct molecular mechanisms underlying cytarabine resistance in human leukemic cells. Cancer Res 2008, 68 (7), 2349–2357. [PubMed: 18381442]

(476). Yamauchi T; Negoro E; Urasaki Y; Nishi R; Hori H; Ueda T Characterization of cytarabine-resistant leukemic cell lines established from five different blood cell lineages using gene expression and proteomic analyses. Int. J. Oncol 2011, 38 (4), 911–919. [PubMed: 21290089]

(477). Lamba JK Genetic factors influencing cytarabine therapy. Pharmacogenomics 2009, 10 (10), 1657–1674. [PubMed: 19842938]

(478). Guo Y; Kock K; Ritter CA; Chen ZS; Grube M; Jedlitschky G; Illmer T; Ayres M; Beck JF; Siegmund W; et al. Expression of ABCC-type nucleotide exporters in blasts of adult acute myeloid leukemia: Relation to long-term survival. Clin. Cancer Res 2009, 15 (5), 1762–1769. [PubMed: 19240178]

(479). Wang JJ; Selawry OS; Vietti TJ; Bodey GP Sr. Prolonged infusion of arabinosyl cytosine in childhood leukemia. Cancer 1970, 25 (1), 1–6. [PubMed: 5262259]

(480). Keane N; Freeman C; Swords R; Giles FJ Elacytarabine: lipid vector technology under investigation in acute myeloid leukemia. Expert Rev. Hematol 2013, 6 (1), 9–24. [PubMed: 23373775]

(481). Walsby EJ; Coles SJ; Knapper S; Burnett AK The topoisomerase II inhibitor voreloxin causes cell cycle arrest and apoptosis in myeloid leukemia cells and acts in synergy with cytarabine. Haematologica 2011, 96 (3), 393–399. [PubMed: 21134979]

(482). Fernandez HF New trends in the standard of care for initial therapy of acute myeloid leukemia. Hematology Am. Soc. Hematol. Educ. Program 2010, 2010, 56–61. [PubMed: 21239771]

(483). Fathi AT; Karp JE New agents in acute myeloid leukemia: beyond cytarabine and anthracyclines. Curr. Oncol. Rep 2009, 11 (5), 346–352. [PubMed: 19679009]

(484). Burke AC; Giles FJ Elacytarabine–lipid vector technology overcoming drug resistance in acute myeloid leukemia. Expert Opin. Invest. Drugs 2011, 20 (12), 1707–1715.

(485). O'Brien S; Rizzieri DA; Vey N; Ravandi F; Krug UO; Sekeres MA; Dennis M; Venditti A; Berry DA; Jacobsen TF; et al. Elacytarabine has single-agent activity in patients with advanced acute myeloid leukaemia. Br. J. Haematol 2012, 158 (5), 581–588. [PubMed: 22702906]

(486). Adema AD; Smid K; Losekoot N; Honeywell RJ; Verheul HM; Myhren F; Sandvold ML; Peters GJ Metabolism and accumulation of the lipophilic deoxynucleoside analogs elacytarabine and CP-4126. Invest. New Drugs 2012, 30 (5), 1908–1916. [PubMed: 22002019]

(487). Bruheim S; Sandvold ML; Maelandsmo GM; Fodstad O Antitumor activity of elacytarabine combined with bevacizumab, cetuximab and trastuzumab in human NSCLC xenografts. Anticancer Res 2013, 33 (9), 3615–3621. [PubMed: 24023287]

(488). Giles F; Rizzieri D; Ravandi F; Swords R; Jacobsen TF; O'Brien S Elacytarabine, a novel 5′-elaidic acid derivative of cytarabine, and idarubicin combination is active in refractory acute myeloid leukemia. Leuk. Res 2012, 36 (4), e71–73. [PubMed: 22226018]

(489). Giles FJ; Vey N; Rizzieri D; Ravandi F; Prebet T; Borthakur G; Jacobsen TF; Hagen S; Nilsson B; O'Brien S Phase I and pharmacokinetic study of elacytarabine, a novel 5′-elaidic acid derivative of cytarabine, in adults with refractory hematological malignancies. Leukemia 2012, 26 (7), 1686–1689. [PubMed: 22222600]

(490). Roboz GJ; Rosenblat T; Arellano M; Gobbi M; Altman JK; Montesinos P; O'Connell C; Solomon SR; Pigneux A; Vey N; et al. International randomized phase III study of elacytarabine versus investigator choice in patients with relapsed/refractory acute myeloid leukemia. J. Clin. Oncol 2014, 32 (18), 1919–1926. [PubMed: 24841975]

(491). Walwick ER; Roberts WK; Dekker CA Cyclisation during the phosphorylation of uridine and cytidine by polyphosphoric acid: A new route to the O-2,2′-cyclonucleosides. Proc. Chem. Soc 1959, 84.

(492). Mehellou Y; Valente R; Mottram H; Walsby E; Mills KI; Balzarini J; McGuigan C Phosphoramidates of 2′-β-D-arabinouridine (AraU) as phosphate prodrugs; design, synthesis, in vitro activity and metabolism. Bioorg. Med. Chem 2010, 18 (7), 2439–2446. [PubMed: 20299228]

(493). Qu G; Guo H; Yang X; Zhang X; Wang D; Niu H; Xia R; Li T; Wang F; Zhang Q, Zeng C CN Patent 101948492, 2011.

(494). Jin C; Wu Z; Jia G; Li H; Wu X CN Patent 1583776, 2005.

(495). Zong M CN Patent 1661030, 2005.

(496). Erion MD; Reddy KR; Boyer SH; Matelich MC; Gomez-Galeno J; Lemus RH; Ugarkar BG; Colby TJ; Schanzer J; Van Poelje PD Design, synthesis, and characterization of a series of cytochrome P(450) 3A-activated prodrugs (HepDirect prodrugs) useful for targeting phosph(on)ate-based drugs to the liver. J. Am. Chem. Soc 2004, 126 (16), 5154–5163. [PubMed: 15099098]

(497). Boyer SH; Sun Z; Jiang H; Esterbrook J; Gomez-Galeno JE; Craigo W; Reddy KR; Ugarkar BG; MacKenna DA; Erion MD Synthesis and characterization of a novel liver-targeted prodrug of cytosine-1-β-D-arabinofuranoside monophosphate for the treatment of hepatocellular carcinoma. J. Med. Chem 2006, 49 (26), 7711–7720. [PubMed: 17181153]

(498). Bentley HR; Cunningham KG; Spring FS 509. Cordycepin, a metabolic product from cultures of cordyceps militaris (Linn.)Link. Part II. The structure of cordycepin. J. Chem. Soc 1951, 2301–2305.

(499). Cheng Z; He W; Zhou X; Lv Q; Xu X; Yang S; Zhao C; Guo L Cordycepin protects against cerebral ischemia/reperfusion injury in vivo and in vitro. Eur. J. Pharmacol 2011, 664 (1–3), 20–28. [PubMed: 21554870]

(500). Sugar AM; McCaffrey RP Antifungal activity of 3′-deoxyadenosine (cordycepin). Antimicrob. Agents Chemother 1998, 42 (6), 1424–1427. [PubMed: 9624488]

(501). Wong YY; Moon A; Duffin R; Barthet-Barateig A; Meijer HA; Clemens MJ; de Moor CH Cordycepin inhibits protein synthesis and cell adhesion through effects on signal transduction. J. Biol. Chem 2010, 285 (4), 2610–2621. [PubMed: 19940154]

(502). Kraupp M; Marz R Nucleobase and nucleoside transport in mammalian cells. Wiener klinische Wochenschrift 1995, 107 (22), 677–680. [PubMed: 8533427]

(503). Holbein S; Wengi A; Decourty L; Freimoser FM; Jacquier A; Dichtl B Cordycepin interferes with 3′ end formation in yeast independently of its potential to terminate RNA chain elongation. RNA 2009, 15 (5), 837–849. [PubMed: 19324962]

(504). Muller WE; Seibert G; Beyer R; Breter HJ; Maidhof A; Zahn RK Effect of cordycepin on nucleic acid metabolism in L5178Y cells and on nucleic acid-synthesizing enzyme systems. Cancer Res 1977, 37 (10), 3824–3833. [PubMed: 332340]

(505). Overgaard-Hansen K The inhibition of 5-phosphoribosyl-1-pyrophosphate formation by cordycepin triphosphate in extracts of Ehrlich ascites tumor cells. Biochim. Biophys. Acta, Spec. Sect. Nucleic Acids Relat. Subj 1964, 80, 504–507.

(506). Rottman F; Guarino AJ The Inhibition of phosphoribosylpyrophosphate amidotransferase activity by cordycepin monophosphate. Biochim. Biophys. Acta, Spec. Sect. Enzymol. Subj 1964, 89, 465–472.

(507). Lee HJ; Burger P; Vogel M; Friese K; Bruning A The nucleoside antagonist cordycepin causes DNA double strand breaks in breast cancer cells. Invest. New Drugs 2012, 30 (5), 1917–1925. [PubMed: 22821173]

(508). Lee SJ; Kim SK; Choi WS; Kim WJ; Moon SK Cordycepin causes p21WAF1-mediated G2/M cell-cycle arrest by regulating c-Jun N-terminal kinase activation in human bladder cancer cells. Arch. Biochem. Biophys 2009, 490 (2), 103–109. [PubMed: 19733546]

(509). Nakamura K; Yoshikawa N; Yamaguchi Y; Kagota S; Shinozuka K; Kunitomo M Antitumor effect of cordycepin (3′-deoxyadenosine) on mouse melanoma and lung carcinoma cells involves adenosine A3 receptor stimulation. Anticancer Res 2006, 26 (1A), 43–47. [PubMed: 16475677]

(510). Tian X; Li Y; Shen Y; Li Q; Wang Q; Feng L Apoptosis and inhibition of proliferation of cancer cells induced by cordycepin. Oncol. Lett 2015, 10 (2), 595–599. [PubMed: 26622539]

(511). Yao WL; Ko BS; Liu TA; Liang SM; Liu CC; Lu YJ; Tzean SS; Shen TL; Liou JY Cordycepin suppresses integrin/FAK signaling and epithelial-mesenchymal transition in hepatocellular carcinoma. Anti-Cancer Agents Med. Chem 2014, 14 (1), 29–34.

(512). Lee WW; Benitez A; Anderson CD; Goodman L; Baker BR Potential anticancer agents. LV. Synthesis of 3′-amino-2′,3′-dideoxyadenosine and related analogs. J. Am. Chem. Soc 1961, 83 (8), 1906–1911.

(513). Hansske F; Robins MJ Regiospecific and stereoselective conversion of ribonucleosides to 3′-deoxynucleosides. A high yield three-stage synthesis of cordycepin from adenosine. Tetrahedron Lett 1985, 26, 4295–4298.

(514). Aman S; Anderson DJ; Connolly TJ; Crittall AJ; Ji GJ From adenosine to 3′-deoxyadenosine: Development and scale up. Org. Process Res. Dev 2000, 4 (6), 601–605.

(515). Grunebaum E; Cohen A; Roifman CM Recent advances in understanding and managing adenosine deaminase and purine nucleoside phosphorylase deficiencies. Curr. Opin. Allergy Clin. Immu 2013, 13 (6), 630–638.

(516). Carson DA; Wasson DB; Taetle R; Yu A Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. Blood 1983, 62 (4), 737–743. [PubMed: 6136305]

(517). Cory JG; Rey DA; Carter GL; Bacon PE Nucleoside 5′-diphosphates as effectors of mammalian ribonucleotide reductase. J. Biol. Chem 1985, 260 (22), 12001–12007. [PubMed: 3900066]

(518). Carson DA; Wasson DB; Kaye J; Ullman B; Martin DWJ; Robins RK; Montgomery JA 2′-deoxycytidine kinase-mediated toxicity of deoxyadenosine analogs toward malignant human lympho-blasts in vitro and toward murine L1210 leukemia in vivo. Proc. Natl. Acad. Sci. U. S. A 1980, 77, 6865–6869. [PubMed: 6256765]

(519). Bryson HM; Sorkin EM Cladribine. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in haematological malignancies. Drugs 1993, 46 (5), 872–894. [PubMed: 7507037]

(520). Ghanem H; Jabbour E; Faderl S; Ghandhi V; Plunkett W; Kantarjian H Clofarabine in leukemia. Expert Rev. Hematol 2010, 3 (1), 15–22. [PubMed: 21082931]

(521). Lindemalm S; Liliemark J; Juliusson G; Larsson R; Albertioni F Cytotoxicity and pharmacokinetics of cladribine metabolite, 2-chloroadenine in patients with leukemia. Cancer Lett 2004, 210 (2), 171–177. [PubMed: 15183532]

(522). King KM; Damaraju VL; Vickers MF; Yao SY; Lang T; Tackaberry TE; Mowles DA; Ng AM; Young JD; Cass CE A comparison of the transportability, and its role in cytotoxicity, of clofarabine, cladribine, and fludarabine by recombinant human nucleoside transporters produced in three model expression systems. Mol. Pharm 2006, 69 (1), 346–353.

(523). Wang L; Karlsson A; Arner ES; Eriksson S Substrate specificity of mitochondrial 2′-deoxyguanosine kinase. Efficient phosphorylation of 2-chlorodeoxyadenosine. J. Biol. Chem 1993, 268 (30), 22847–22852. [PubMed: 7901204]

(524). Huang MC; Ashmun RA; Avery TL; Kuehl M; Blakley RL Effects of cytotoxicity of 2-chloro-2′-deoxyadenosine and 2-bromo-2′-deoxyadenosine on cell growth, clonogenicity, DNA synthesis, and cell cycle kinetics. Cancer Res 1986, 46 (5), 2362–2368. [PubMed: 2421877]

(525). Parker WB; Bapat AR; Shen JX; Townsend AJ; Cheng YC Interaction of 2-halogenated dATP analogs (F, Cl, and Br) with human DNA polymerases, DNA primase, and ribonucleotide reductase. Mol. Pharmacol 1988, 34 (4), 485–491. [PubMed: 3050447]

(526). Tanabe K; Hiraoka W; Kuwabara M; Sato F; Matsuda A; Ueda T Induction of DNA double-strand breaks in Chinese hamster V79 cells by 2-chlorodeoxyadenosine. Chem.-Biol. Interact 1989, 71 (2–3), 167–175. [PubMed: 2574637]

(527). Wataya Y; Watanabe K; Yoshida S; Hiramoto-Yoshioka A dNTP imbalance and DNA double strand breaks in mouse FM3A cells and the mechanism of cell death. Nucleic Acids Symp. Ser 1989, 21, 11–12.

(528). Ghai V; Sharma K; Abbi KK; Shimko S; Epner EM Current approaches to epigenetic therapy for the treatment of mantle cell lymphoma. Adv. Exp. Med. Biol 2013, 779, 257–266. [PubMed: 23288643]

(529). Lubecka-Pietruszewska K; Kaufman-Szymczyk A; Stefanska B; Cebula-Obrzut B; Smolewski P; Fabianowska-Majewska K Clofarabine, a novel adenosine analogue, reactivates DNA methylation-silenced tumour suppressor genes and inhibits cell growth in breast cancer cells. Eur. J. Pharmacol 2014, 723, 276–287. [PubMed: 24296317]

(530). Stefanska B; Rudnicka K; Bednarek A; Fabianowska-Majewska K Hypomethylation and induction of retinoic acid receptor $\beta 2$ by concurrent action of adenosine analogues and natural compounds in breast cancer cells. Eur. J. Pharmacol 2010, 638 (1–3), 47–53. [PubMed: 20447390]

(531). Conrad DM; Robichaud MR; Mader JS; Boudreau RT; Richardson AM; Giacomantonio CA; Hoskin DW 2-Chloro-2′-deoxyadenosine-induced apoptosis in T leukemia cells is mediated via a caspase-3-dependent mitochondrial feedback amplification loop. Int. J. Oncol 2008, 32 (6), 1325–1333. [PubMed: 18497995]

(532). Kopadze T; Dobert M; Leussink VI; Dehmel T; Kieseier BC Cladribine impedes in vitro migration of mononuclear cells: a possible implication for treating multiple sclerosis. Eur. J. Neurol 2009, 16 (3), 409–412. [PubMed: 19175384]

(533). Mansson E; Spasokoukotskaja T; Sallstrom J; Eriksson S; Albertioni F Molecular and biochemical mechanisms of fludarabine and cladribine resistance in a human promyelocytic cell line. Cancer Res 1999, 59 (23), 5956–5963. [PubMed: 10606241]

(534). Scheible H; Laisney M; Wimmer E; Javornik A; Dolgos H Comparison of the in vitro and in vivo metabolism of cladribine (Leustatin, Movectro) in animals and human. Xenobiotica 2013, 43 (12), 1084–1094. [PubMed: 23627543]

(535). Takenaka K; Morgan JA; Scheffer GL; Adachi M; Stewart CF; Sun D; Leggas M; Ejendal KF; Hrycyna CA; Schuetz JD Substrate overlap between Mrp4 and Abcg2/Bcrp affects purine analogue drug cytotoxicity and tissue distribution. Cancer Res 2007, 67 (14), 6965–6972. [PubMed: 17638908]

(536). Spurgeon S; Yu M; Phillips JD; Epner EM Cladribine: not just another purine analogue? Expert Opin. Invest. Drugs 2009, 18 (8), 1169–1181.

(537). Singh V; Prajeeth CK; Gudi V; Benardais K; Voss EV; Stangel M 2-Chlorodeoxyadenosine (cladribine) induces apoptosis in human monocyte-derived dendritic cells. Clin. Exp. Immunol 2013, 173 (2), 288–297. [PubMed: 23607690]

(538). Gerrie AS; Zypchen LN; Connors JM Fludarabine and rituximab for relapsed or refractory hairy cell leukemia. Blood 2012, 119 (9), 1988–1991. [PubMed: 22223825]

(539). Beutler E; Sipe JC; Romine JS; Koziol JA; McMillan R; Zyroff J The treatment of chronic progressive multiple sclerosis with cladribine. Proc. Natl. Acad. Sci. U. S. A 1996, 93, 1716–1720. [PubMed: 8643695]

(540). Pardanani A Systemic mastocytosis in adults: 2013 update on diagnosis, risk stratification, and management. Am. J. Hematol 2013, 88 (7), 612–624. [PubMed: 23720340]

(541). Lajolo C; Campisi G; Deli G; Littarru C; Guiglia R; Giuliani M Langerhans's cell histiocytosis in old subjects: two rare case reports and review of the literature. Gerodontology 2012, 29 (2), e1207–1214. [PubMed: 22612839]

(542). Oh J; O'Connor PW Safety, Tolerability, and Efficacy of Oral Therapies for Relapsing-Remitting Multiple Sclerosis. CNS Drugs 2013, 27 (8), 591–609. [PubMed: 23801528]

(543). Venner H Synthese der den natürlichen entsprechenden 2-desoxy-nucleoside des adenins, guanins und hypoxanthins. Chem. Ber 1960, 93 (1), 140–149.

(544). Ikehara M; Tada H Studies of nucleosides and nucleotides. XXIV. Purine cyclonucleosides. I. 8,2′-cyclonucleoside derived from 2-chloro-8-mercapto-9-β-D-xylofuranosyladenine. J. Am. Chem. Soc 1965, 87 (3), 606–610. [PubMed: 14263385]

(545). Christensen LF; Broom AD; Robins MJ; Bloch A Synthesis and biological activity of selected 2, 6-disubstituted (2-deoxy-α-and-β-D-erythro-pentofuranosyl) purines. J. Med. Chem 1972, 15 (7), 735–739. [PubMed: 4625489]

(546). Zhong M; Nowak I; Robins MJ Regiospecific and highly stereoselective coupling of 6-(substituted-imidazol-1-yl)purines with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride. Sodium-salt glycosylation in binary solvent mixtures: improved synthesis of cladribine. J. Org. Chem 2006, 71 (20), 7773–7779. [PubMed: 16995685]

(547). Hoffer M α-Thymidin. Chem. Ber 1960, 93 (12), 2777–2781.

(548). Jaworski JS Looking for a contribution of the non-equilibrium solvent polarization to the activation barrier of the SN2 reaction. J. Phys. Org. Chem 2002, 15 (6), 319–323.

(549). Henschke JP; Zhang X; Huang X; Mei L; Chu G; Hu K; Wang Q; Zhu G; Wu M; Kuo C; Chen Y A stereoselective process for the manufacture of a 2′-deoxy-β-D-ribonucleoside using the Vorbruggen glycosylation. Org. Process Res. Dev 2013, 17 (11), 1419–1429.

(550). Woo PWK; Dion HW; Lange SM; Dahl LF; Durham LJ A novel adenosine and ara-A deaminase inhibitor, (R)-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-d] [1,3]-diazepin-8-ol. J. Heterocycl. Chem 1974, 11, 641–643.

(551). Chen SF; Stoeckler JD; Parks RE Jr. Transport of deoxycoformycin in human erythrocytes. Measurement by adenosine deaminase titration and radioisotope assays. Biochem. Pharmacol 1984, 33 (24), 4069–4079. [PubMed: 6334522]

(552). Hunt SW 3rd; Hoffee PA Adenosine deaminase from deoxycoformycin-sensitive and -resistant rat hepatoma cells. Purification and characterization. J. Biol. Chem 1982, 257 (23), 14239–14244. [PubMed: 6815190]

(553). Merkler DJ; Brenowitz M; Schramm VL The rate constant describing slow-onset inhibition of yeast AMP deaminase by coformycin analogues is independent of inhibitor structure. Biochemistry 1990, 29 (36), 8358–8364. [PubMed: 2252896]

(554). Kajander EO; Kubota M; Willis EH; Carson DA S-adenosylmethionine metabolism as a target for adenosine toxicity. Adv. Exp. Med. Biol 1986, 131 (Pt B), 221–226.

(555). Renshaw J; Harrap KR In vivo inhibition of mouse liver methyltransferase enzymes following treatment with 2′-deoxycoformycin and 2′-deoxyadenosine. Adv. Exp. Med. Biol 1986, 131 (Pt B), 673–675.

(556). Hermes M; Osswald H; Kloor D Role of S-adenosylhomocysteine hydrolase in adenosine-induced apoptosis in HepG2 cells. Exp. Cell Res 2007, 313 (2), 264–283. [PubMed: 17097637]

(557). Grever MR; Doan CA; Kraut EH Pentostatin in the treatment of hairy-cell leukemia. Best Pract. Res. Clin. Haematol 2003, 16 (1), 91–99. [PubMed: 12670468]

(558). Kurzrock R Therapy of T cell lymphomas with pentostatin. Ann. N. Y. Acad. Sci 2001, 941, 200–205. [PubMed: 11594574]

(559). Anglin JL; Song Y A medicinal chemistry perspective for targeting histone H3 lysine-79 methyltransferase DOT1L. J. Med. Chem 2013, 56 (22), 8972–8983. [PubMed: 23879463]

(560). Showalter HD; Bunge RH; French JC; Hurley TR; Leeds RL; Leja B; McDonnell PD; Edmunds CR Improved production of pentostatin and identification of fermentation comet-abolites. J. Antibiot 1992, 45 (12), 1914–1918.

(561). Baker DC; Putt SR A total synthesis of pentostatin, the potent inhibitor of adenosine deaminase. J. Am. Chem. Soc 1979, 101 (20), 6127–6128.

(562). Chan E; Putt SR; Showalter HDH; Baker DC Total synthesis of (8R)-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol (pentostatin), the potent inhibitor of adenosine deaminase. J. Org. Chem 1982, 47, 3457–3464.

(563). Zhang H-R; Wang W US Patent 20090012288, 2009.

(564). Phiasivongsa P; Redkar S WO Patent 2005027838, 2005.

(565). Majda K; Lubecka K; Kaufman-Szymczyk A; Fabianowska-Majewska K Clofarabine (2-chloro-2′-fluoro-2′-deoxyarabinosylade-nine)-biochemical aspects of anticancer activity. Acta. Polym. Pharm 2011, 68 (4), 459–466.

(566). Montgomery JA; Shortnacy-Fowler AT; Clayton SD; Riordan JM; Secrist JA 3rd Synthesis and biologic activity of 2′-fluoro-2-halo derivatives of 9-β-D-arabinofuranosyladenine. J. Med. Chem 1992, 35 (2), 397–401. [PubMed: 1732556]

(567). Parker WB; Allan PW; Hassan AE; Secrist JA 3rd; Sorscher EJ; Waud WR Antitumor activity of 2-fluoro-2′-deoxyadenosine against tumors that express Escherichia coli purine nucleoside phosphorylase. Cancer Gene Ther 2003, 10 (1), 23–29. [PubMed: 12489025]

(568). Bonate PL; Arthaud L; Cantrell WR Jr.; Stephenson K; Secrist JA 3rd; Weitman S Discovery and development of clofarabine: a nucleoside analogue for treating cancer. Nat. Rev. Drug Discovery 2006, 5 (10), 855–863. [PubMed: 17016426]

(569). Takahashi T; Kanazawa J; Akinaga S; Tamaoki T; Okabe M Antitumor activity of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine, a novel deoxyadenosine analog, against human colon tumor xenografts by oral administration. Cancer Chemother. Pharmacol 1999, 43 (3), 233–240. [PubMed: 9923554]

(570). Carson DA; Wasson DB; Esparza LM; Carrera CJ; Kipps TJ; Cottam HB Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2′-arabino-fluoro-2′-deoxyadenosine. Proc. Natl. Acad. Sci. U. S. A 1992, 89 (7), 2970–2974. [PubMed: 1348362]

(571). Parker WB; Shaddix SC; Chang CH; White EL; Rose LM; Brockman RW; Shortnacy AT; Montgomery JA; Secrist JA 3rd; Bennett LL Jr. Effects of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5′-triphosphate. Cancer Res 1991, 51 (9), 2386–2394. [PubMed: 1707752]

(572). Lotfi K; Mansson E; Spasokoukotskaja T; Pettersson B; Liliemark J; Peterson C; Eriksson S; Albertioni F Biochemical pharmacology and resistance to 2-chloro-2′-arabino-fluoro-2′-deoxyadenosine, a novel analogue of cladribine in human leukemic cells. Clin. Cancer Res 1999, 5 (9), 2438–2444. [PubMed: 10499616]

(573). Xie C; Plunkett W Metabolism and actions of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine in human lymphoblastoid cells. Cancer Res 1995, 55 (13), 2847–2852. [PubMed: 7540950]

(574). Xie KC; Plunkett W Deoxynucleotide pool depletion and sustained inhibition of ribonucleotide reductase and DNA synthesis after treatment of human lymphoblastoid cells with 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine. Cancer Res 1996, 56 (13), 3030–3037. [PubMed: 8674058]

(575). Genini D; Adachi S; Chao Q; Rose DW; Carrera CJ; Cottam HB; Carson DA; Leoni LM Deoxyadenosine analogs induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly affecting the mitochondria. Blood 2000, 96 (10), 3537–3543. [PubMed: 11071652]

(576). Locke F; Agarwal R; Kunnavakkam R; van Besien K; Larson RA; Odenike O; Godley LA; Liu H; Le Beau MM; Gurbuxani S; et al. A novel clofarabine bridge strategy facilitates allogeneic transplantation in patients with relapsed/refractory leukemia and high-risk myelodysplastic syndromes. Bone Marrow Transplant 2013, 48 (11), 1437–1443. [PubMed: 23771005]

(577). Tiley S; Claxton D Clofarabine in the treatment of acute myeloid leukemia in older adults. Ther. Adv. Hematol 2013, 4 (1), 5–13. [PubMed: 23610610]

(578). Abramson JS; Takvorian RW; Fisher DC; Feng Y; Jacobsen ED; Brown JR; Barnes JA; Neuberg DS; Hochberg EP Oral clofarabine for relapsed/refractory non-Hodgkin lymphomas: results of a phase 1 study. Leuk. Lymphoma 2013, 54 (9), 1915–1920. [PubMed: 23289359]

(579). Faderl S; Garcia-Manero G; Estrov Z; Ravandi F; Borthakur G; Cortes JE; O'Brien S; Gandhi V; Plunkett W; Byrd A; et al. Oral clofarabine in the treatment of patients with higher-risk myelodysplastic syndrome. J. Clin. Oncol 2010, 28 (16), 2755–2760. [PubMed: 20421540]

(580). Montgomery JA; Shortnacy AT; Carson DA; Secrist JA 3rd 9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)guanine: a metabolically stable cytotoxic analogue of 2′-deoxyguanosine. J. Med. Chem 1986, 29 (11), 2389–2392. [PubMed: 3783596]

(581). Reichman U; Watanabe KA; Fox JJ A practical synthesis of 2-deoxy-2-fluoro-D-arabinofuranose derivatives. Carbohydr. Res 1975, 42 (2), 233–240. [PubMed: 1139559]

(582). Bauta WE; Schulmeier BE; Burke B; Puente JF; Cantrell WR; Lovett D; Goebel J; Anderson B; Ionescu D; Guo R A new process for antineoplastic agent clofarabine. Org. Process Res. Dev 2004, 8, 889–896.

(583). Tann CH; Brodfuehrer PR; Brundidge SP; Sapino C; Howell HG Fluorocarbohydrates in synthesis. An efficient synthesis of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (β-FIAU) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (β-FMAU). J. Org. Chem 1985, 50 (19), 3644–3647.

(584). Brodfuehrer PR; Sapino CJ; Howell HG A stereo-controlled synthesis of 1,3,5-tri-O-benzoyl-α-D-ribofuranose. J. Org. Chem 1985, 50 (14), 2597–2598.

(585). Gandhi V; Keating MJ; Bate G; Kirkpatrick P Nelarabine. Nat. Rev. Drug Discovery 2006, 5 (1), 17–18. [PubMed: 16485343]

(586). Osborne WR; Chen SH; Giblett ER; Biggar WD; Ammann AA; Scott CR Purine nucleoside phosphorylase deficiency. Evidence for molecular heterogeneity in two families with enzyme-deficient members. J. Clin. Invest 1977, 60 (3), 741–746. [PubMed: 408378]

(587). Curbo S; Kalsson A In Deoxynucleoside Analogs in Cancer Therapy; Humana Press: Totowa, NJ, 2006; pp 215–224.

(588). Gokbuget N; Basara N; Baurmann H; Beck J; Bruggemann M; Diedrich H; Guldenzoph B; Hartung G; Horst HA; Huttmann A; et al. High single-drug activity of nelarabine in relapsed T-lymphoblastic leukemia/lymphoma offers curative option with subsequent stem cell transplantation. Blood 2011, 118 (13), 3504–3511. [PubMed: 21715318]

(589). Ravandi F; Gandhi V Novel purine nucleoside analogues for T-cell-lineage acute lymphoblastic leukaemia and lymphoma. Expert Opin. Invest. Drugs 2006, 15 (12), 1601–1613.

(590). Mahmoudian M; Eaddy J; Dawson M Enzymic acylation of 506U78 (2-amino-9-β-D-arabinofuranosyl-6- methoxy-9H-purine), a powerful new anti-leukaemic agent. Biotechnol. Appl. Biochem 1999, 29 (Pt 3), 229–233. [PubMed: 10334953]

(591). Balakrishnan K; Nimmanapalli R; Ravandi F; Keating MJ; Gandhi V Forodesine, an inhibitor of purine nucleoside phosphorylase, induces apoptosis in chronic lymphocytic leukemia cells. Blood 2006, 108 (7), 2392–2398. [PubMed: 16778146]

(592). Robak T; Lech-Maranda E; Korycka A; Robak E Purine nucleoside analogs as immunosuppressive and antineoplastic agents: mechanism of action and clinical activity. Curr. Med. Chem 2006, 13 (26), 3165–3189. [PubMed: 17168705]

(593). Rodriguez CO Jr.; Mitchell BS; Ayres M; Eriksson S; Gandhi V Arabinosylguanine is phosphorylated by both cytoplasmic 2′-deoxycytidine kinase and mitochondrial deoxyguanosine kinase. Cancer Res 2002, 62 (11), 3100–3105. [PubMed: 12036920]

(594). Rodriguez CO Jr.; Gandhi V Arabinosylguanine-induced apoptosis of T-lymphoblastic cells: incorporation into DNA is a necessary step. Cancer Res 1999, 59 (19), 4937–4943. [PubMed: 10519407]

(595). Rodriguez CO Jr.; Stellrecht CM; Gandhi V Mechanisms for T-cell selective cytotoxicity of arabinosylguanine. Blood 2003, 102 (5), 1842–1848. [PubMed: 12750168]

(596). Gravatt LC; Chaffee S; Hebert ME; Halperin EC; Friedman HS; Kurtzberg J Efficacy and toxicity of 9-β-D-arabinofuranosylguanine (araG) as an agent to purge malignant T cells from murine bone marrow: application to an in vivo T-leukemia model. Leukemia 1993, 7 (8), 1261–1267. [PubMed: 8350627]

(597). Cohen MH; Johnson JR; Massie T; Sridhara R; McGuinn WD Jr.; Abraham S; Booth BP; Goheer MA; Morse D; Chen XH; et al. Approval summary: Nelarabine for the treatment of T-cell lymphoblastic leukemia/lymphoma. Clin. Cancer Res 2006, 12 (18), 5329–5335. [PubMed: 17000665]

(598). Krenitsky TA; Koszalka GW; Jones LA; Averett DR; Moorman AR EP Patent 0294114, 1988.

(599). Zong Z; Wang X; Zhao J; Chen L; Wei J CN Patent 101402662, 2009.

(600). Hanna NB; Ramasamy K; Robins RK; Revankar GR A convenient synthesis of 2′-deoxy-6-thioguanosine, ara-guanine, ara-6-thioguanine and certain related purine nucleosides by the

stereospecific sodium salt glycosylation procedure. J. Heterocycl. Chem 1988, 25 (6), 1899–1903.

(601). Blumbergs P; Kalamas RL; Khan MS WO Patent 1991008215, 1991.

(602). Sagar S; Kaur M; Minneman KP Antiviral lead compounds from marine sponges. Mar. Drugs 2010, 8 (10), 2619–2638. [PubMed: 21116410]

(603). Gandhi V; Chen W; Ayres M; Rhie JK; Madden TL; Newman RA Plasma and cellular pharmacology of 8-chloro-adenosine in mice and rats. Cancer Chemother. Pharmacol 2002, 50 (2), 85–94. [PubMed: 12172971]

(604). Niitsu N; Umeda M; Honma Y Myeloid and monocytoid leukemia cells have different sensitivity to differentiation-inducing activity of deoxyadenosine analogs. Leuk. Res 2000, 24 (1), 1–9. [PubMed: 10634639]

(605). Avramis VI; Plunkett W 2-fluoro-ATP: A toxic metabolite of 9-β-D-arabinosyl-2-fluoroadenine. Biochem. Biophys. Res. Commun 1983, 113 (1), 35–43. [PubMed: 6860342]

(606). Rivero A; Rapado I; Tomas JF; Montalban C; de Ona R; Paz-Carreira J; Canales M; Martinez R; Sanchez-Godoy P; de Sevilla AF; et al. Relationship between 2′-deoxycytidine kinase (DCK) genotypic variants and fludarabine toxicity in patients with follicular lymphoma. Leuk. Res 2011, 35 (4), 431–437. [PubMed: 21030078]

(607). Brockman RW; Cheng YC; Schabel FM Jr.; Montgomery JA Metabolism and chemotherapeutic activity of 9-β-D-arabinofuranosyl-2-fluoroadenine against murine leukemia L1210 and evidence for its phosphorylation by 2′-deoxycytidine kinase. Cancer Res 1980, 40 (10), 3610–3615. [PubMed: 6254636]

(608). Lotfi K; Karlsson K; Fyrberg A; Juliusson G; Jonsson V; Peterson C; Eriksson S; Albertioni F The pattern of deoxycytidineand deoxyguanosine kinase activity in relation to messenger RNA expression in blood cells from untreated patients with B-cell chronic lymphocytic leukemia. Biochem. Pharmacol 2006, 71 (6), 882–890. [PubMed: 16436271]

(609). Lukenbill J; Kalaycio M Fludarabine: A review of the clear benefits and potential harms. Leuk. Res 2013, 37 (9), 986–994. [PubMed: 23787174]

(610). Huang P; Sandoval A; Van Den Neste E; Keating MJ; Plunkett W Inhibition of RNA transcription: a biochemical mechanism of action against chronic lymphocytic leukemia cells by fludarabine. Leukemia 2000, 14 (8), 1405–1413. [PubMed: 10942236]

(611). Pepper C; Lowe H; Fegan C; Thurieau C; Thurston DE; Hartley JA; Delavault P Fludarabine-mediated suppression of the excision repair enzyme ERCC1 contributes to the cytotoxic synergy with the DNA minor groove crosslinking agent SJG-136 (NSC 694501) in chronic lymphocytic leukaemia cells. Br. J. Cancer 2007, 97 (2), 253–259. [PubMed: 17579621]

(612). Ferracin M; Zagatti B; Rizzotto L; Cavazzini F; Veronese A; Ciccone M; Saccenti E; Lupini L; Grilli A; De Angeli C; et al. MicroRNAs involvement in fludarabine refractory chronic lymphocytic leukemia. Mol. Cancer 2010, 9, 123. [PubMed: 20504344]

(613). Moussay E; Palissot V; Vallar L; Poirel HA; Wenner T; El Khoury V; Aouali N; Van Moer K; Leners B; Bernardin F; et al. Determination of genes and microRNAs involved in the resistance to fludarabine in vivo in chronic lymphocytic leukemia. Mol. Cancer 2010, 9, 115. [PubMed: 20487546]

(614). Molica S Progress in the treatment of elderly/unfit chronic lymphocytic leukemia patients: results of the German CLL-11 trial. Expert Rev. Anticancer Ther 2015, 15, 9–15. [PubMed: 25479582]

(615). Montgomery JA; Hewson K Nucleosides of 2-fluoroadenine. J. Med. Chem 1969, 12 (3), 498–504. [PubMed: 5788168]

(616). Barker R; Fletcher HG Jr. 2,3,5-tri-O-benzyl-D-ribosyl and -L-arabinosyl bromides. J. Org. Chem 1961, 26 (11), 4605–4609.

(617). Glaudemans CPJ; Fletcher HG Jr. Syntheses with partially benzylated sugars. III. A simple pathway to a “cis- nucleoside,” 9-β-D-arabinofuranosyladenine (Spongoadenosine). J. Org. Chem 1963, 28 (11), 3004–3006.

(618). Montgomery JA US Patent 4188378, 1980.

(619). Montgomery JA; Shortnacy AT US Patent 4357324, 1982.

(620). Blumbergs P; Khan MS; Kalamas RL US Patent 5110919, 1992.

(621). Blumbergs P US Patent 5296589, 1994.

(622). Kshirsagar SW; Deshpande MS; Sonawane SP; Maikap GC; Gurjar MK Simple modification to obtain high quality fludarabine. Org. Process Res. Dev 2012, 16 (5), 840–842.

(623). Farina P; Petrucciani L; Colombo P; Caprioli G EP Patent 1464708, 2004.

(624). Stellrecht CM; Ayres M; Arya R; Gandhi V A unique RNA-directed nucleoside analog is cytotoxic to breast cancer cells and depletes cyclin E levels. Breast Cancer Res. Treat 2010, 121 (2), 355–364. [PubMed: 19641990]

(625). Katsaros D; Tortora G; Tagliaferri P; Clair T; Ally S; Neckers L; Robins RK; Cho-Chung YS Site-selective cyclic AMP analogs provide a new approach in the control of cancer cell growth. FEBS Lett 1987, 223 (1), 97–103. [PubMed: 2822483]

(626). Tortora G; Ciardiello F; Ally S; Clair T; Salomon DS; Cho-Chung YS Site-selective 8-chloroadenosine 3′,5′-cyclic monophosphate inhibits transformation and transforming growth factor alpha production in Ki-ras-transformed rat fibroblasts. FEBS Lett 1989, 242 (2), 363–367. [PubMed: 2536620]

(627). Ally S; Tortora G; Clair T; Grieco D; Merlo G; Katsaros D; Ogreid D; Doskeland SO; Jahnsen T; Cho-Chung YS Selective modulation of protein kinase isozymes by the site-selective analog 8-chloroadenosine 3′,5′-cyclic monophosphate provides a biological means for control of human colon cancer cell growth. Proc. Natl. Acad. Sci. U. S. A 1988, 85 (17), 6319–6322. [PubMed: 3413098]

(628). Halgren RG; Traynor AE; Pillay S; Zell JL; Heller KF; Krett NL; Rosen ST 8Cl-cAMP cytotoxicity in both steroid sensitive and insensitive multiple myeloma cell lines is mediated by 8Cladenosine. Blood 1998, 92 (8), 2893–2898. [PubMed: 9763575]

(629). Lang TT; Young JD; Cass CE Interactions of nucleoside analogs, caffeine, and nicotine with human concentrative nucleoside transporters 1 and 2 stably produced in a transport-defective human cell line. Mol. Pharm 2004, 65 (4), 925–933.

(630). Balakrishnan K; Stellrecht CM; Genini D; Ayres M; Wierda WG; Keating MJ; Leoni LM; Gandhi V Cell death of bioenergetically compromised and transcriptionally challenged CLL lymphocytes by chlorinated ATP. Blood 2005, 105 (11), 4455–4462. [PubMed: 15718423]

(631). Chen LS; Sheppard TL Chain termination and inhibition of Saccharomyces cerevisiae poly(A) polymerase by C-8-modified ATP analogs. J. Biol. Chem 2004, 279 (39), 40405–40411. [PubMed: 15265873]

(632). Stellrecht CM; Rodriguez CO Jr.; Ayres M; Gandhi V RNA-directed actions of 8-chloro-adenosine in multiple myeloma cells. Cancer Res 2003, 63 (22), 7968–7974. [PubMed: 14633728]

(633). Stellrecht CM; Phillip CJ; Cervantes-Gomez F; Gandhi V Multiple myeloma cell killing by depletion of the MET receptor tyrosine kinase. Cancer Res 2007, 67 (20), 9913–9920. [PubMed: 17942923]

(634). Yang SY; Jia XZ; Feng LY; Li SY; An GS; Ni JH; Jia HT Inhibition of topoisomerase II by 8-chloro-adenosine triphosphate induces DNA double-stranded breaks in 8-chloro-adenosine-exposed human myelocytic leukemia K562 cells. Biochem. Pharmacol 2009, 77 (3), 433–443. [PubMed: 19014910]

(635). Dennison JB; Balakrishnan K; Gandhi V Preclinical activity of 8-chloroadenosine with mantle cell lymphoma: Roles of energy depletion and inhibition of DNA and RNA synthesis. Br. J. Haematol 2009, 147 (3), 297–307. [PubMed: 19709085]

(636). Ahn YH; Jung JM; Hong SH 8-Chloro-cyclic AMP-induced growth inhibition and apoptosis is mediated by p38 mitogen-activated protein kinase activation in HL60 cells. Cancer Res 2005, 65 (11), 4896–4901. [PubMed: 15930311]

(637). Stellrecht CM; Vangapandu HV; Le XF; Mao W; Shentu S ATP directed agent, 8-chloro-adenosine, induces AMP activated protein kinase activity, leading to autophagic cell death in breast cancer cells. J. Hematol. Oncol 2014, 7, 23. [PubMed: 24628795]

(638). McBrayer SK; Yarrington M; Qian J; Feng G; Shanmugam M; Gandhi V; Krett NL; Rosen ST Integrative gene expression profiling reveals G6PD-mediated resistance to RNA-directed nucleoside analogues in B-cell neoplasms. PLoS One 2012, 7 (7), e41455. [PubMed: 22848499]

(639). Shanmugam M; McBrayer SK; Qian J; Raikoff K; Avram MJ; Singhal S; Gandhi V; Schumacker PT; Krett NL; Rosen ST Targeting glucose consumption and autophagy in myeloma with the novel nucleoside analogue 8-aminoadenosine. J. Biol. Chem 2009, 284 (39), 26816–26830. [PubMed: 19648108]

(640). Brentnall HJ; Hutchinson DW Preparation of 8-chloroadenosine and its phosphate esters. Tetrahedron Lett 1972, 13 (25), 2595–2596.

(641). Ryu EK; MacCoss M New procedure for the chlorination of pyrimidine and purine nucleosides. J. Org. Chem 1981, 46 (13), 2819–2823.

(642). Woo NT; Jin SY; Cho DJ; Kim NS; Bae EH; Jung JH; Ham WH; Jung YH The facile and efficient synthesis of 8-chloroadenosine 3′,5′-cyclic monophosphate by phosphorylative cyclization of 8-chloroadenosine and its characterization by(1)H and (13)C NMR spectroscopy. Arch. Pharmacal Res 1997, 20 (2), 176–179.

(643). Schmidt CL; Townsend LB Purine nucleosides. XXXI. The directive effect which certain exocyclic substituents at C-8 of adenine have on the site of ribosylation. J. Org. Chem 1972, 37 (14), 2300–2302. [PubMed: 5040378]

(644). Morris PE; Kamath VP The design of forodesin HCl and other purine nucleoside phosphorylase inhibitors; Wiley-VCH: Weinheim, 2008; pp 451–472.

(645). Al-Kali A; Gandhi V; Ayoubi M; Keating M; Ravandi F Forodesine: review of preclinical and clinical data. Future Oncol 2010, 6 (8), 1211–1217. [PubMed: 20799866]

(646). Homminga I; Zwaan CM; Manz CY; Parker C; Bantia S; Smits WK; Higginbotham F; Pieters R; Meijerink JP In vitro efficacy of forodesine and nelarabine (ara-G) in pediatric leukemia. Blood 2011, 118 (8), 2184–2190. [PubMed: 21730354]

(647). Ogura M; Tsukasaki K; Nagai H; Uchida T; Oyama T; Suzuki T; Taguchi J; Maruyama D; Hotta T; Tobinai K Phase I study of BCX1777 (forodesine) in patients with relapsed or refractory peripheral T/natural killer-cell malignancies. Cancer Sci 2012, 103 (7), 1290–1295. [PubMed: 22448814]

(648). Balakrishnan K; Ravandi F; Bantia S; Franklin A; Gandhi V Preclinical and clinical evaluation of forodesine in pediatric and adult B-cell acute lymphoblastic leukemia. Clin. Lymphoma Myeloma Leuk 2013, 13 (4), 458–466. [PubMed: 23773454]

(649). Evans GB; Furneaux RH; Gainsford GJ; Schramm VL; Tyler PC Synthesis of transition state analogue inhibitors for purine nucleoside phosphorylase and N-riboside hydrolases. Tetrahedron 2000, 56 (19), 3053–3062.

(650). Batra H; Moriarty RM; Penmasta R; Sharma V; Stanciuc G; Staszewski JP; Tuladhar SM; Walsh DA; Datla S; Krishnaswamy S A concise, efficient and production-scale synthesis of a protected l-lyxonolactone derivative: An important aldonolactone core. Org. Process Res. Dev 2006, 10, 484–486.

(651). Clinch K; Evans GB; Fleet GWJ; Furneaux RH; Johnson SW; Lenz DH; Mee SPH; Rands PR; Schramm VL; Taylor Ringia EA; Tyler PC Syntheses and bio-activities of the L-enantiomers of two potent transition state analogue inhibitors of purine nucleoside phosphorylases. Org. Biomol. Chem 2006, 4 (6), 1131–1139. [PubMed: 16525558]

(652). Winslow CD; Morris PE Jr.. US Patent 6972331, 2005.

(653). Evans GB; Furneaux RH; Hutchison TL; Kezar HS; Morris PE Jr.; Schramm VL; Tyler PC Addition of lithiated 9-deazapurine derivatives to a carbohydrate cyclic imine: convergent synthesis of the aza-C-nucleoside immucillins. J. Org. Chem 2001, 66 (17), 5723–5730. [PubMed: 11511245]

(654). Kamath VP; Xue J; Juarez-Brambila JJ; Morris PE Jr. Alternative route towards the convergent synthesis of a human purine nucleoside phosphorylase inhibitor—forodesine HCl. Tetrahedron Lett 2009, 50 (37), 5198–5200.

(655). Resek JE; Meyers AI Unsaturation of ketones, nitriles and lactams with methyl phenylsulfinate. Tetrahedron Lett 1995, 36 (39), 7051–7054.

(656). Biocryst Pharmaceuticals. WO Patent 2016110527, 2016.

(657). Schram KH; Townsend LB The synthesis of 6-amino-4-methyl-8-(β-D-ribofuranosyl) (4-H,8-H)pyrrolo-[4,3,2-de]pyrimido-[4,5-c]pyridazine, a new tricyclic nucleoside. Tetrahedron Lett 1971, 12 (49), 4757–4760.

(658). Kucera LS; Iyer NP; Puckett SH; Buckheit RW Jr.; Westbrook L; Toyer BR; White EL; Germany-Decker JM; Shannon WM; Chen RC; et al. Activity of triciribine and triciribine-5′-monophosphate against human immunodeficiency virus types 1 and 2. AIDS Res. Hum. Retroviruses 1993, 9 (4), 307–314. [PubMed: 7685612]

(659). Shen W; Kim J-S; Hilfinger J Expedient total synthesis of triciribine and its prodrugs. Synth. Commun 2012, 42 (3), 358–374.

(660). Welker ME; Kulik G Recent syntheses of PI3K/Akt/mTOR signaling pathway inhibitors. Bioorg. Med. Chem 2013, 21 (14), 4063–4091. [PubMed: 23735831]

(661). Owonikoko TK; Khuri FR Targeting the PI3K/AKT/mTOR pathway. Am. Soc. Clin. Oncol. Educ. Book 2013, 2013, 395–401.

(662). Garrett CR; Coppola D; Wenham RM; Cubitt CL; Neuger AM; Frost TJ; Lush RM; Sullivan DM; Cheng JQ; Sebti SM Phase I pharmacokinetic and pharmacodynamic study of triciribine phosphate monohydrate, a small-molecule inhibitor of AKT phosphorylation, in adult subjects with solid tumors containing activated AKT. Invest. New Drugs 2011, 29 (6), 1381–1389. [PubMed: 20644979]

(663). Wotring LL; Townsend LB; Jones LM; Borysko KZ; Gildersleeve DL; Parker WB Dual mechanisms of inhibition of DNA synthesis by triciribine. Cancer Res 1990, 50 (16), 4891–4899. [PubMed: 2379153]

(664). Sagatys E; Garrett CR; Boulware D; Kelley S; Malafa M; Cheng JQ; Sebti S; Coppola D Activation of the serine/threonine protein kinase Akt during the progression of Barrett neoplasia. Hum. Pathol 2007, 38 (10), 1526–1531. [PubMed: 17640711]

(665). Costa C; Pereira S; Lima L; Peixoto A; Fernandes E; Neves D; Neves M; Gaiteiro C; Tavares A; Gil da Costa RM; et al. Abnormal protein glycosylation and activated PI3K/Akt/mTOR pathway: Role in bladder cancer prognosis and targeted therapeutics. PLoS One 2015, 10 (11), e0141253. [PubMed: 26569621]

(666). Houede N; Pourquier P Targeting the genetic alterations of the PI3K-AKT-mTOR pathway: Its potential use in the treatment of bladder cancers. Pharmacol. Ther 2015, 145, 1–18. [PubMed: 24929024]

(667). Hoffman K; Holmes FA; Fraschini G; Esparza L; Frye D; Raber MN; Newman RA; Hortobagyi GN Phase I-II study: Triciribine (tricyclic nucleoside phosphate) for metastatic breast cancer. Cancer Chemother. Pharmacol 1995, 37 (3), 254–258.

(668). Wang Q; Li SH; Wang H; Xiao Y; Sahin O; Brady SW; Li P; Ge H; Jaffee EM; Muller WJ; et al. Concomitant targeting of tumor cells and induction of T-cell response synergizes to effectively inhibit trastuzumab-resistant breast cancer. Cancer Res 2012, 72 (17), 4417–4428. [PubMed: 22773664]

(669). Townsend LB; Lewis AF; Roti-Roti LW US Patent 4123524, 1978.

(670). Soucy TA; Dick LR; Smith PG; Milhollen MA; Brownell JE The NEDD8 conjugation pathway and its relevance in cancer biology and therapy. Genes Cancer 2010, 1 (7), 708–716. [PubMed: 21779466]

(671). Yang X; Brownell JE; Xu Q; Zhu F; Ma J; Loke HK; Rollins N; Soucy TA; Minissale JJ; Thomas MP; et al. Absolute quantification of E1, ubiquitin-like proteins and Nedd8-MLN4924 adduct by mass spectrometry. Cell Biochem. Biophys 2013, 67 (1), 139–147. [PubMed: 23754621]

(672). Luo Z; Pan Y; Jeong LS; Liu J; Jia L Inactivation of the Cullin (CUL)-RING E3 ligase by the NEDD8-activating enzyme inhibitor MLN4924 triggers protective autophagy in cancer cells. Autophagy 2012, 8 (11), 1677–1679. [PubMed: 22874562]

(673). Jin HS; Liao L; Park Y; Liu YC Neddylation pathway regulates T-cell function by targeting an adaptor protein Shc and a protein kinase Erk signaling. Proc. Natl. Acad. Sci. U. S. A 2013, 110 (2), 624–629. [PubMed: 23267066]

(674). Chang FM; Reyna SM; Granados JC; Wei SJ; Innis-Whitehouse W; Maffi SK; Rodriguez E; Slaga TJ; Short JD Inhibition of neddylation represses lipopolysaccharide-induced proinflammatory cytokine production in macrophage cells. J. Biol. Chem 2012, 287 (42), 35756–35767. [PubMed: 22927439]

(675). Eriksen BC; Hagen B; Eik-Nes SH; Molne K; Mjolnerod OK; Romslo I Long-term effectiveness of the Burch colposuspension in female urinary stress incontinence. Acta Obstet. Gynecol. Scand 1990, 69 (1), 45–50. [PubMed: 2346079]

(676). Blank JL; Liu XJ; Cosmopoulos K; Bouck DC; Garcia K; Bernard H; Tayber O; Hather G; Liu R; Narayanan U; et al. Novel DNA damage checkpoints mediating cell death induced by the NEDD8-activating enzyme inhibitor MLN4924. Cancer Res 2013, 73 (1), 225–234. [PubMed: 23100467]

(677). Milhollen MA; Traore T; Adams-Duffy J; Thomas MP; Berger AJ; Dang L; Dick LR; Garnsey JJ; Koenig E; Langston SP; et al. MLN4924, a NEDD8-activating enzyme inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment of NF-κB-dependent lymphoma. Blood 2010, 116 (9), 1515–1523. [PubMed: 20525923]

(678). Toth JI; Yang L; Dahl R; Petroski MD A gatekeeper residue for NEDD8-activating enzyme inhibition by MLN4924. Cell Rep 2012, 1 (4), 309–316. [PubMed: 22832224]

(679). Zhao Y; Xiong X; Jia L; Sun Y Targeting Cullin-RING ligases by MLN4924 induces autophagy via modulating the HIF1-REDD1-TSC1-mTORC1-DEPTOR axis. Cell Death Dis 2012, 3, e386. [PubMed: 22951983]

(680). Nawrocki ST; Kelly KR; Smith PG; Espitia CM; Possemato A; Beausoleil SA; Milhollen M; Blakemore S; Thomas M; Berger A; et al. Disrupting protein NEDDylation with MLN4924 is a novel strategy to target cisplatin resistance in ovarian cancer. Clin. Cancer Res 2013, 19 (13), 3577–3590. [PubMed: 23633453]

(681). Nawrocki ST; Griffin P; Kelly KR; Carew JS MLN4924: a novel first-in-class inhibitor of NEDD8-activating enzyme for cancer therapy. Expert Opin. Invest. Drugs 2012, 21 (10), 1563–1573.

(682). Choi WJ; Moon HR; Kim HO; Yoo BN; Lee JA; Shin DH; Jeong LS Preparative and stereoselective synthesis of the versatile intermediate for carbocyclic nucleosides: effects of the bulky protecting groups to enforce facial selectivity. J. Org. Chem 2004, 69 (7), 2634–2636. [PubMed: 15049678]

(683). Lee HW; Nam SK; Choi WJ; Kim HO; Jeong LS Stereoselective synthesis of MLN4924, an inhibitor of NEDD8-activating enzyme. J. Org. Chem 2011, 76 (9), 3557–3561. [PubMed: 21417215]

(684). Langston SP; Olhava EJ; Vyskocil S WO Patent 2007092213, 2007.

(685). An GI; Rhee H A facile synthesis of cis-4-amino-2-cyclopentene-1-methanol, a key intermediate for the synthesis of carbocyclic nucleosides. Nucleosides, Nucleotides Nucleic Acids 2002, 21 (1), 65–72. [PubMed: 11991149]

(686). Armitage I; Elliott EL; Hicks F; Langston M; McCarron A; McCubbin QJ; O'Brien E; Stirling M; Zhu L Process development and GMP production of a potent NAE inhibitor Pevonedistat. Org. Process Res. Dev 2015, 19 (9), 1299–1307.

(687). Nguyen AT; Zhang Y The diverse functions of Dot1 and H3K79 methylation. Genes Dev 2011, 25 (13), 1345–1358. [PubMed: 21724828]

(688). Anglin JL; Deng L; Yao Y; Cai G; Liu Z; Jiang H; Cheng G; Chen P; Dong S; Song Y Synthesis and structure-activity relationship investigation of adenosine-containing inhibitors of histone methyltransferase DOT1L. J. Med. Chem 2012, 55 (18), 8066–8074. [PubMed: 22924785]

(689). Krivtsov AV; Armstrong SA MLL translocations, histone modifications and leukaemia stem-cell development. Nat. Rev. Cancer 2007, 7 (11), 823–833. [PubMed: 17957188]

(690). Krivtsov AV; Feng Z; Lemieux ME; Faber J; Vempati S; Sinha AU; Xia X; Jesneck J; Bracken AP; Silverman LB; et al. H3K79 methylation profiles define murine and human MLL-AF4 leukemias. Cancer Cell 2008, 14 (5), 355–368. [PubMed: 18977325]

(691). Muntean AG; Hess JL The pathogenesis of mixed-lineage leukemia. Annu. Rev. Pathol.: Mech. Dis 2012, 7, 283–301.

(692). Tamai H; Inokuchi K 11q23/MLL acute leukemia: Update of clinical aspects. J. Clin. Exp. Hematop 2010, 50 (2), 91–98. [PubMed: 21123966]

(693). Hilden JM; Dinndorf PA; Meerbaum SO; Sather H; Villaluna D; Heerema NA; McGlennen R; Smith FO; Woods WG; Salzer WL; et al. Analysis of prognostic factors of acute lymphoblastic

leukemia in infants: Report on CCG 1953 from the Children's Oncology Group. Blood 2006, 108 (2), 441–451. [PubMed: 16556894]

(694). Yu W; Chory EJ; Wernimont AK; Tempel W; Scopton A; Federation A; Marineau JJ; Qi J; Barsyte-Lovejoy D; Yi J; et al. Catalytic site remodelling of the DOT1L methyltransferase by selective inhibitors. Nat. Commun 2012, 3, 1288. [PubMed: 23250418]

(695). Daigle SR; Olhava EJ; Therkelsen CA; Majer CR; Sneeringer CJ; Song J; Johnston LD; Scott MP; Smith JJ; Xiao Y; et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell 2011, 20 (1), 53–65. [PubMed: 21741596]

(696). Daigle SR; Olhava EJ; Therkelsen CA; Basavapathruni A; Jin L; Boriack-Sjodin PA; Allain CJ; Klaus CR; Raimondi A; Scott MP; et al. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood 2013, 122 (6), 1017–1025. [PubMed: 23801631]

(697). Waters NJ; Daigle SR; Rehlaender BN; Basavapathruni A; Campbell CT; Jensen TB; Truitt BF; Olhava EJ; Pollock RM; Stickland KA; et al. Exploring drug delivery for the DOT1L inhibitor pinometostat (EPZ-5676): Subcutaneous administration as an alternative to continuous IV infusion, in the pursuit of an epigenetic target. J. Controlled Release 2015, 220 (Pt B), 758–765.

(698). Basavapathruni A; Olhava EJ; Daigle SR; Therkelsen CA; Jin L; Boriack-Sjodin PA; Allain CJ; Klaus CR; Raimondi A; Scott MP; et al. Nonclinical pharmacokinetics and metabolism of EPZ-5676, a novel DOT1L histone methyltransferase inhibitor. Biopharm. Drug Dispos 2014, 35 (4), 237–252. [PubMed: 24415392]

(699). Waters NJ; Smith SA; Olhava EJ; Duncan KW; Burton RD; O'Neill J; Rodrigue ME; Pollock RM; Moyer MP; Chesworth R Metabolism and disposition of the DOT1L inhibitor, pinometostat (EPZ-5676), in rat, dog and human. Cancer Chemother. Pharmacol 2016, 77 (1), 43–62. [PubMed: 26645404]

(700). Lipka DB; Kuck D; Kliem C; Gerhauser C Substituted purine and 7-deazapurine compounds as modulators of epigenetic enzymes: a patent evaluation (WO2012075381). Expert Opin. Ther. Pat 2013, 23 (4), 537–543. [PubMed: 23448241]

(701). Olhava EJ; Chesworth R; Kuntz KW; Richon VM; Pollck RM; Daigle SR WO Patent 2012075381, 2012.

(702). Olhava EJ WO Patent 2014152566, 2014.

(703). Bar-Yehuda S; Stemmer SM; Madi L; Castel D; Ochaion A; Cohen S; Barer F; Zabutti A; Perez-Liz G; Del Valle L; et al. The A3 adenosine receptor agonist CF102 induces apoptosis of hepatocellular carcinoma via de-regulation of the Wnt and NF-kappaB signal transduction pathways. Int. J. Oncol 2008, 33 (2), 287–295. [PubMed: 18636149]

(704). Cohen S; Stemmer SM; Zozulya G; Ochaion A; Patoka R; Barer F; Bar-Yehuda S; Rath-Wolfson L; Jacobson KA; Fishman P CF102 an A3 adenosine receptor agonist mediates anti-tumor and anti-inflammatory effects in the liver. J. Cell. Physiol 2011, 226 (9), 2438–2447. [PubMed: 21660967]

(705). Young HW; Molina JG; Dimina D; Zhong H; Jacobson M; Chan LN; Chan TS; Lee JJ; Blackburn MR A3 adenosine receptor signaling contributes to airway inflammation and mucus production in adenosine deaminase-deficient mice. J. Immunol 2004, 173 (2), 1380–1389. [PubMed: 15240734]

(706). Muller CE; Jacobson KA Recent developments in adenosine receptor ligands and their potential as novel drugs. Biochim. Biophys. Acta, Biomembr 2011, 1808 (5), 1290–1308.

(707). Madi L; Cohen S; Ochayin A; Bar-Yehuda S; Barer F; Fishman P Overexpression of A3 adenosine receptor in peripheral blood mononuclear cells in rheumatoid arthritis: involvement of nuclear factor-kappaB in mediating receptor level. J. Rheumatol 2007, 34 (1), 20–26. [PubMed: 17216675]

(708). Fishman P; Bar-Yehuda S; Liang BT; Jacobson KA Pharmacological and therapeutic effects of A3 adenosine receptor agonists. Drug Discovery Today 2012, 17 (7–8), 359–366. [PubMed: 22033198]

(709). Stemmer SM; Benjaminov O; Medalia G; Ciuraru NB; Silverman MH; Bar-Yehuda S; Fishman S; Harpaz Z; Farbstein M; Cohen S; et al. CF102 for the treatment of hepatocellular carcinoma: a phase I/II, open-label, dose-escalation study. Oncologist 2013, 18 (1), 25–26. [PubMed: 23299770]

(710). Kim HO; Ji XD; Siddiqi SM; Olah ME; Stiles GL; Jacobson KA 2-substitution of N6-benzyladenosine-5′-uronamides enhances selectivity for A3 adenosine receptors. J. Med. Chem 1994, 37 (21), 3614–3621. [PubMed: 7932588]

(711). Hou X; Lee HW; Tosh DK; Zhao LX; Jeong LS Alternative and improved syntheses of highly potent and selective A3 adenosine receptor agonists, Cl-IB-MECA and thio-Cl-IB-MECA. Arch. Pharmacal Res 2007, 30 (10), 1205–1209.

(712). Beutner KR; Friedman DJ; Forszpaniak C; Andersen PL; Wood MJ Valaciclovir compared with acyclovir for improved therapy for herpes zoster in immunocompetent adults. Antimicrob. Agents Chemother 1995, 39 (7), 1546–1553. [PubMed: 7492102]

(713). Elion GB Mechanism of action and selectivity of acyclovir. Am. J. Med 1982, 73 (1A), 7–13. [PubMed: 6285736]

(714). Kieback DG Adenovirus-mediated thymidine kinase gene therapy induces apoptosis in human epithelial ovarian cancer cells and damages PARP-1. In Vivo 2009, 23 (1), 77–80. [PubMed: 19368128]

(715). Mesnil M; Yamasaki H Bystander effect in herpes simplex virus-thymidine kinase/ganciclovir cancer gene therapy: role of gapjunctional intercellular communication. Cancer Res 2000, 60 (15), 3989–3999. [PubMed: 10945596]

(716). Hasenburg A; Tong XW; Rojas-Martinez A; Nyberg-Hoffman C; Kieback CC; Kaplan AL; Kaufman RH; Ramzy I; Aguilar-Cordova E; Kieback DG Thymidine kinase (TK) gene therapy of solid tumors: valacyclovir facilitates outpatient treatment. Anticancer Res 1999, 19 (3B), 2163–2165. [PubMed: 10472325]

(717). Soderberg-Naucler C; Rahbar A; Stragliotto G Survival in patients with glioblastoma receiving valganciclovir. N. Engl. J. Med 2013, 369 (10), 985–986. [PubMed: 24004141]

(718). Lawler SE Cytomegalovirus and glioblastoma; controversies and opportunities. J. Neuro-Oncol 2015, 123 (3), 465–471.

(719). Cobbs CS Cytomegalovirus and brain tumor: epidemiology, biology and therapeutic aspects. Curr. Opin. Oncol 2013, 25 (6), 682–688. [PubMed: 24097102]

(720). Martin JC; Dvorak CA; Smee DF; Matthews TR; Verheyden JP 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine: a new potent and selective antiherpes agent. J. Med. Chem 1983, 26 (5), 759–761. [PubMed: 6302255]

(721). Babu JS; Bay PC; Kumar Y; Khanduri CH WO Patent 2004048380, 2004.

(722). Arzeno HB; Humphreys ER US Patent 5756736, 1998.

(723). Khanduri CH; Kumar Y; Panda AK; Raina S; Sharma MK WO Patent 2005092891, 2005.

(724). Krenitsky TA; Beauchamp LM EP Patent 0308065, 1989.

(725). Etinger MY; Yudovich LM; Yuzefovich M; Nisnevich GA; Dolitzky BZ; Pertsikov B; Tishin B; Blasberger D US Patent 6849737, 2005.

(726). Vetukuri PRVNKV; Vedantham R; Mathad VT; Padi PR; Ramasamy VA A concise route to valacyclovir hydrochloride. Helv. Chim. Acta 2011, 94 (4), 592–596.

(727). Sidwell RW; Huffman JH; Khare GP; Allen LB; Witkowski JT; Robins RK Broad-spectrum antiviral activity of Virazole: 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. Science 1972, 177 (4050), 705–706. [PubMed: 4340949]

(728). Sidwell RW; Khare GP; Allen LB; Huffman JG; Witkowski JT; Simon LN; Robins RK In vitro and in vivo effect of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) on types 1 and 3 parainfulenza virus infections. Chemotherapy 2004, 21 (3–4), 205–220.

(729). James JS Ribavirin approved for hepatitis C combination treatment. AIDS Treat. News 1998, No. No 297, 7.

(730). Eriksson B; Helgstrand E; Johansson NG; Larsson A; Misiorny A; Noren JO; Philipson L; Stenberg K; Stening G; Stridh S; et al. Inhibition of influenza virus ribonucleic acid polymerase by ribavirin triphosphate. Antimicrob. Agents Chemother 1977, 11 (6), 946–951. [PubMed: 879760]

(731). Sintchak MD; Nimmesgern E The structure of inosine 5′-monophosphate dehydrogenase and the design of novel inhibitors. Immunopharmacology 2000, 47 (2–3), 163–184. [PubMed: 10878288]

(732). Jayaram HN; Cooney DA; Grusch M; Krupitza G Consequences of IMP dehydrogenase inhibition, and its relationship to cancer and apoptosis. Curr. Med. Chem 1999, 6 (7), 561–574. [PubMed: 10390601]

(733). Borden KL Targeting the oncogene eIF4E in cancer: From the bench to clinical trials. Clin. Invest. Med 2011, 34 (6), E315. [PubMed: 22129918]

(734). Borden KL; Culjkovic-Kraljacic B Ribavirin as an anti-cancer therapy: acute myeloid leukemia and beyond? Leuk. Lymphoma 2010, 51 (10), 1805–1815. [PubMed: 20629523]

(735). Volpon L; Osborne MJ; Zahreddine H; Romeo AA; Borden KL Conformational changes induced in the eukaryotic translation initiation factor eIF4E by a clinically relevant inhibitor, ribavirin triphosphate. Biochem. Biophys. Res. Commun 2013, 434 (3), 614–619. [PubMed: 23583375]

(736). Holm N; Byrnes K; Johnson L; Abreo F; Sehon K; Alley J; Meschonat C; Md QC; Li BD A prospective trial on initiation factor 4E (eIF4E) overexpression and cancer recurrence in node-negative breast cancer. Ann. Surg. Oncol 2008, 15 (11), 3207–3215. [PubMed: 18719964]

(737). Coleman LJ; Peter MB; Teall TJ; Brannan RA; Hanby AM; Honarpisheh H; Shaaban AM; Smith L; Speirs V; Verghese ET; et al. Combined analysis of eIF4E and 4E-binding protein expression predicts breast cancer survival and estimates eIF4E activity. Br. J. Cancer 2009, 100 (9), 1393–1399. [PubMed: 19367274]

(738). Pettersson F; Yau C; Dobocan MC; Culjkovic-Kraljacic B; Retrouvey H; Puckett R; Flores LM; Krop IE; Rousseau C; Cocolakis E; et al. Ribavirin treatment effects on breast cancers overexpressing eIF4E, a biomarker with prognostic specificity for luminal B-type breast cancer. Clin. Cancer Res 2011, 17 (9), 2874–2884. [PubMed: 21415224]

(739). Witkowski JT; Robins RK; Sidwell RW; Simon LN Design, synthesis, and broad spectrum antiviral activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide and related nucleosides. J. Med. Chem 1972, 15 (11), 1150–1154. [PubMed: 4347550]

(740). Banfi A; Dall'Oro B; Frigerio M; Mancini A US Patent 7285659, 2007.

(741). Li YS; Zhang JJ; Mei LQ; Tan CX An improved procedure for the preparation of ribavirin. Org. Prep. Proced. Int 2012, 44 (4), 387–391.

(742). Hennen WJ; Wong CH A new method for the enzymic synthesis of nucleosides using purine nucleoside phosphorylase. J. Org. Chem 1989, 54 (19), 4692–4695.

(743). Taverna-Porro M; Bouvier LA; Pereira CA; Montserrat JM; Irivarren AM Chemoenzymatic preparation of nucleosides from furanoses. Tetrahedron Lett 2008, 49 (16), 2642–2645.

(744). Galinanes M; Bullough D; Mullane KM; Hearse DJ Sustained protection by acadesine against ischemia- and reperfusion-induced injury. Studies in the transplanted rat heart. Circulation 1992, 86 (2), 589–597. [PubMed: 1638724]

(745). Favero CB; Mandell JW A pharmacological activator of AMP-activated protein kinase (AMPK) induces astrocyte stellation. Brain Res 2007, 1168, 1–10. [PubMed: 17706943]

(746). Sengupta TK; Leclerc GM; Hsieh-Kinser TT; Leclerc GJ; Singh I; Barredo JC Cytotoxic effect of 5-aminoimidazole-4-carboxamide-1-β−4-ribofuranoside (AICAR) on childhood acute lymphoblastic leukemia (ALL) cells: implication for targeted therapy. Mol. Cancer 2007, 6, 46. [PubMed: 17623090]

(747). Cronstein BN; Kamen BA 5-aminoimidazole-4-carbox-amide-1-β−4-ribofuranoside (AICA-riboside) as a targeting agent for therapy of patients with acute lymphoblastic leukemia: are we there and are there pitfalls? J. Pediatr. Hematol./Oncol 2007, 29 (12), 805–807.

(748). Montraveta A; Xargay-Torrent S; Lopez-Guerra M; Rosich L; Perez-Galan P; Salaverria I; Bea S; Kalko SG; de Frias M; Campas C; et al. Synergistic anti-tumor activity of acadesine (AICAR) in combination with the anti-CD20 monoclonal antibody rituximab in in vivo and in vitro models of mantle cell lymphoma. Oncotarget 2014, 5 (3), 726–739. [PubMed: 24519895]

(749). Van Den Neste E; Cazin B; Janssens A; Gonzalez-Barca E; Terol MJ; Levy V; Perez de Oteyza J; Zachee P; Saunders A; de Frias M; et al. Acadesine for patients with relapsed/refractory chronic lymphocytic leukemia (CLL): a multicenter phase I/II study. Cancer Chemother. Pharmacol 2013, 71 (3), 581–591. [PubMed: 23228986]

(750). Nara T; Misawa M JP Patent 46033196, 1971.

(751). Sakai S; Niwa K; Sasaki H; Hirose Y; Kinoshita K JP Patent 46028821, 1971.

(752). Kanemitsu O; Akimoto T; Ito M; Mimura S JP Patent 43020717, 1968.

(753). Baddiley J; Buchanan JG; Hardy FE; Stewart J Chemical studies in the biosynthesis of purine nucleotides. Part III. The synthesis of 5-amino-1-(β-D-ribofuranosyl)glyoxaline-4-carboxyamide and 4-amino-1-(β-D-ribofuranosyl)glyoxaline-5-carboxyamide. J. Chem. Soc 1959, 0, 2893–2901.

(754). Kissman HM; Pidacks C; Baker BR Puromycin. Synthetic studies. XI. D-ribofuranosyl derivatives of 6-dimethylaminopurine. J. Am. Chem. Soc 1955, 77 (1), 18–24.

(755). Windaus A; Langenbeck W Über die 4(5)-nitro-imidazol-5(4)-carbonsäure. Ber. Dtsch. Chem. Ges. B 1923, 56 (3), 683–686.

(756). Kohyama N; Yamamoto Y A facile synthesis of AICAR from inosine. Synthesis 2003, 17, 2639–2642.

(757). Coats SJ; Garnier-Amblard EC; Amblard F; Ehteshami M; Amiralaei S; Zhang H; Zhou L; Boucle SR; Lu X; Bondada L; et al. Chutes and ladders in hepatitis C nucleoside drug development. Antiviral Res 2014, 102, 119–147. [PubMed: 24275341]

(758). Ji X; Wu Y; Yan J; Mehrens J; Yang H; DeLucia M; Hao C; Gronenborn AM; Skowronski J; Ahn J; et al. Mechanism of allosteric activation of SAMHD1 by dGTP. Nat. Struct. Mol. Biol 2013, 20 (11), 1304–1309. [PubMed: 24141705]

(759). Behrendt R; Schumann T; Gerbaulet A; Nguyen LA; Schubert N; Alexopoulou D; Berka U; Lienenklaus S; Peschke K; Gibbert K; et al. Mouse SAMHD1 has antiretroviral activity and suppresses a spontaneous cell-intrinsic antiviral response. Cell Rep 2013, 4 (4), 689–696. [PubMed: 23972988]

(760). Hollenbaugh JA; Tao S; Lenzi GM; Ryu S; Kim DH; Diaz-Griffero F; Schinazi RF; Kim B dNTP pool modulation dynamics by SAMHD1 protein in monocyte-derived macrophages. Retrovirology 2014, 11, 63. [PubMed: 25158827]

(761). de Silva S; Wang F; Hake TS; Porcu P; Wong HK; Wu L Downregulation of SAMHD1 expression correlates with promoter DNA methylation in Sezary syndrome patients. J. Invest. Dermatol 2014, 134 (2), 562–565. [PubMed: 23884314]

(762). Sjoblom T; Jones S; Wood LD; Parsons DW; Lin J; Barber TD; Mandelker D; Leary RJ; Ptak J; Silliman N; et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006, 314 (5797), 268–274. [PubMed: 16959974]

(763). Rentoft M; Lindell K; Tran P; Chabes AL; Buckland RJ; Watt DL; Marjavaara L; Nilsson AK; Melin B; Trygg J; et al. Heterozygous colon cancer-associated mutations of SAMHD1 have functional significance. Proc. Natl. Acad. Sci. U. S. A 2016, 113 (17), 4723–4728. [PubMed: 27071091]

(764). Kohnken R; Kodigepalli KM; Wu L Regulation of deoxynucleotide metabolism in cancer: novel mechanisms and therapeutic implications. Mol. Cancer 2015, 14, 176. [PubMed: 26416562]

(765). Rampazzo C; Tozzi MG; Dumontet C; Jordheim LP The druggability of intracellular nucleotide-degrading enzymes. Cancer Chemother. Pharmacol 2016, 77 (5), 883–893. [PubMed: 26614508]

(766). Porta R; Benaglia M; Puglisi A Flow chemistry: Recent developments in the synthesis of pharmaceutical products. Org. Process Res. Dev 2016, 20 (1), 2–25.

(767). Rodrigues T; Schneider P; Schneider G Accessing new chemical entities through microfluidic systems. Angew. Chem., Int. Ed 2014, 53 (23), 5750–5758.

(768). Hessel V; Kralisch D; Kockmann N; Noel T; Wang Q Novel process windows for enabling, accelerating, and uplifting flow chemistry. ChemSusChem 2013, 6 (5), 746–789. [PubMed: 23606410]

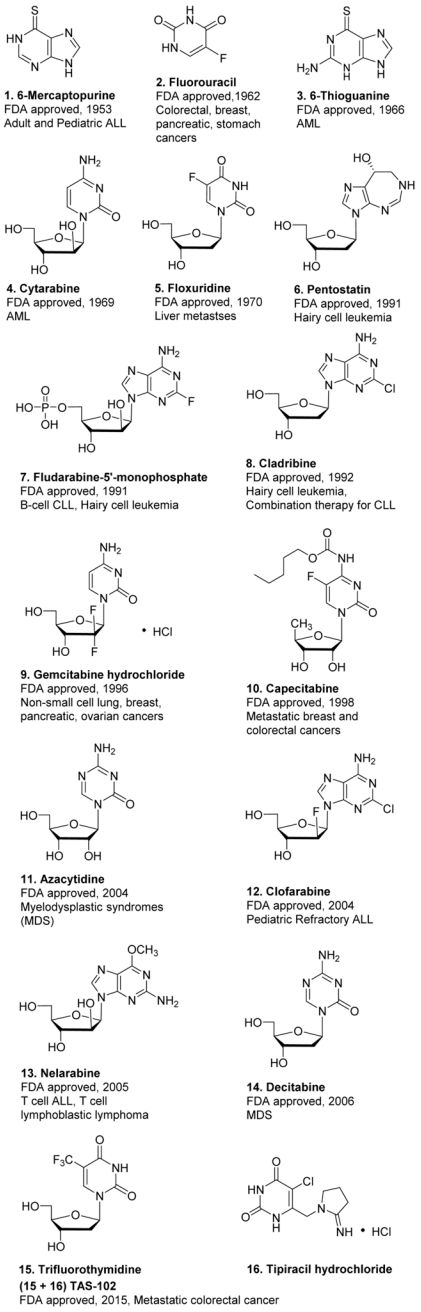
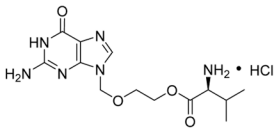
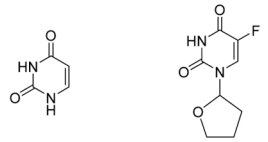


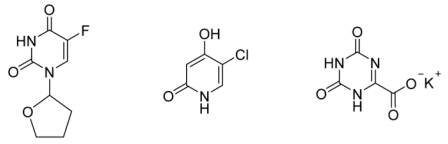
Figure 1.
FDA-approved anticancer nucleoside analogs.



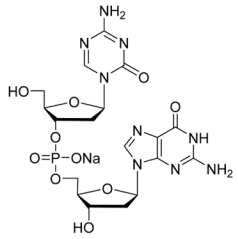
17. Valacyclovir hydrochloride
Phase III, Combination therapy for localized prostate cancer
Phase I/II, Combination therapy for recurrent prostate cancer
Phase I, Combination therapy for pediatric brain tumors



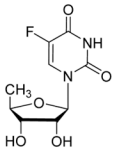
18. Uracil **19. Tegafur**
(18 + 19) Tegafur-uracil
Used in Japan and Taiwan, Advanced GI cancers
Phase II/III, Gastric cancer
Phase II, Colon cancer, combination therapy for hepatocellular carcinoma



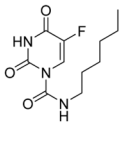
19. Tegafur **20. Gimeracil** **21. Oteracil**
(19 + 20 + 21) TS-1
Phase III, Combination therapy with cisplatin for advanced gastric cancer
Multiple Phase I/II, Combination therapy



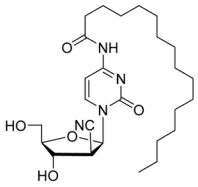
22. SGI-110 (Dinucleotide of **14**)
Phase III, AML
Phase I/II,
Metastatic colorectal and
ovarian cancer, MDS, AML, HCC



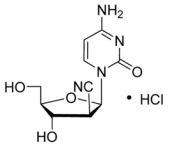
23. Doxifluridine
Phase III,
Combination therapy
for GI cancer



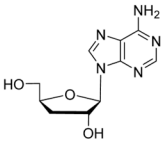
24. Carmofur
Used in China, Japan
and Finland
Colorectal cancer



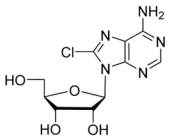
25. Sapacitabine
Phase III, newly diagnosed AML in elderly
Multiple Phase I/II, AML, CLL, non-small
cell lung cancers, solid tumors



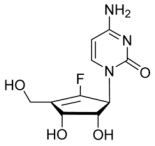
(HCl salt of CNDAC)
26. DFP-10917
Phae I/II,
Relapsed or refractory AML



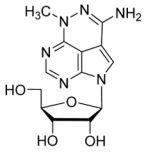
27. Cordycepin
Phase I/II,
Combination therapy for
TdT-positive leukemia



28. 8-Chloro-adenosine
Phase I,
Chronic lymphocytic leukemia



29. RX-3117
Phase I/II, Solid tumor
Phase I,
Advanced Malignancies



30. Triciribine
Phase I/II, ovarian cancer
Phase I,
Combination therapy
for ovarian cancer

31. Flucytosine
Used in gene therapy,
Phase I/II, Combination
therapy for solid tumors
Phase I, brain tumors

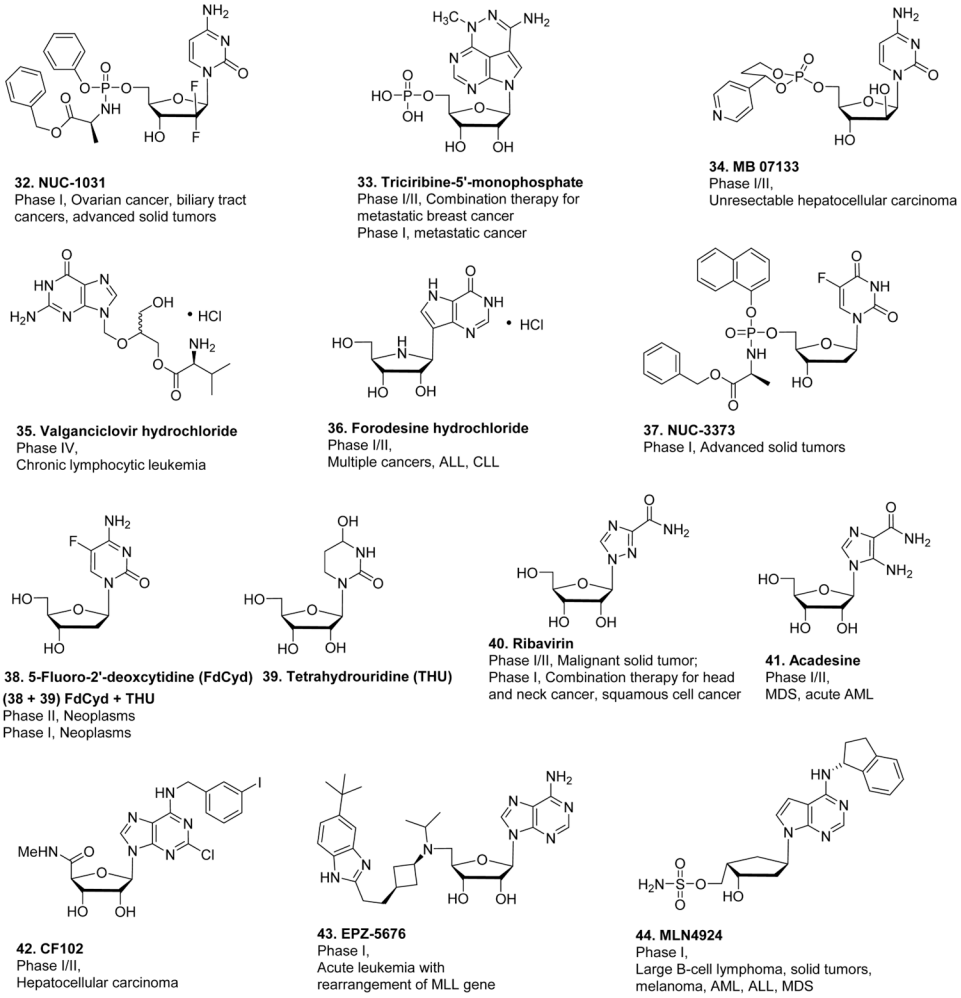


Figure 2.

Various nucleoside analogs in clinical trials or used outside of the U.S.

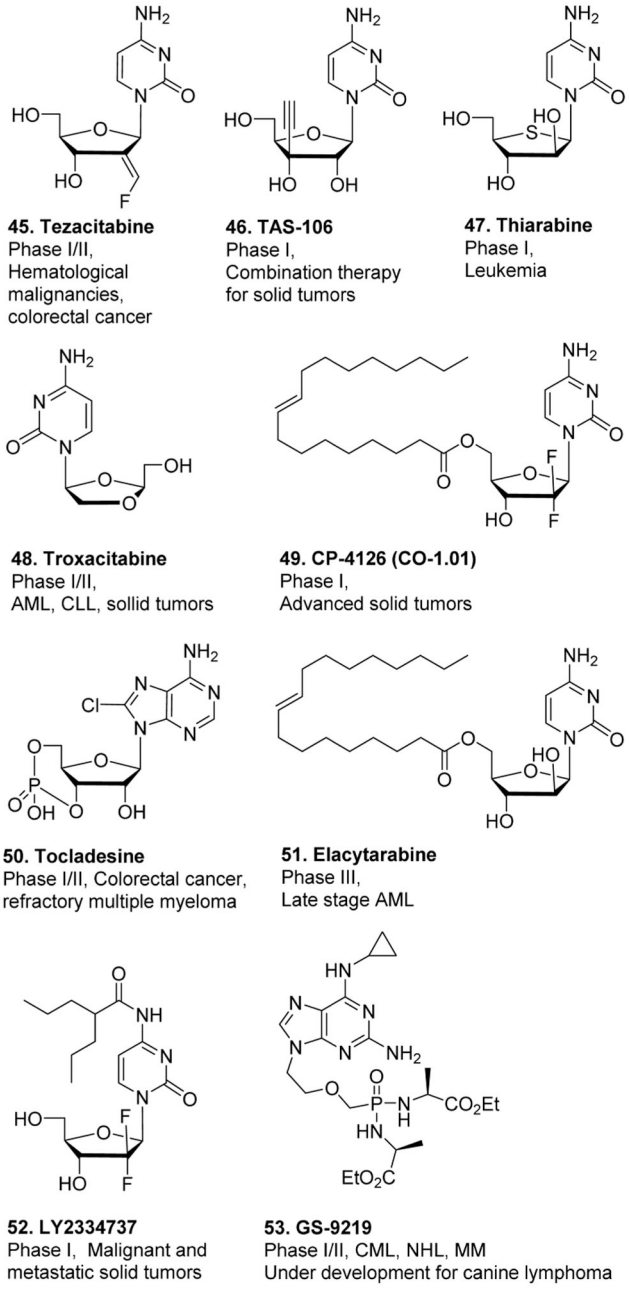


Figure 3.

Various nucleoside analogs that have stalled in clinical trials.

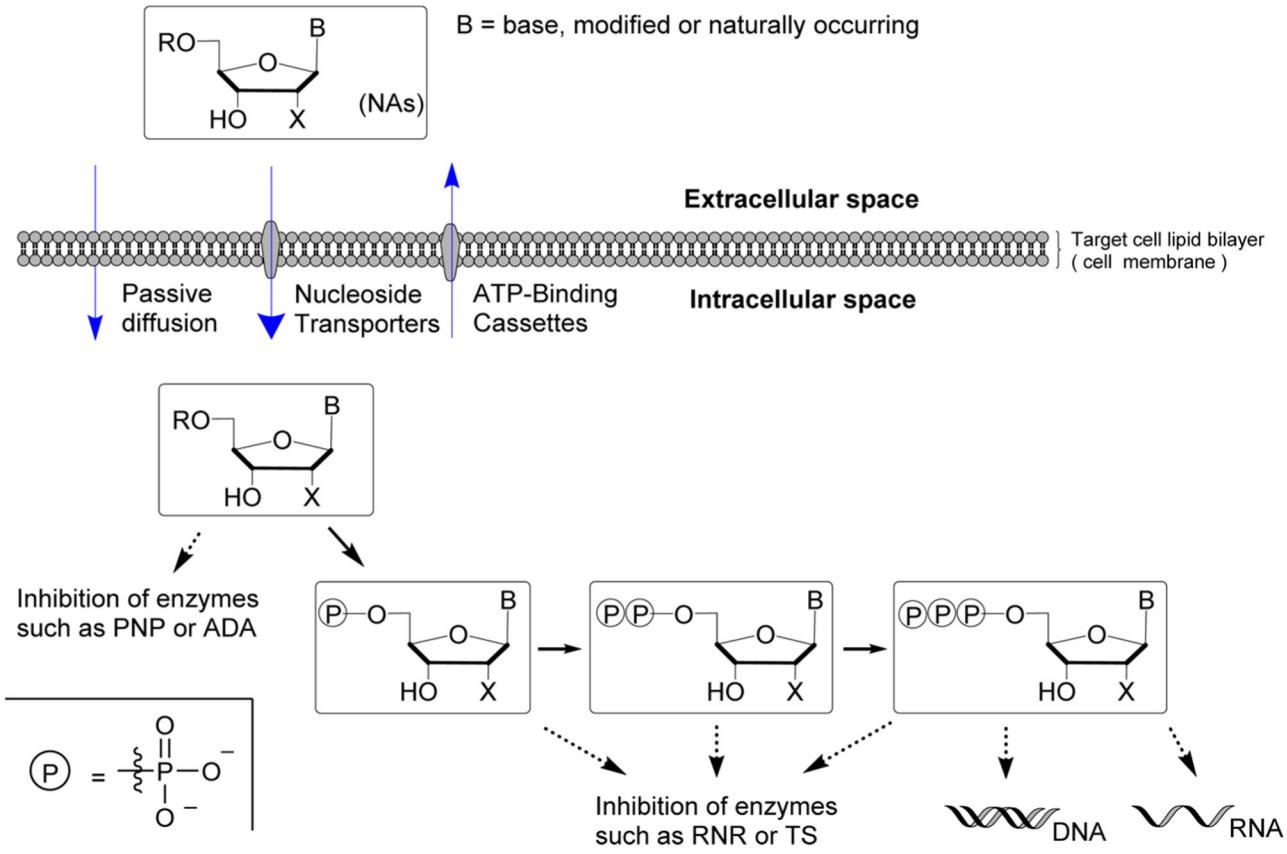


Figure 4.
General biological mechanism of action of anticancer nucleoside analogs.

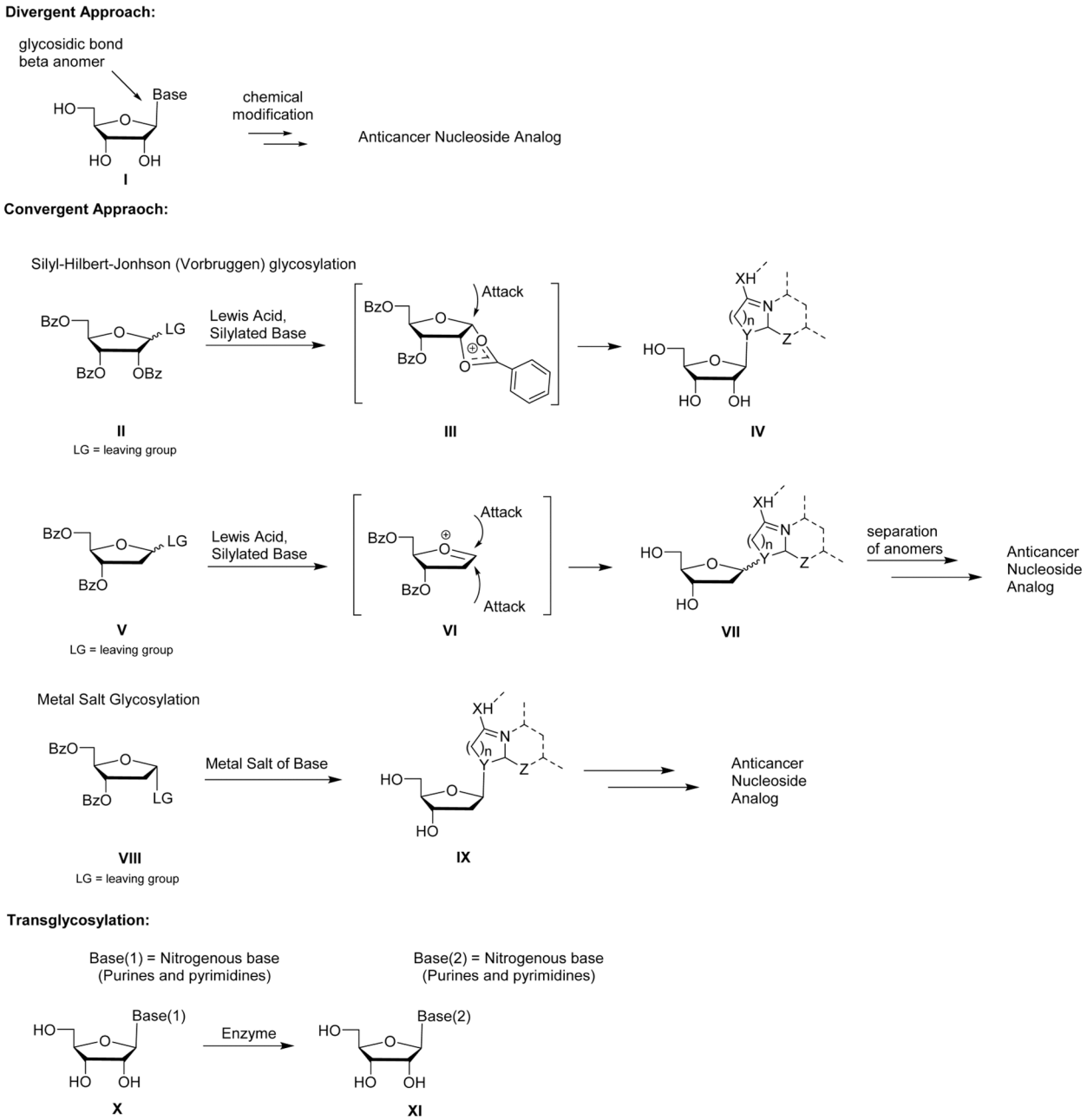


Figure 5.
General synthetic approaches to anticancer nucleoside analogs.

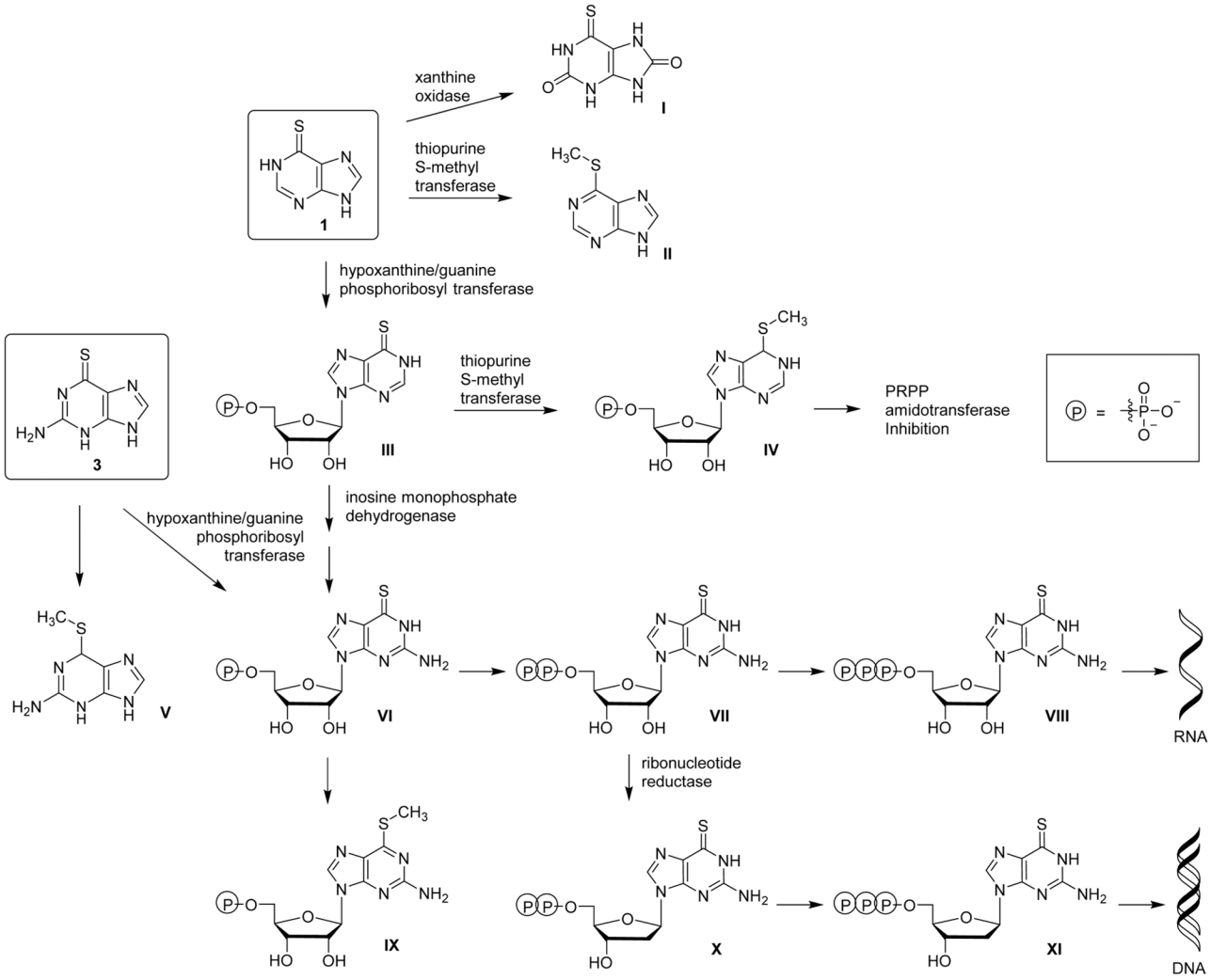


Figure 6.
Metabolism of 6-MP **1** and **6**-TG 3.

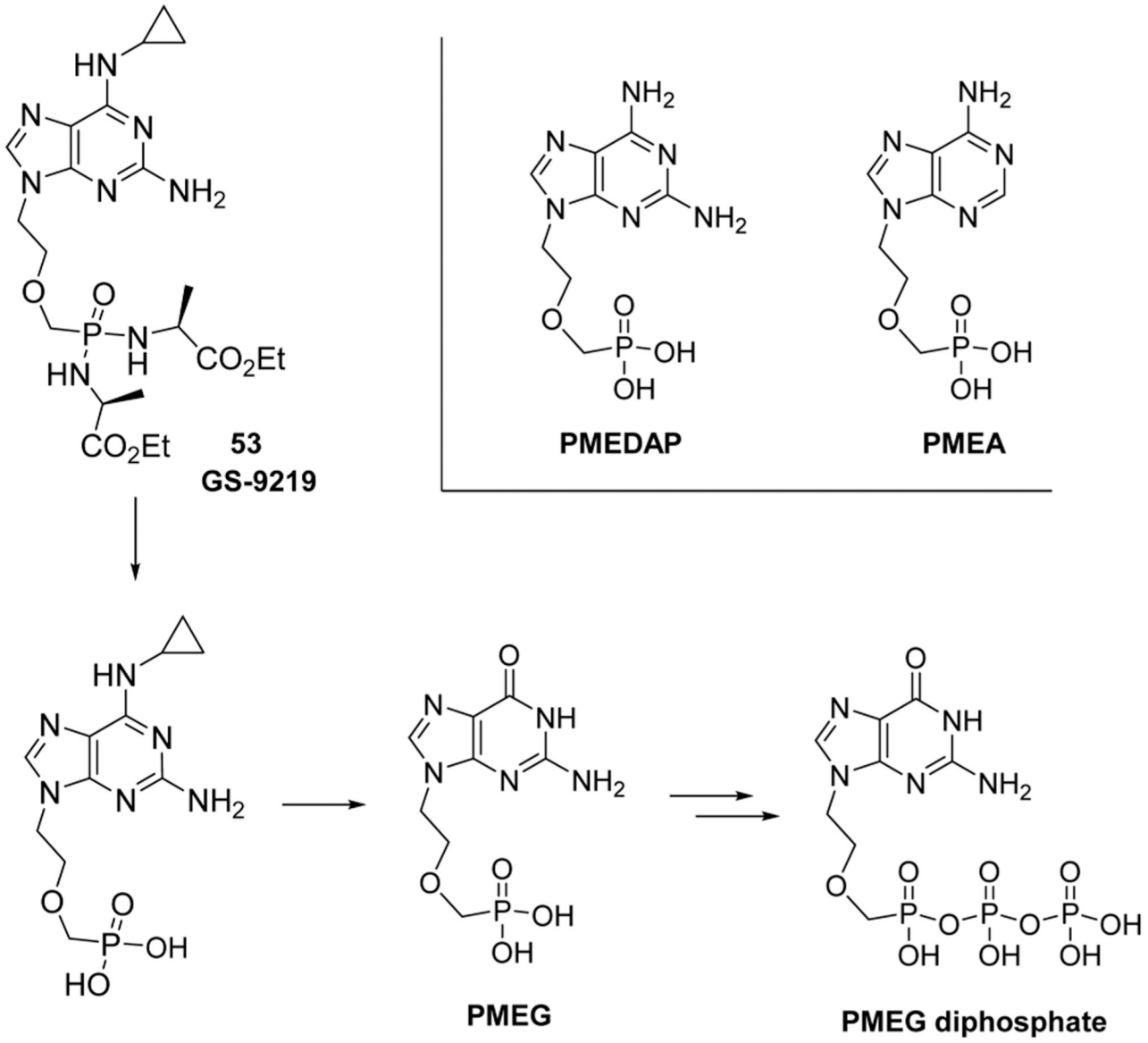


Figure 7.
Structures of PMEG, PMEDAP, and PMEA, and proposed cellular metabolism of GS-9219 53.

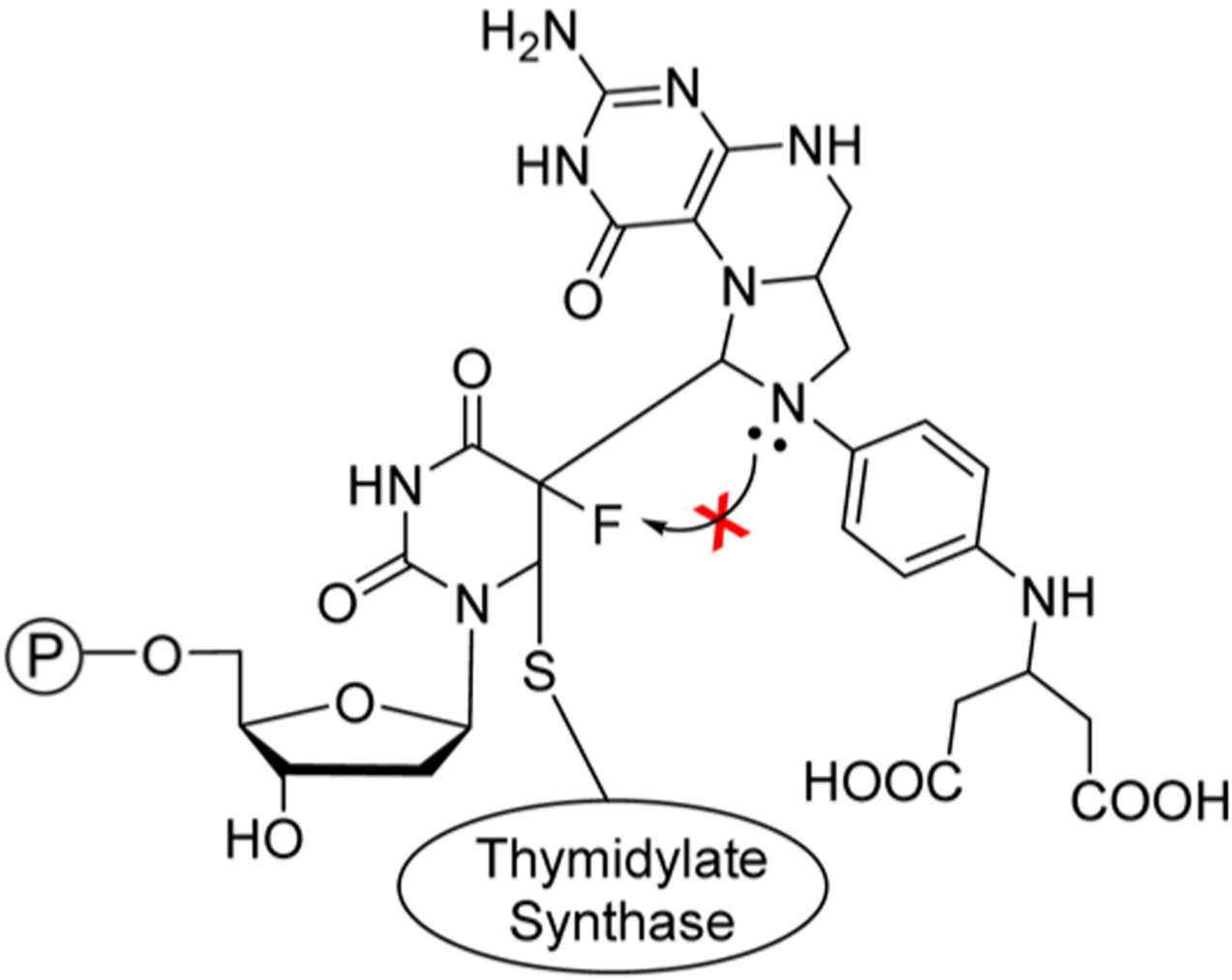


Figure 8.
Complex of 5,10-methylenetetrahydrofolate and thymidylate synthase with 5-FU-5′-monophosphate.

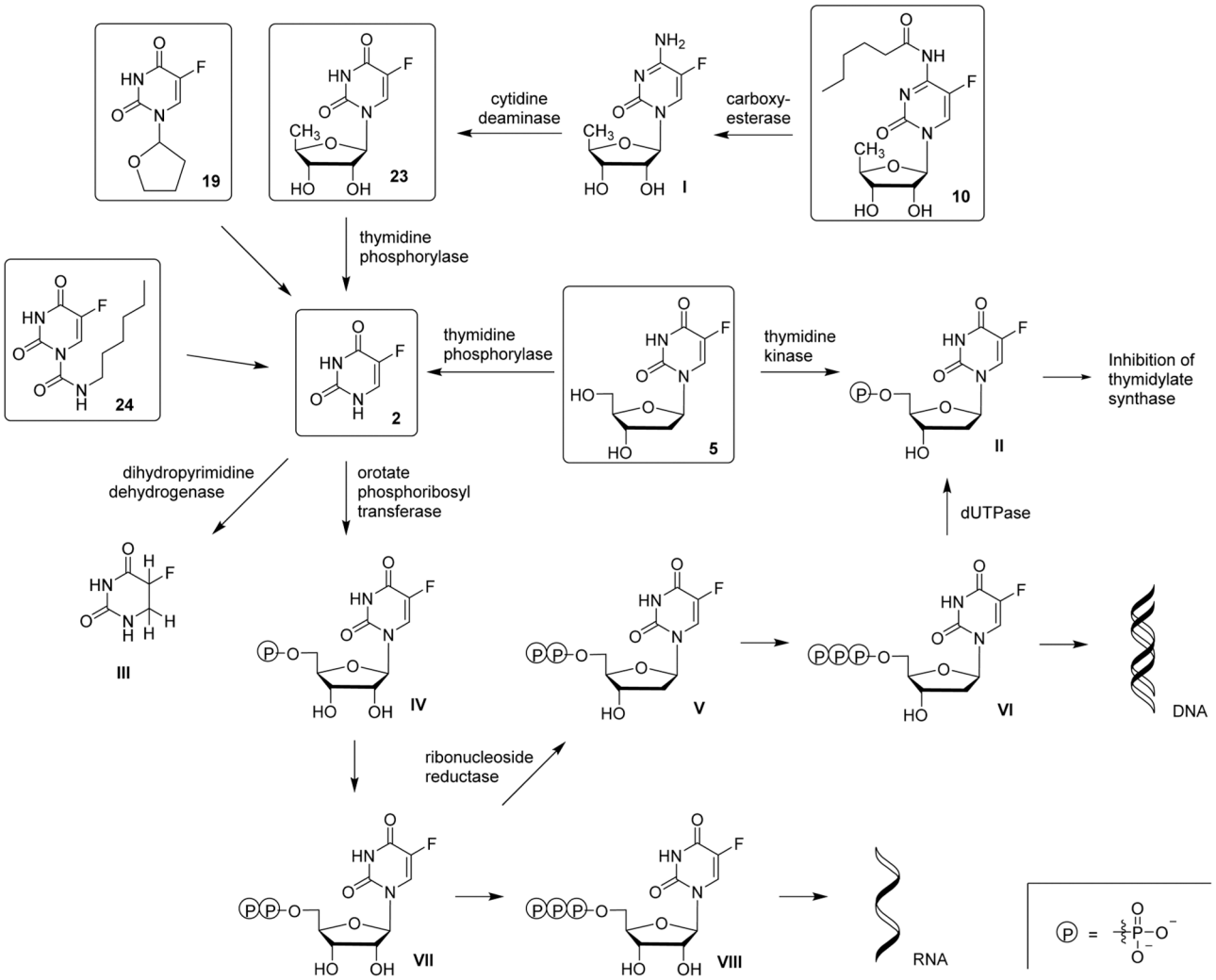


Figure 9.
Metabolism of 5-FU 2 and derivatives.

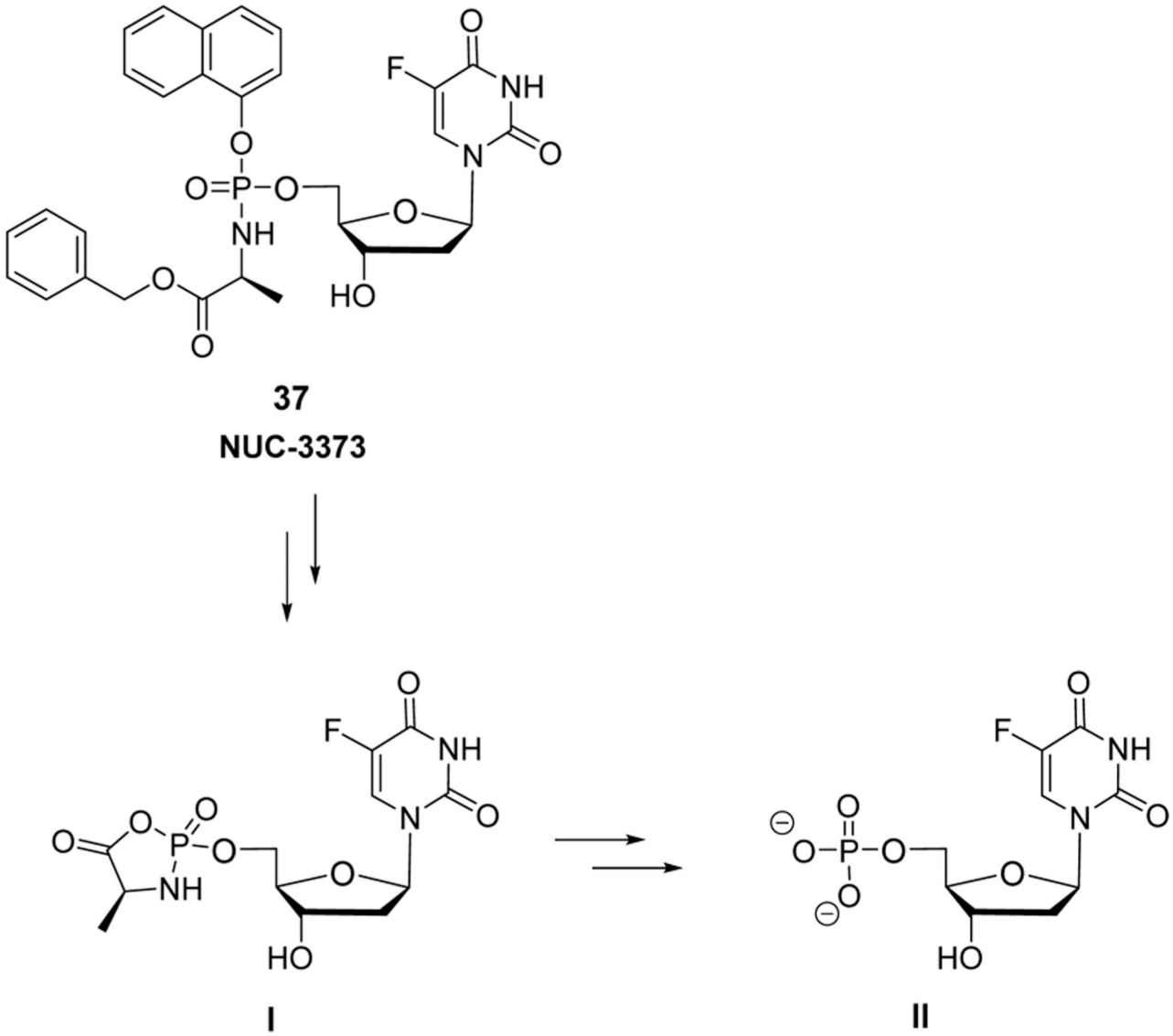


Figure 10.
Proposed mechanism for phosphoramidate decomposition of NUC-3373 **37**.

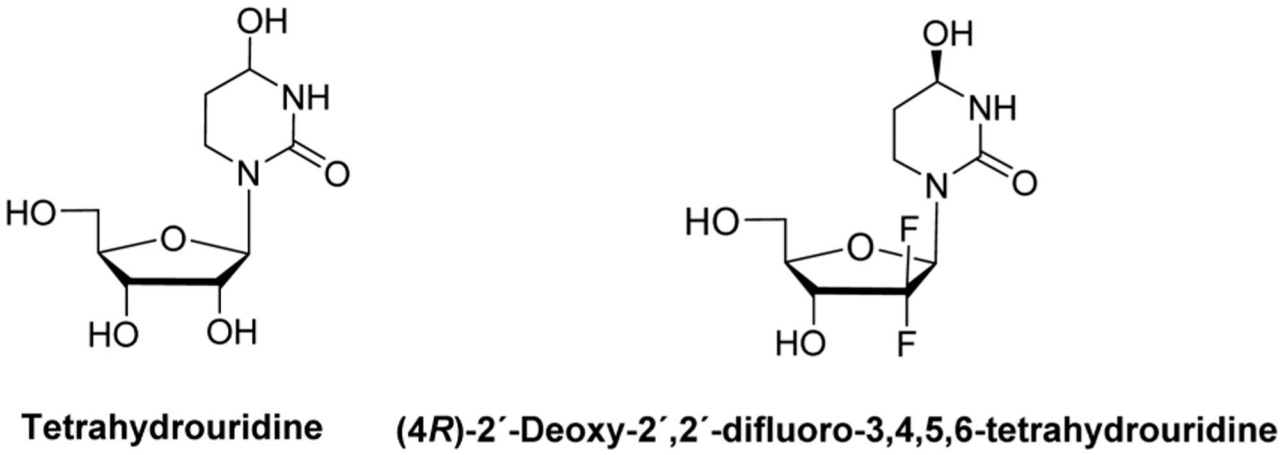


Figure 11.
Structures of tetrahydrouridine **39** and (4*R*)-2′-deoxy-2′,2′-difluoro-3,4,5,6-tetrahydrouridine.

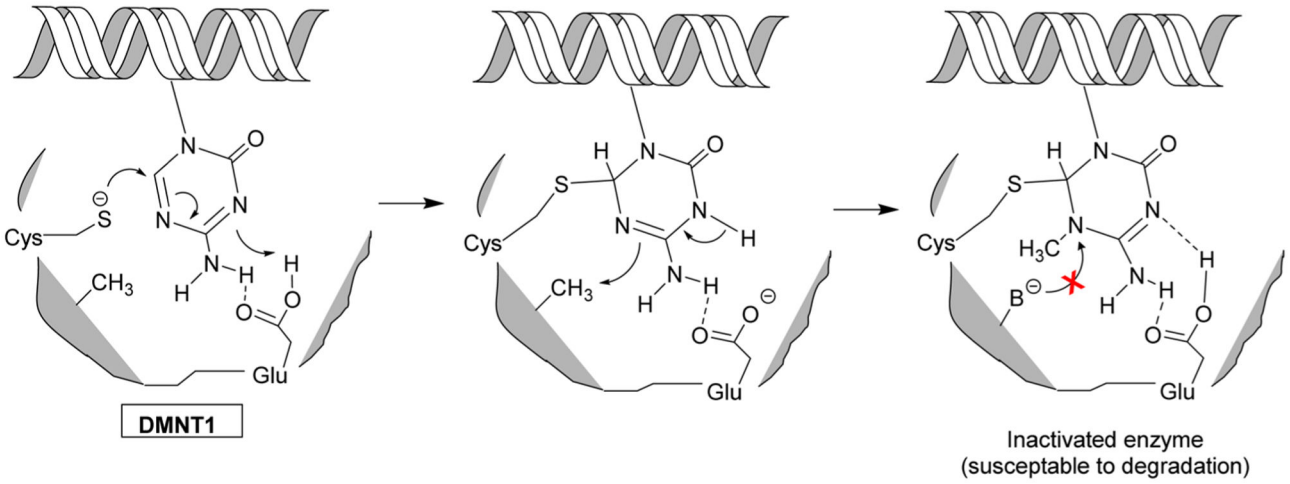


Figure 12.

Covalent trapping paradigm: mechanism-based DNMT1 degradation by decitabine **14**.

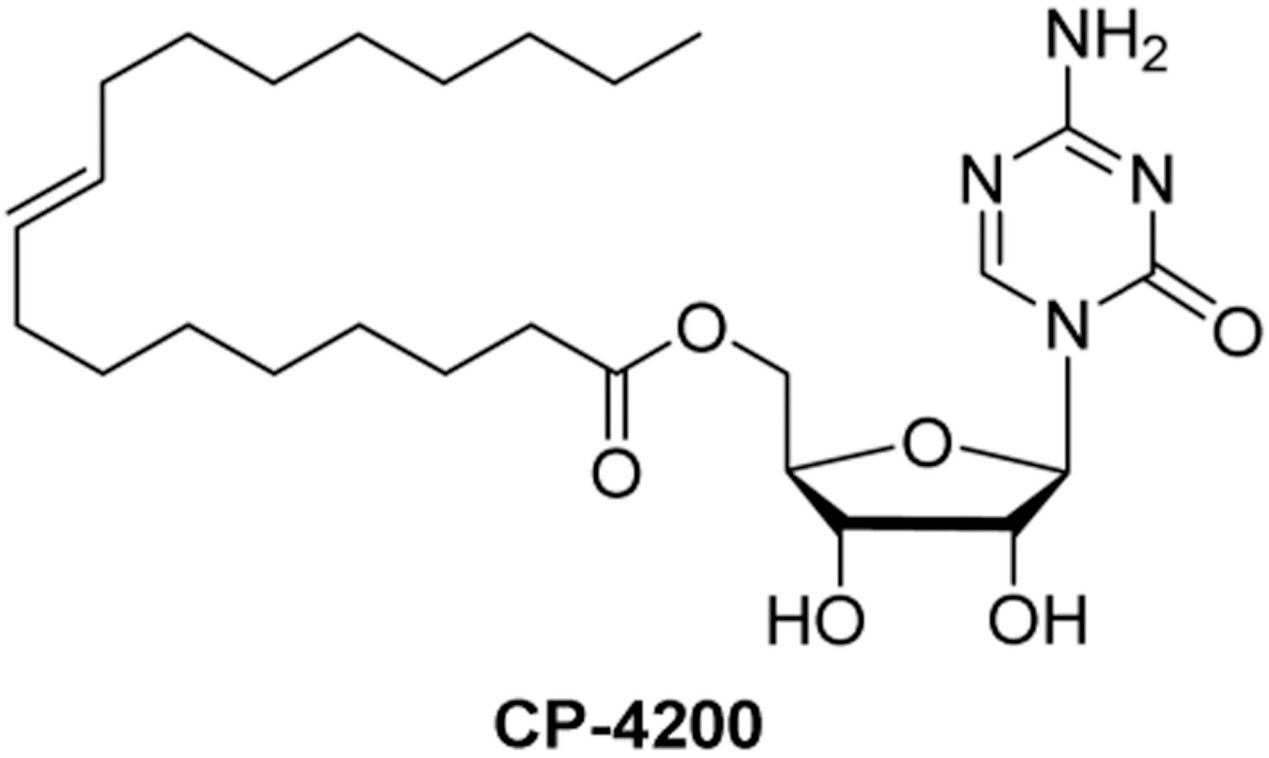


Figure 13.
Chemical structure of CP-4200.

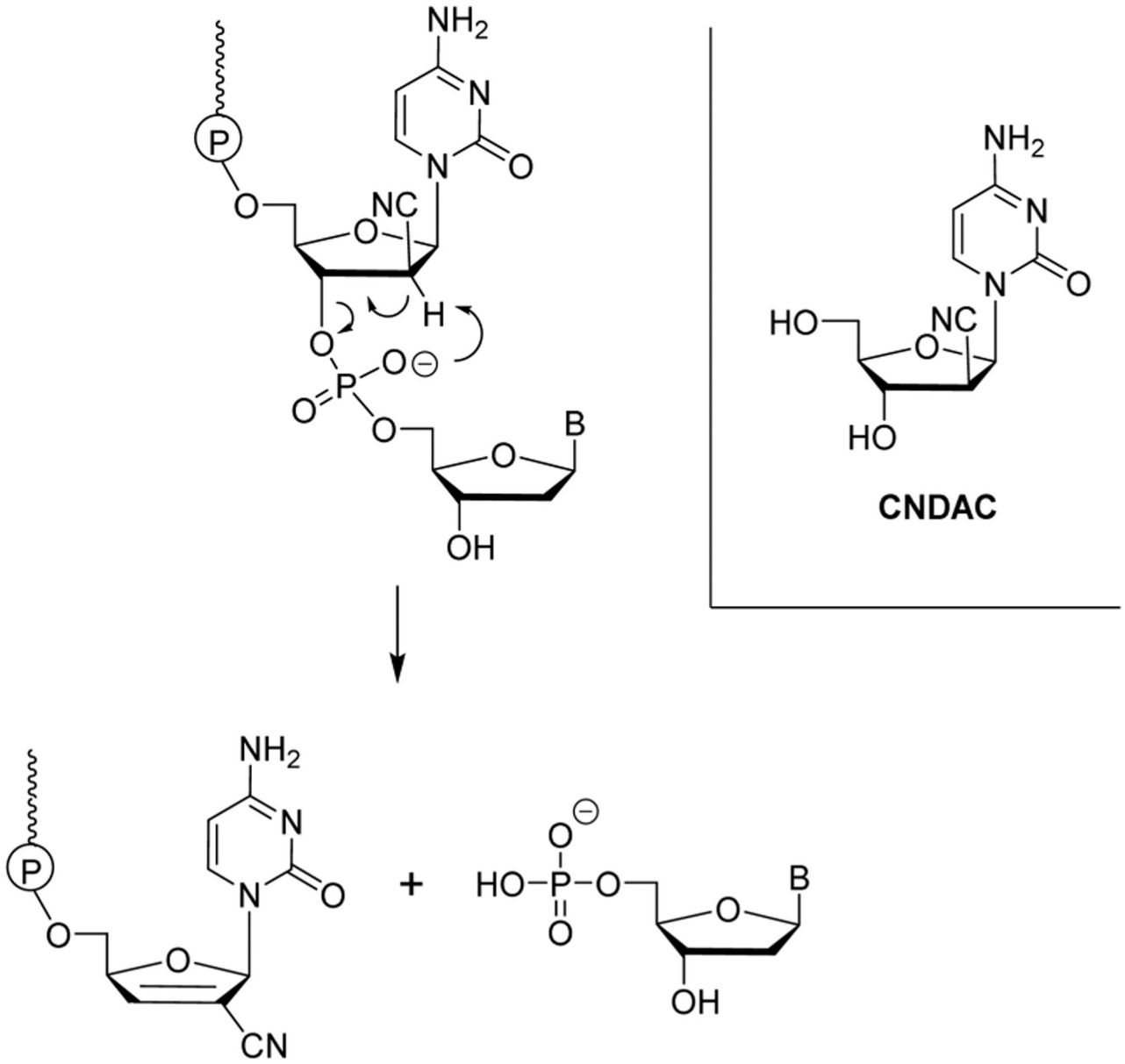


Figure 14.
CNDAC structure and hypothesized mechanism of DNA strand break with incorporated CNDAC.

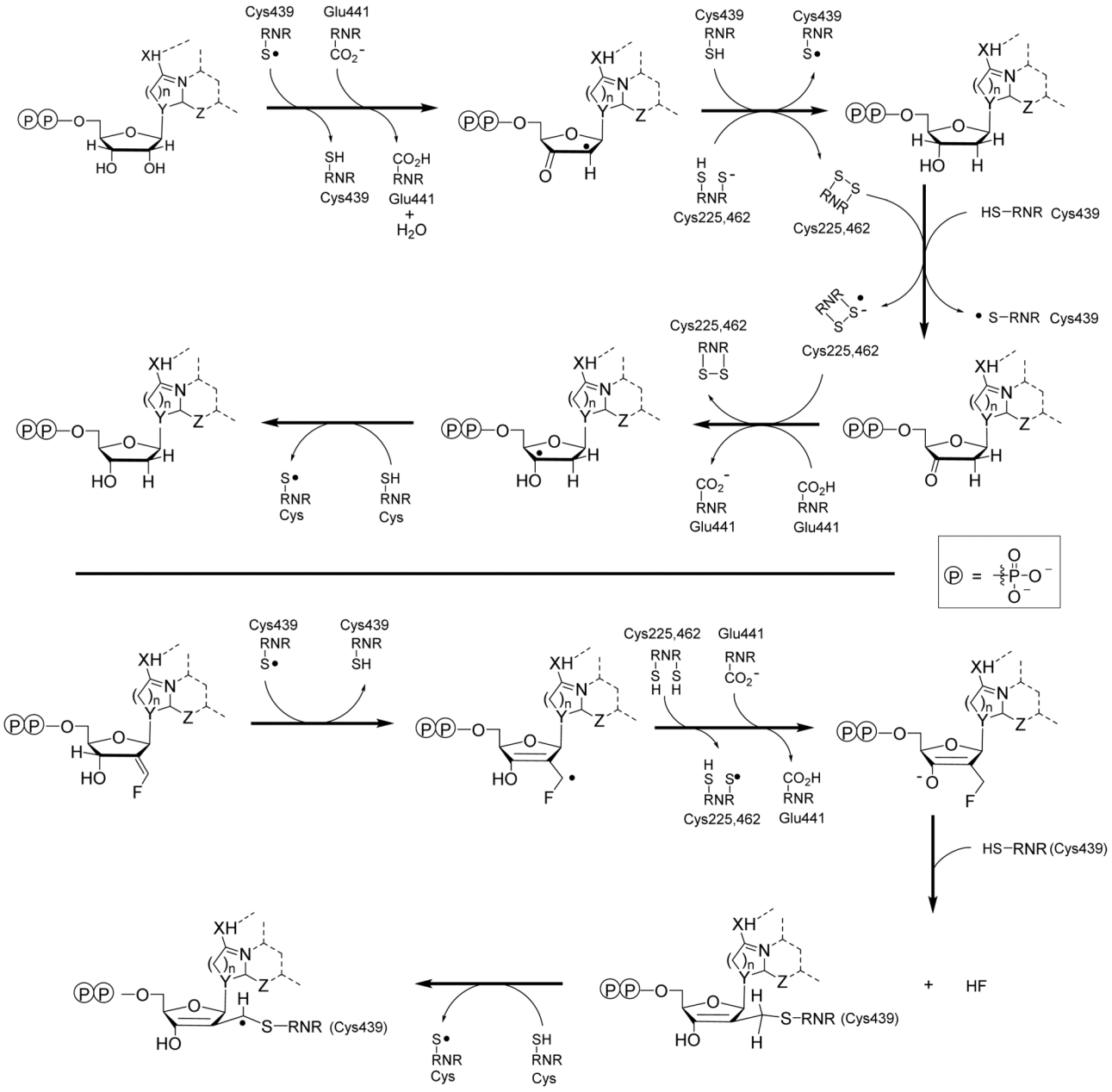


Figure 15.

Proposed mechanism of catalytic reduction of nucleoside-5′-diphosphates by ribonucleotide reductase (upper), and proposed mechanism of ribonucleotide reductase inhibition by tezacitabine-5′-diphosphate (lower).

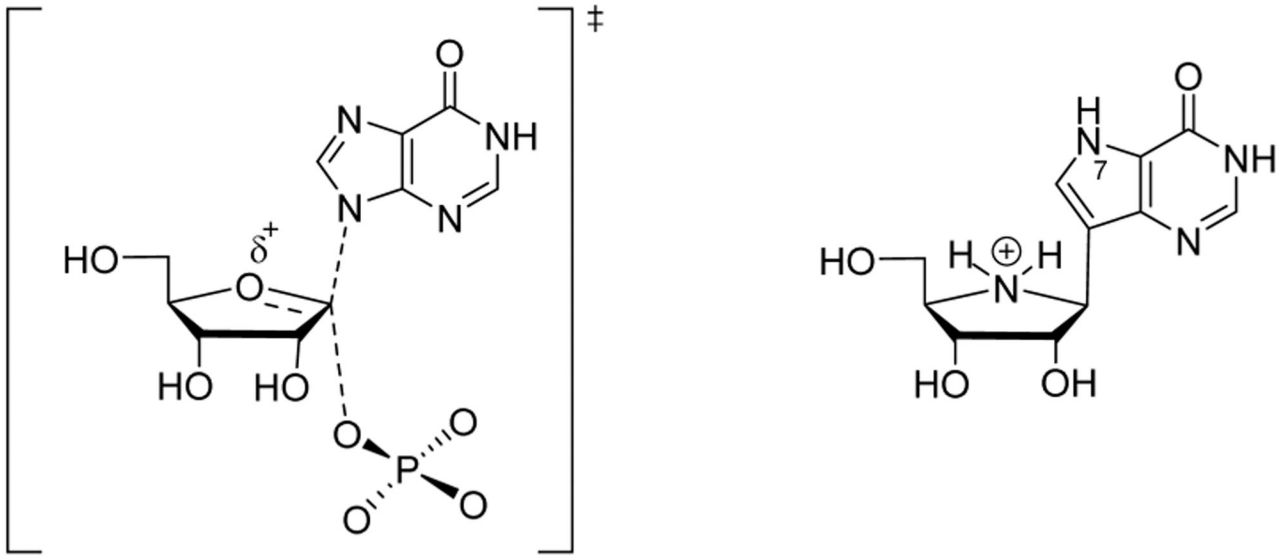


Figure 16.

Transition state model for the phosphorolysis of inosine by purine nucleoside phosphorylase and the protonated form of forodesine.

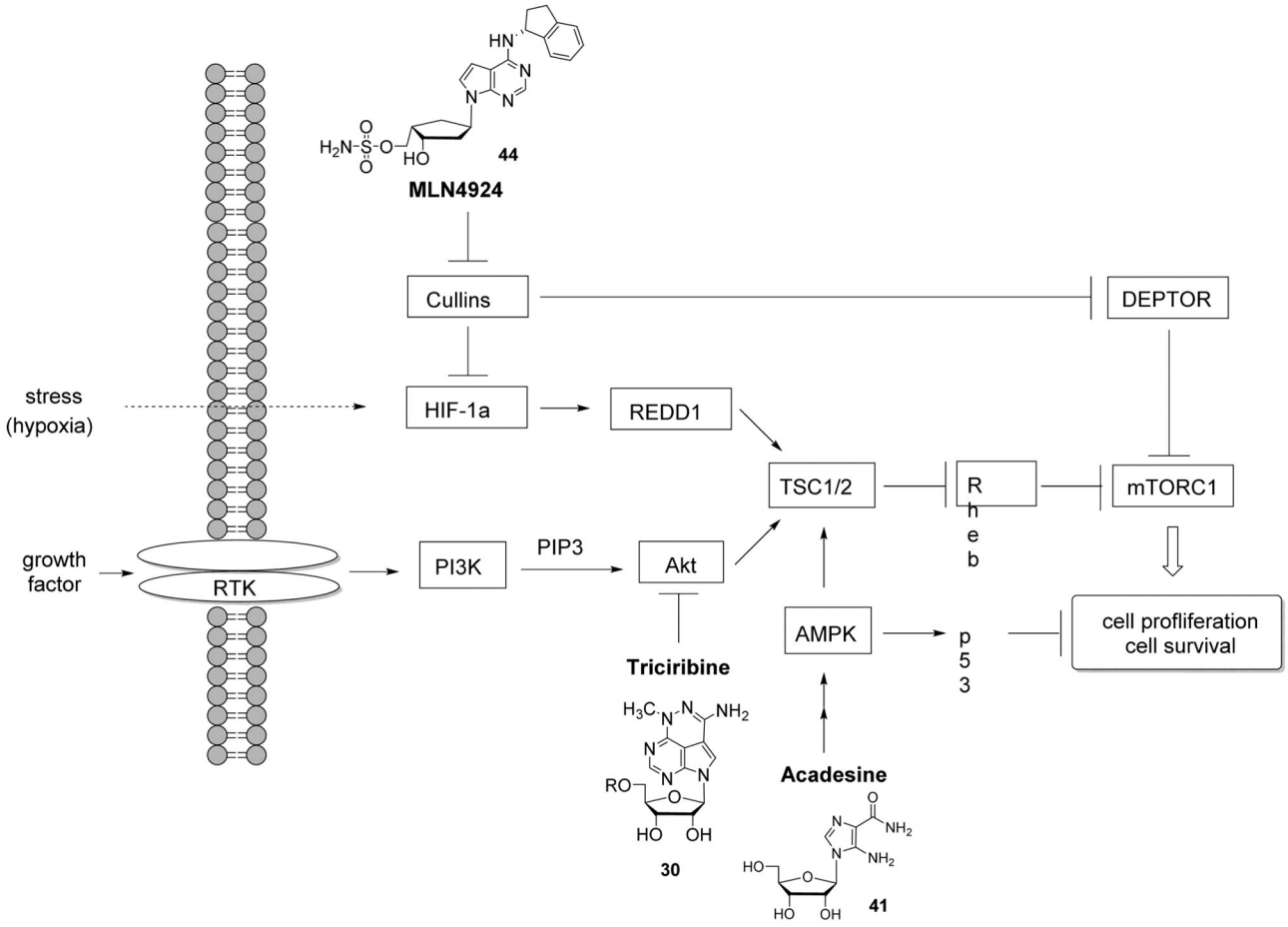


Figure 17.

Proposed biological mechanisms of actions of triciribine **30** as an Akt inhibitor (see Section 7.1, Triciribine Biology); MLN4924 **44** as a Cullins inhibitor (see Section 7.3, MLN4924 Biology); and acadesine **41** as an AMPK activator (see Section 8.5, Acadesine Biology).

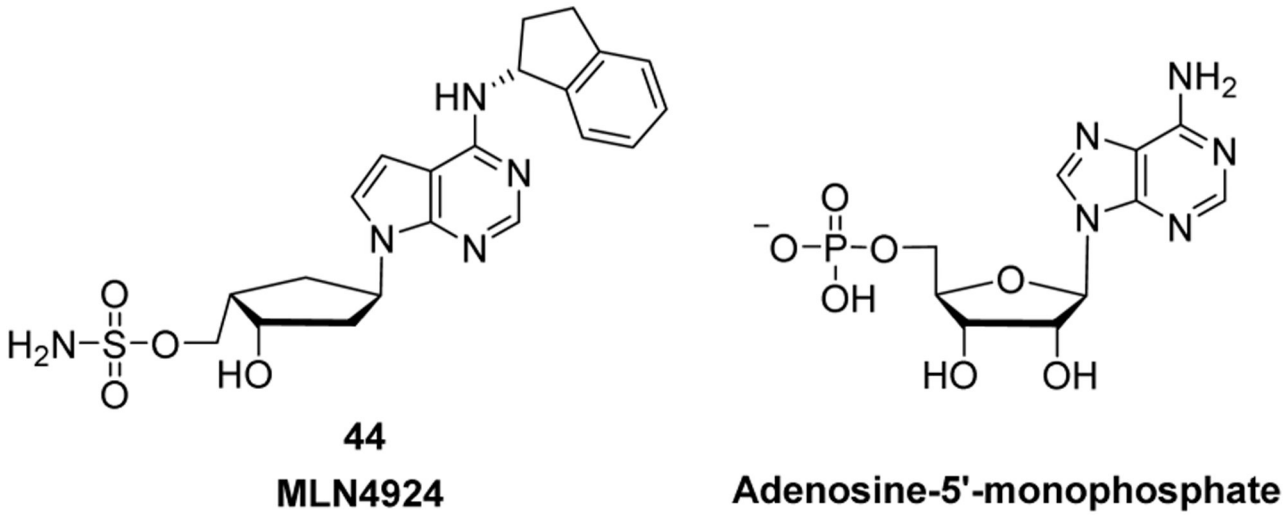


Figure 18.
Structures of MLN4924 44 and adenosine-5′-monophosphate.

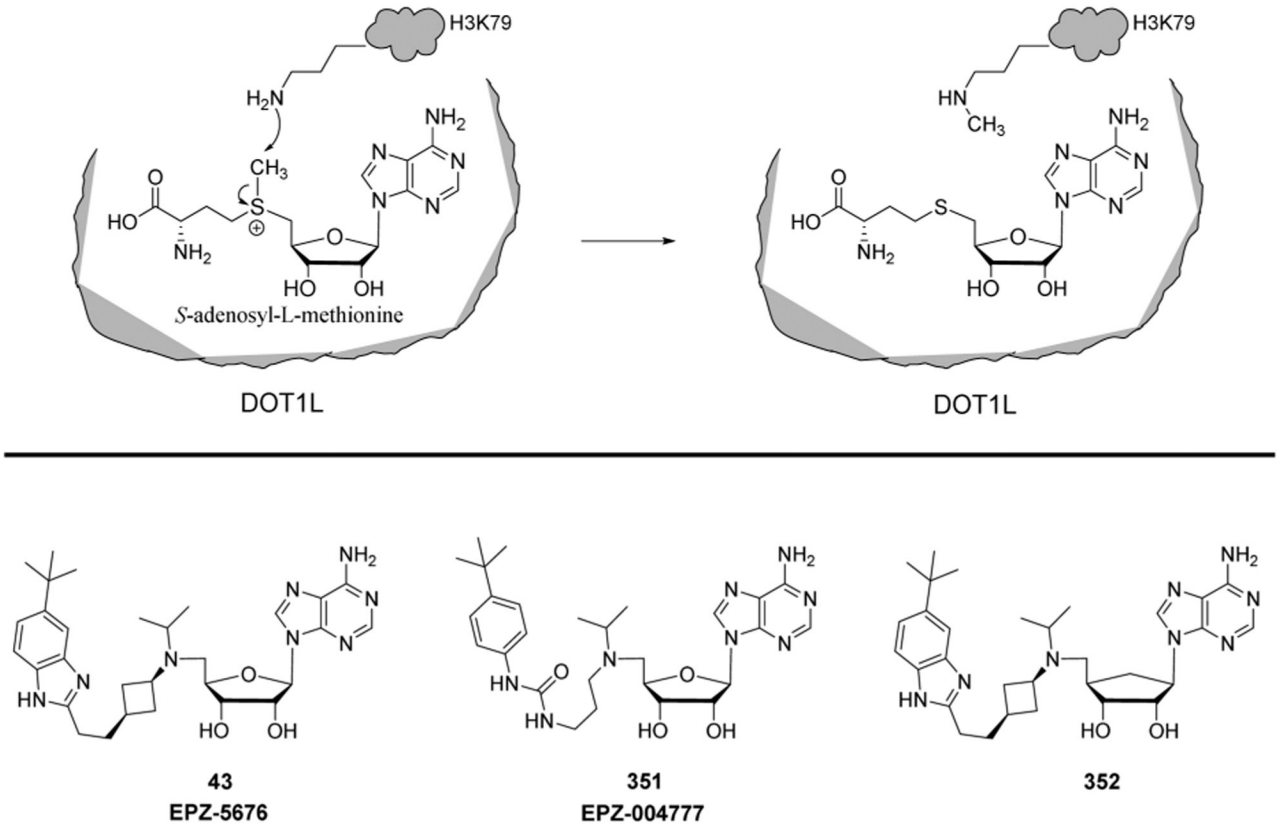
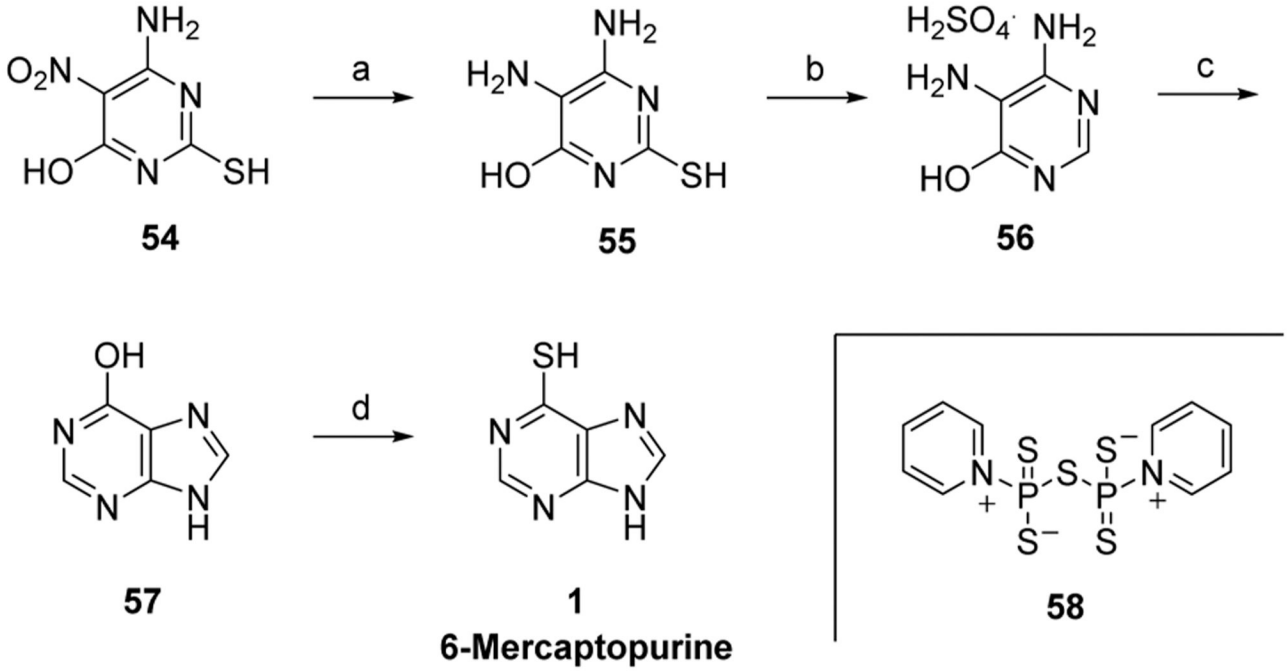
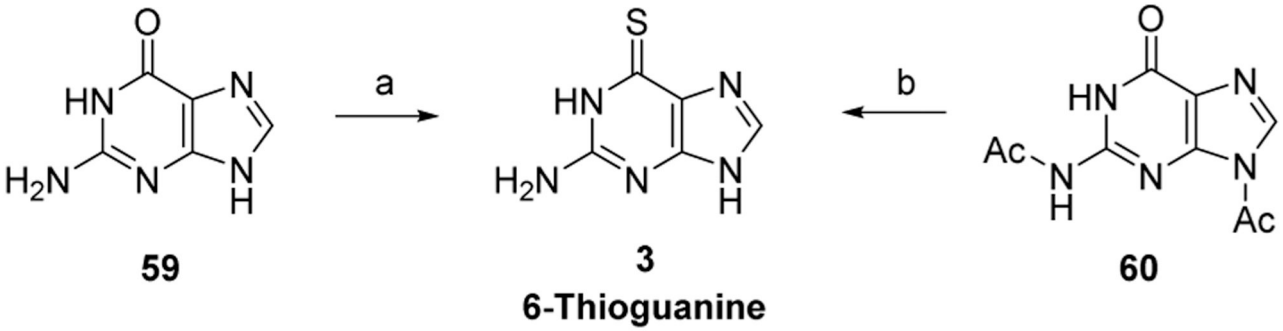


Figure 19.
DOT1L-catalyzed methylation of H3K79.



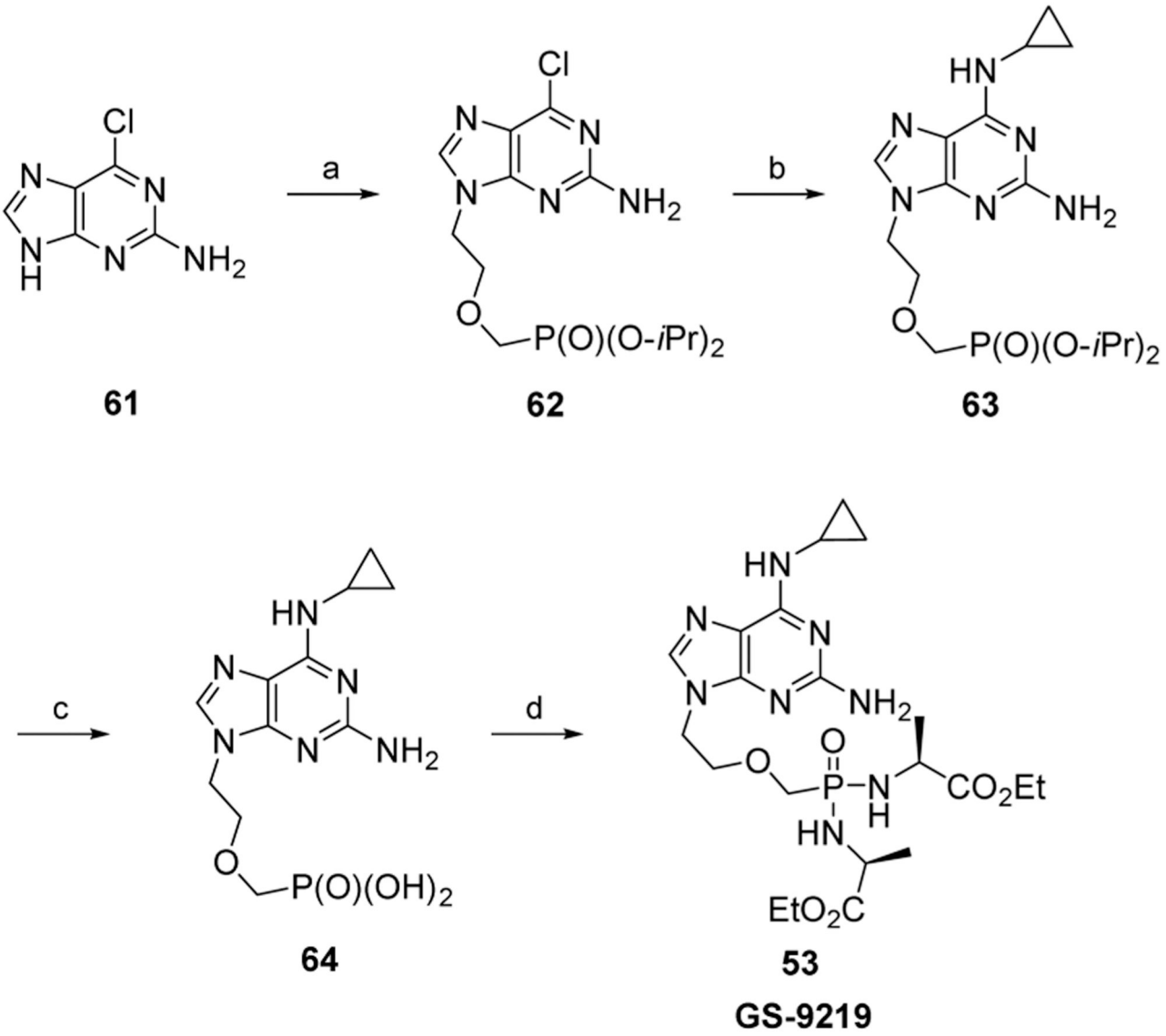
Scheme 1. Synthesis of 6-Mercaptopurine 1[a]

[a]Reagents and conditions: (a) $Na_2S_2O_4$, H_2O, 60–70 °C, 80–85%; (b) Raney Ni, Na_2CO_3, H_2O, reflux, 2 h then H_2SO_4, 88%; (c) HCO_2H, reflux, 2 h then aq NaOH, AcOH, 93%; (d) P_2S_5, tetralin, 190–200 °C, 12 h, 40%; or **58**, $DMSO_2$, 165–170 °C, 85%.



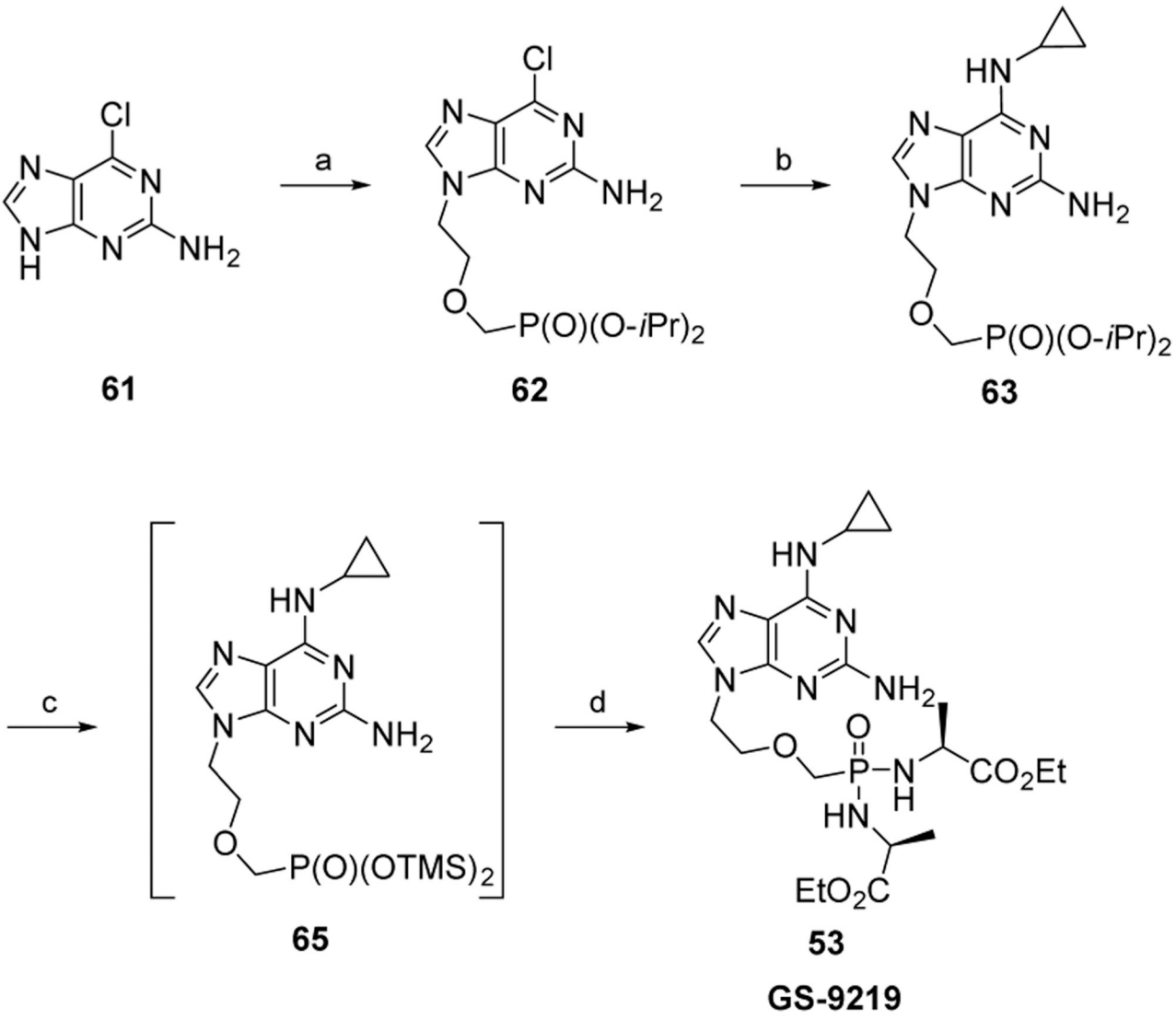
Scheme 2. Synthesis of 6-Thioguanine 3[a]

[a]Reagents and conditions: (a) P_2S_5, pyr, reflux, 2.5 h, 32%; (b) P_2S_5, pyridine hydrochloride, pyr, 110 °C, 4 h, then HCl, H_2O, pH 4, recrystallization, 75%.



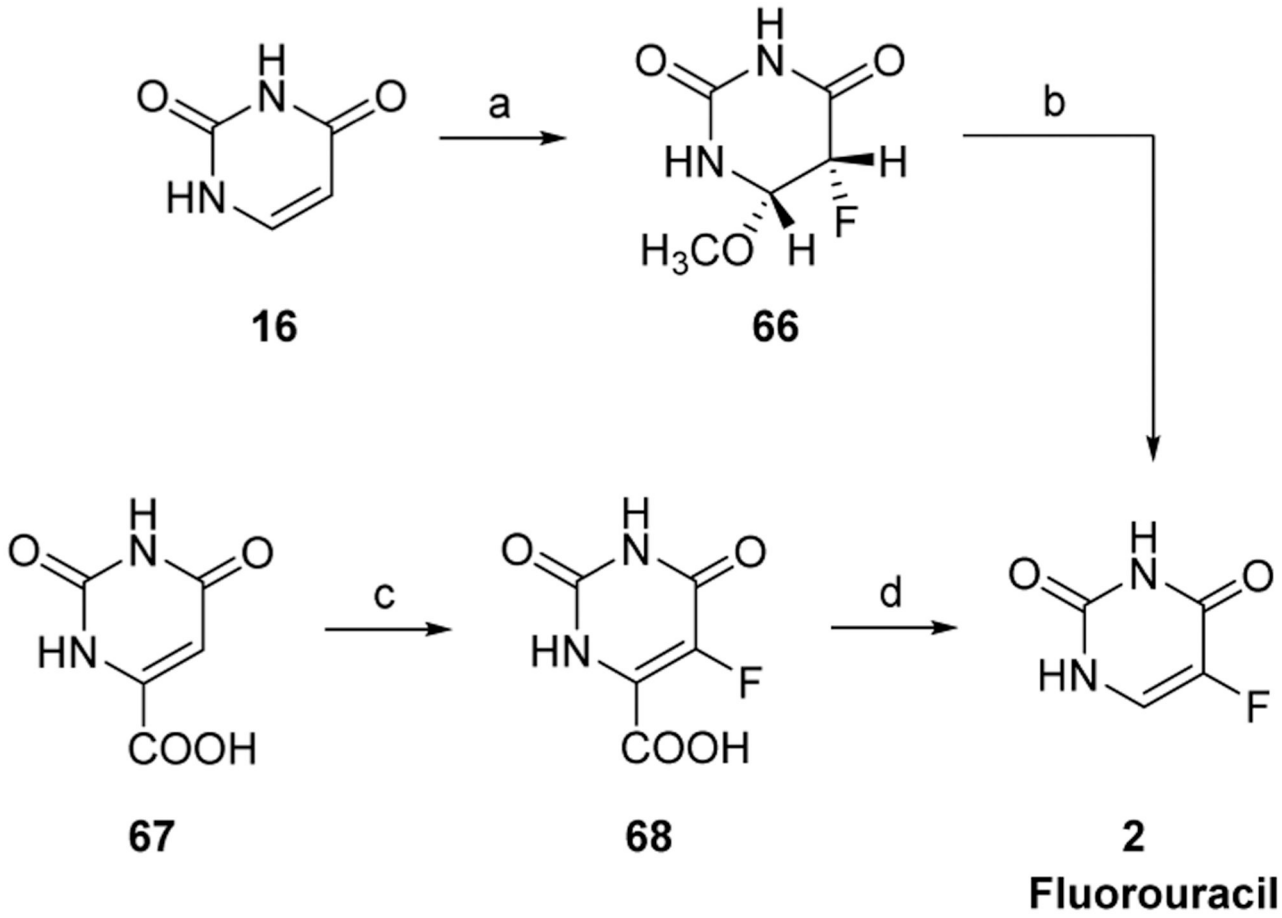
Scheme 3. Synthesis of GS-9219 53[a]

[a]Reagents and conditions: (a) $HO(CH_2)_2OCH_2PO(O\text{-}iPr)_2$, DIAD, PPh_3, DMF, −15 °C to rt, 4 h, 63%; (b) cyclopropylamine, CH_3CN, 100 °C, 4 h, 90%; (c) TMSBr, CH_3CN, rt, overnight, 90%; (d) D-alanine ethyl ester HCl, 2,2′-dithiodipyridine, PPh_3, Et_3N, pyr, 60 °C, overnight, 50%.



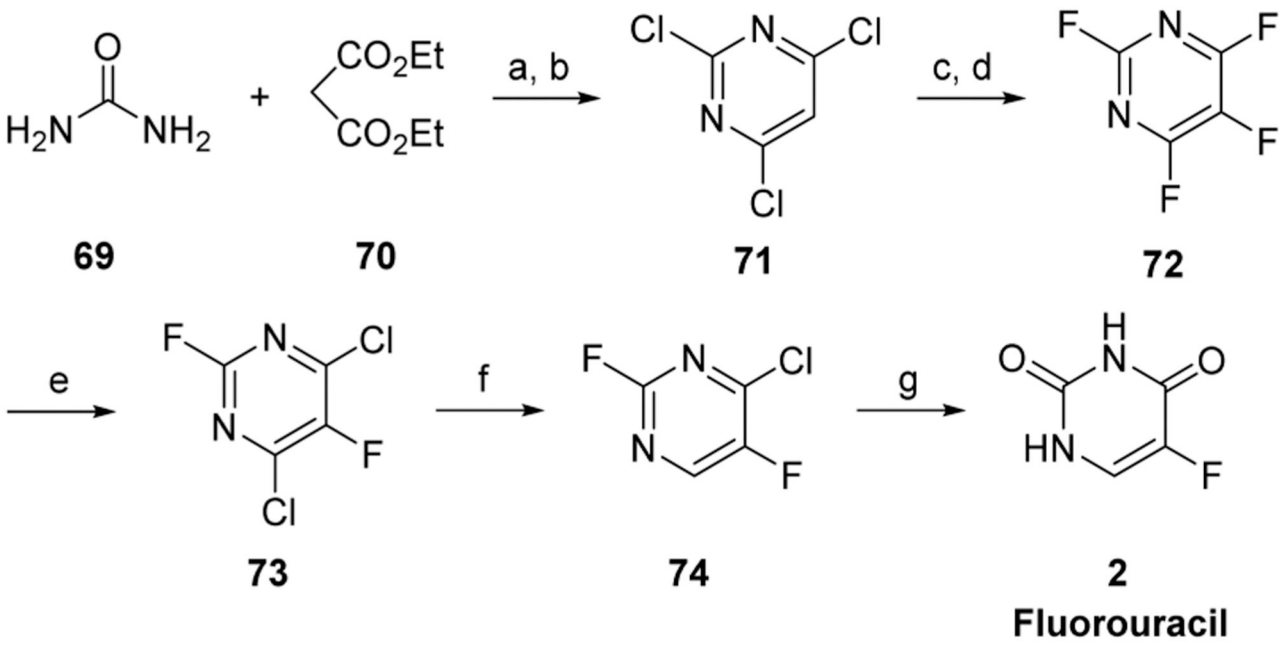
Scheme 4. Synthesis of GS-9219 53[a]

[a]Reagents and conditions: (a) $Cl(CH_2)_2OCH_2P(O)(O\text{-}iPr)_2$, Cs_2CO_3, DMF, 80 °C, 8 h, 56%; (b) cyclopropylamine, EtOH, reflux, 3 h, 65%; (c) TMSBr, CH_3CN, rt, overnight; (d) D-alanine ethyl ester, aldrithiol-2, PPh_3, Et_3N, pyr, 50 °C, 3 h, 92–98% (over two steps).



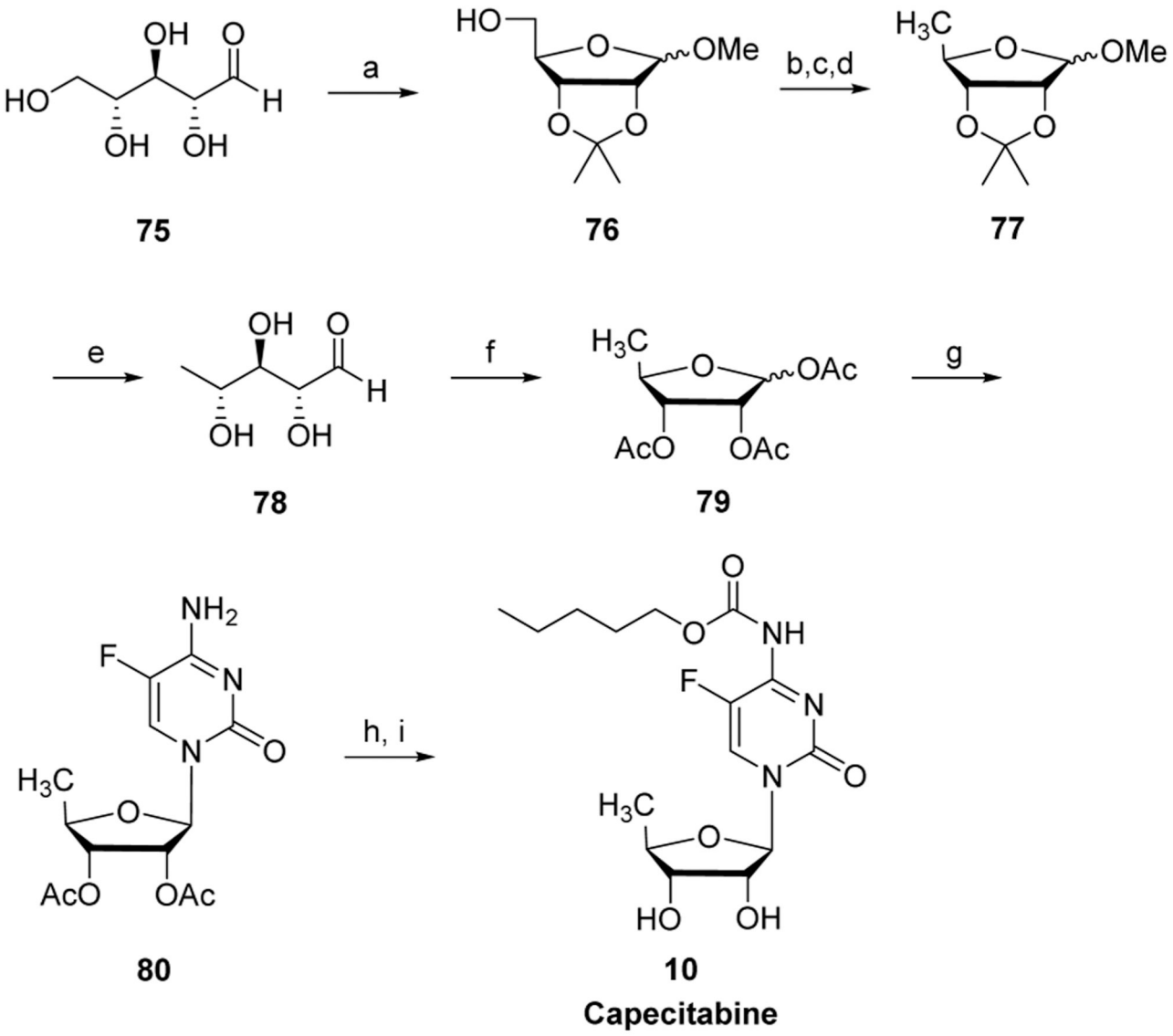
Scheme 5. Synthesis of Fluorouracil 2[a]

[a]Reagents and conditions: (a) CF_3OF/CCl_3F, MeOH, −78 °C, 5 min; (b) Et_3N, MeOH, H_2O, rt, 11 min, 90% (over two steps); (c) F_2 (g), TFA, 0 °C to 10 °C, 3 h, 80%; (d) triethyleneglycol dimethyl ether, 130 °C to 200 °C, 20 min, 90%.



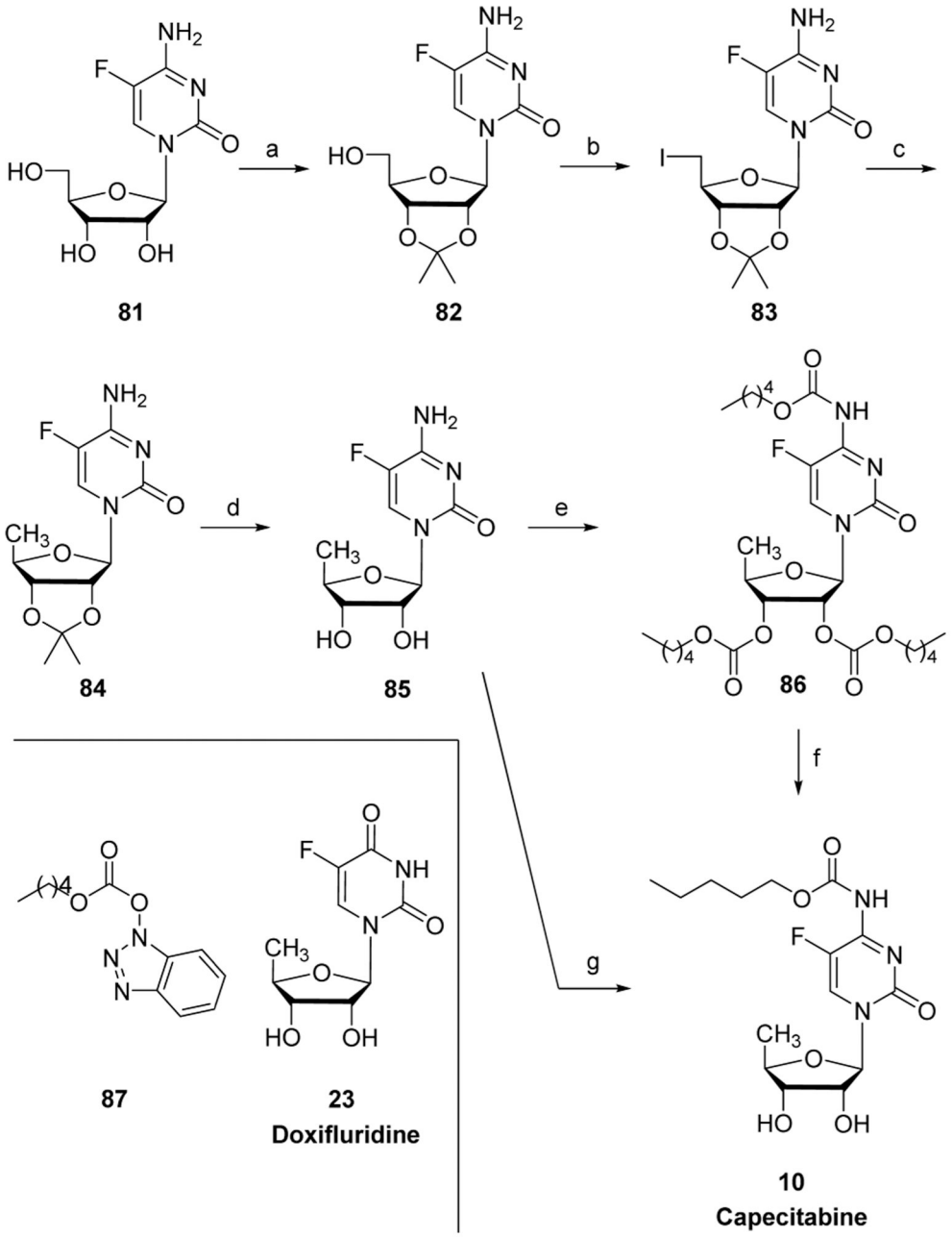
Scheme 6. Synthesis of Fluorouracil 2[a]

[a]Reagents and conditions: (a) Na, MeOH; (b) $POCl_3$, $PhNMe_2$; (c) proton sponge hydrofluoride; (d) NaF, F_2, $CFCl_3$; (e) HCl (g), 160 °C, 4 h, 38%; (f) Pd/C, H_2, Et_3N, EtOAc, 3.5 h, 82%; (g) aq NaOH, 80 °C, 4 h, 93%.



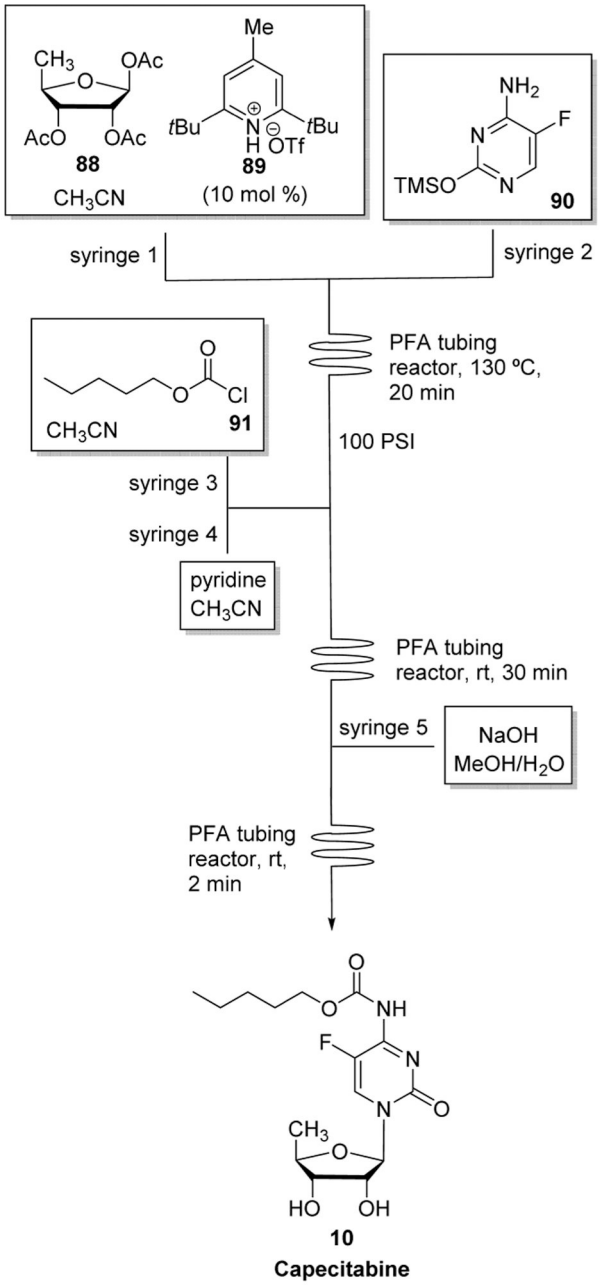
Scheme 7. Synthesis of Capecitabine 10[a]

[a]Reagents and conditions: (a) HCl, MeOH/Me_2CO, 78%; (b) MsCl, pyr; (c) NaI, DMF; (d) Pd/C, H_2, 27%; (e) aq HCl, 100 °C, 97%; (f) Ac_2O, pyr, 64%; (g) 5-fluorocytosine, HMDS, $SnCl_4$, DCM, 76%; (h) *n*-pentyl chloroformate, pyr; (i) aq NaOH, MeOH.

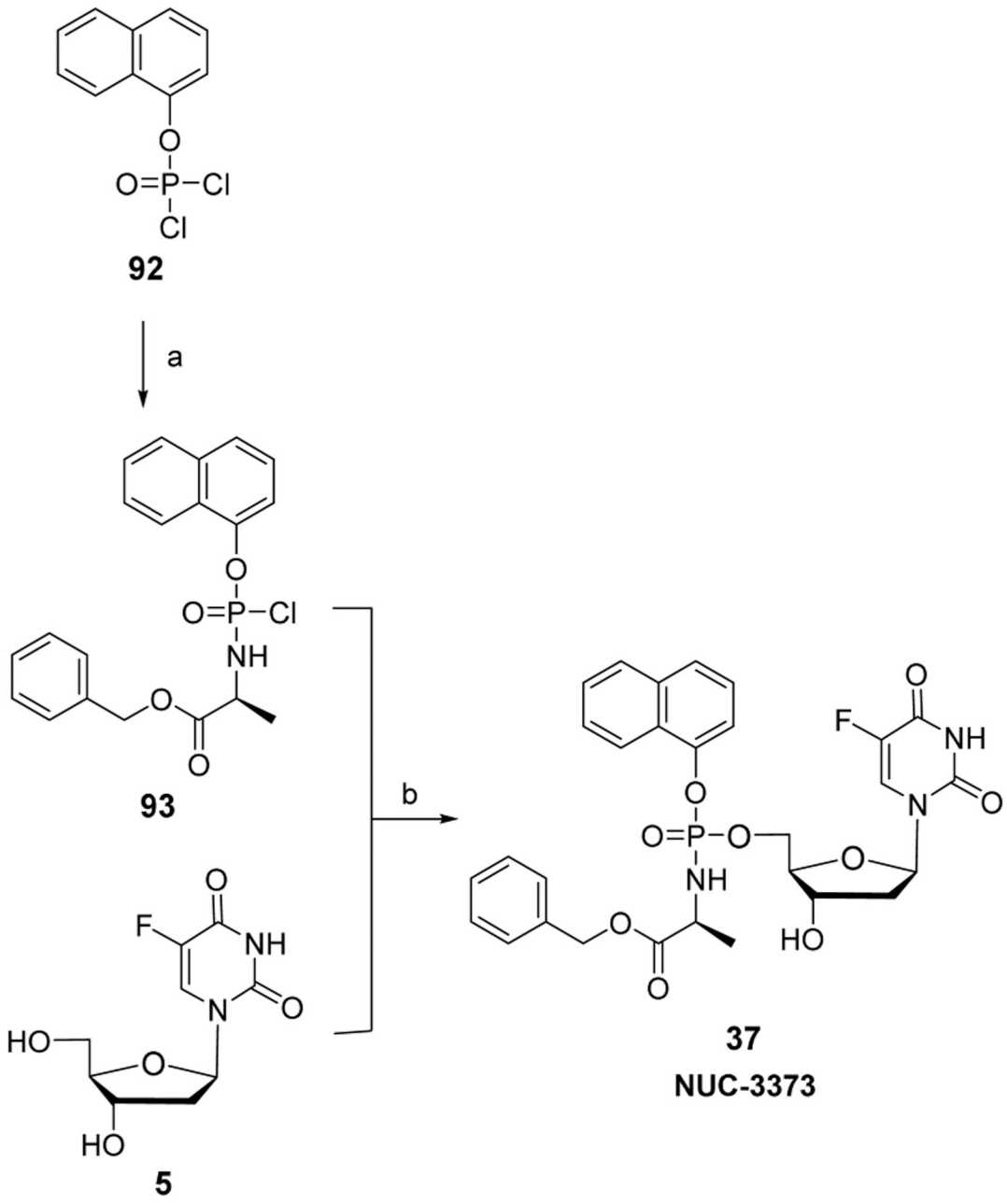


Scheme 8. Synthesis of Doxifluridine 23 and Capecitabine 10[a]

[a]Reagents and conditions: (a) 2,2-dimethoxypropane, TsOH, Me_2CO, rt, 2 h, 95%; (b) methyltriphenoxyphosphonium iodide, DMF, rt, 2.5 h, then MeOH, rt, 1 h, 74%; (c) Et_3N, H_2, Pd/C, MeOH, rt, 1.5 h, 93%; (d) TFA, 40 min, 78%; (e) $CH_3(CH_2)_4OCOCl$, pyr, DCM, −5 °C to rt, overnight, 92%; (f) NaOH, MeOH, −10 °C, 15 min, then concd HCl, 87%; (g) **87**, THF, reflux, 66%.

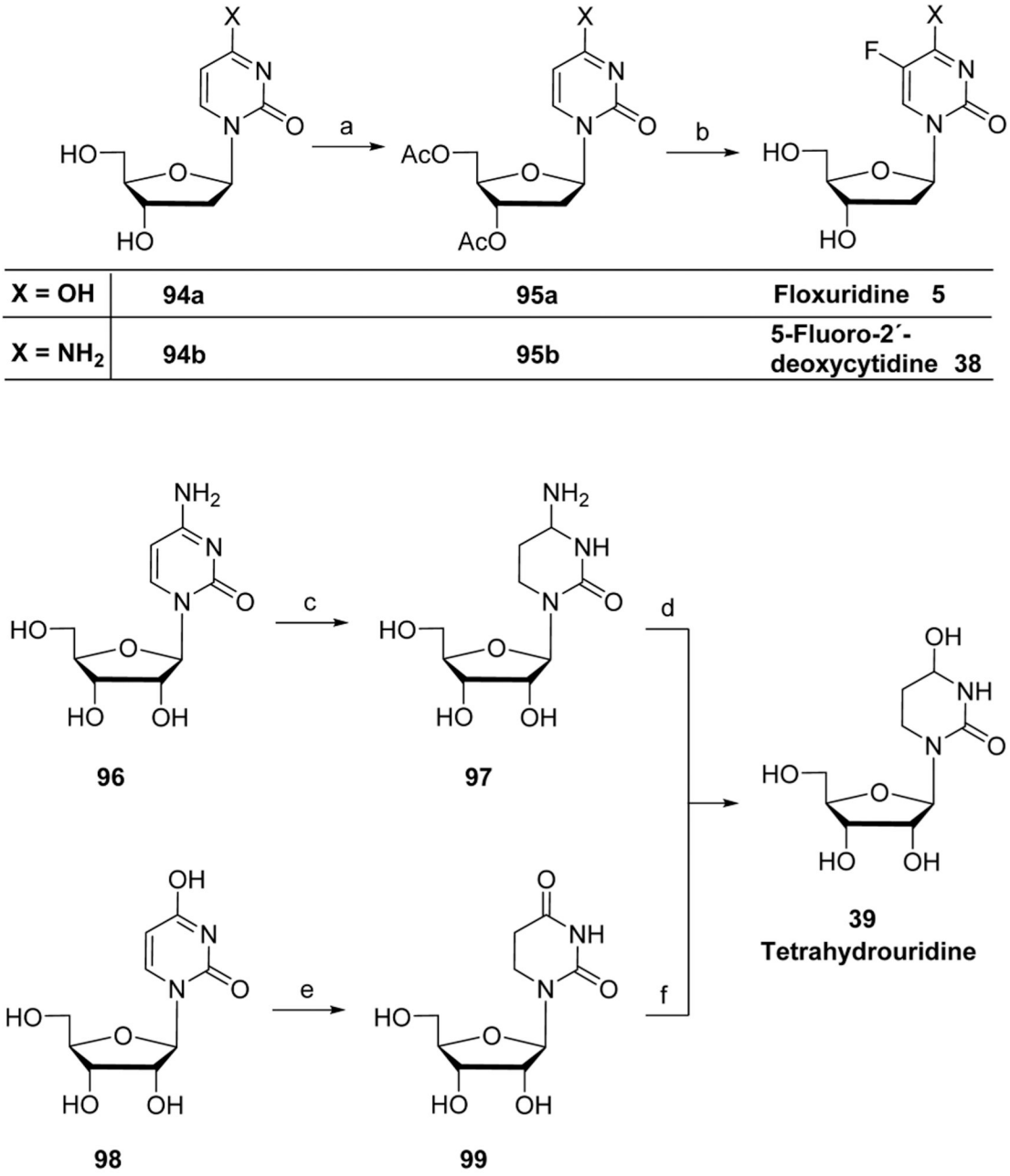


Scheme 9.
Flow Synthesis of Capecitabine 10



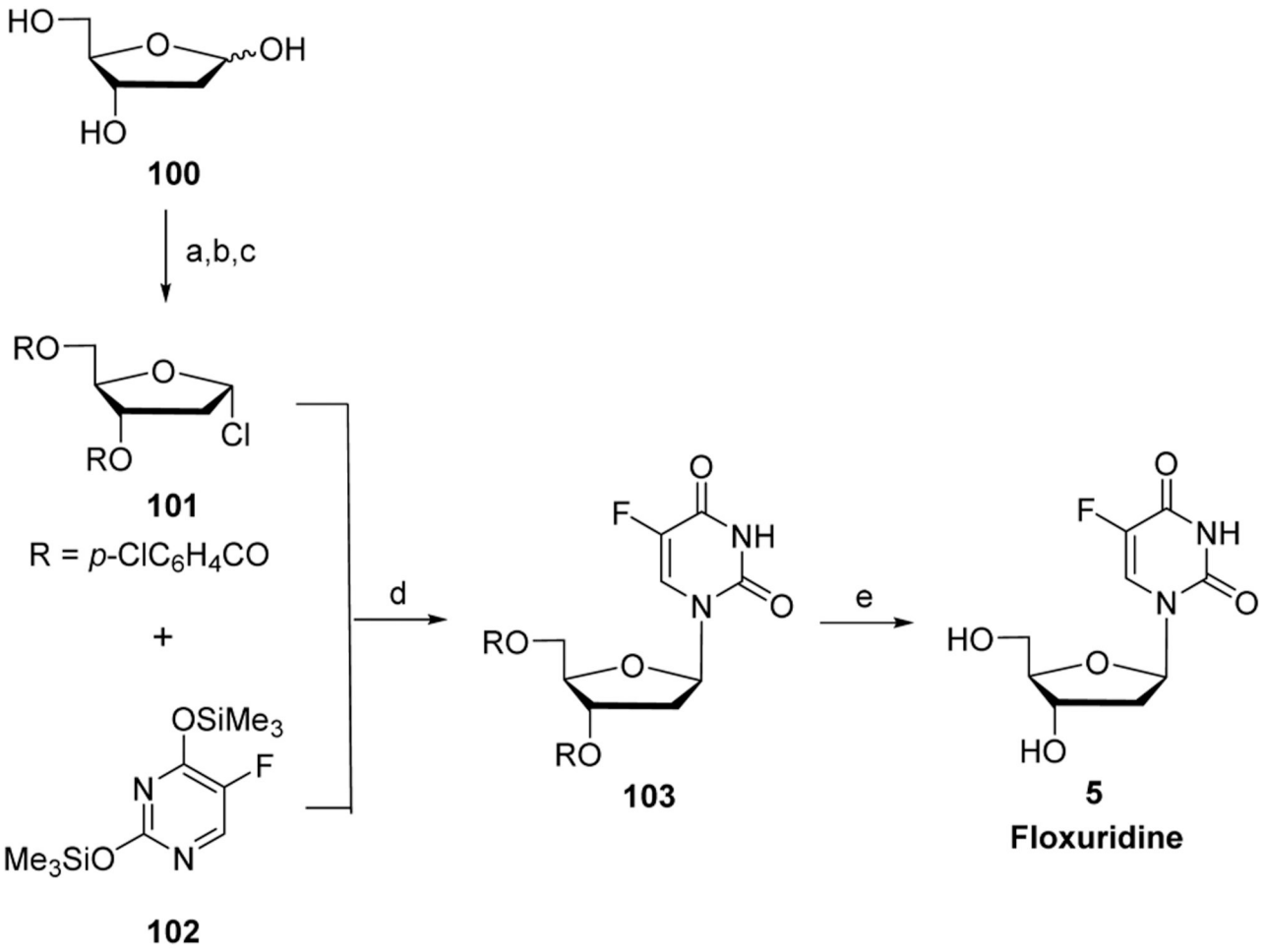
Scheme 10. Synthesis of NUC-3373 37[a]

[a]Reagents and conditions: (a) L-alanine benzyl ester salt, Et_3N, DCM, −78 °C, 1–3 h, 74%; (b) *t*-BuMgCl, THF, rt, 18 h, 8%.



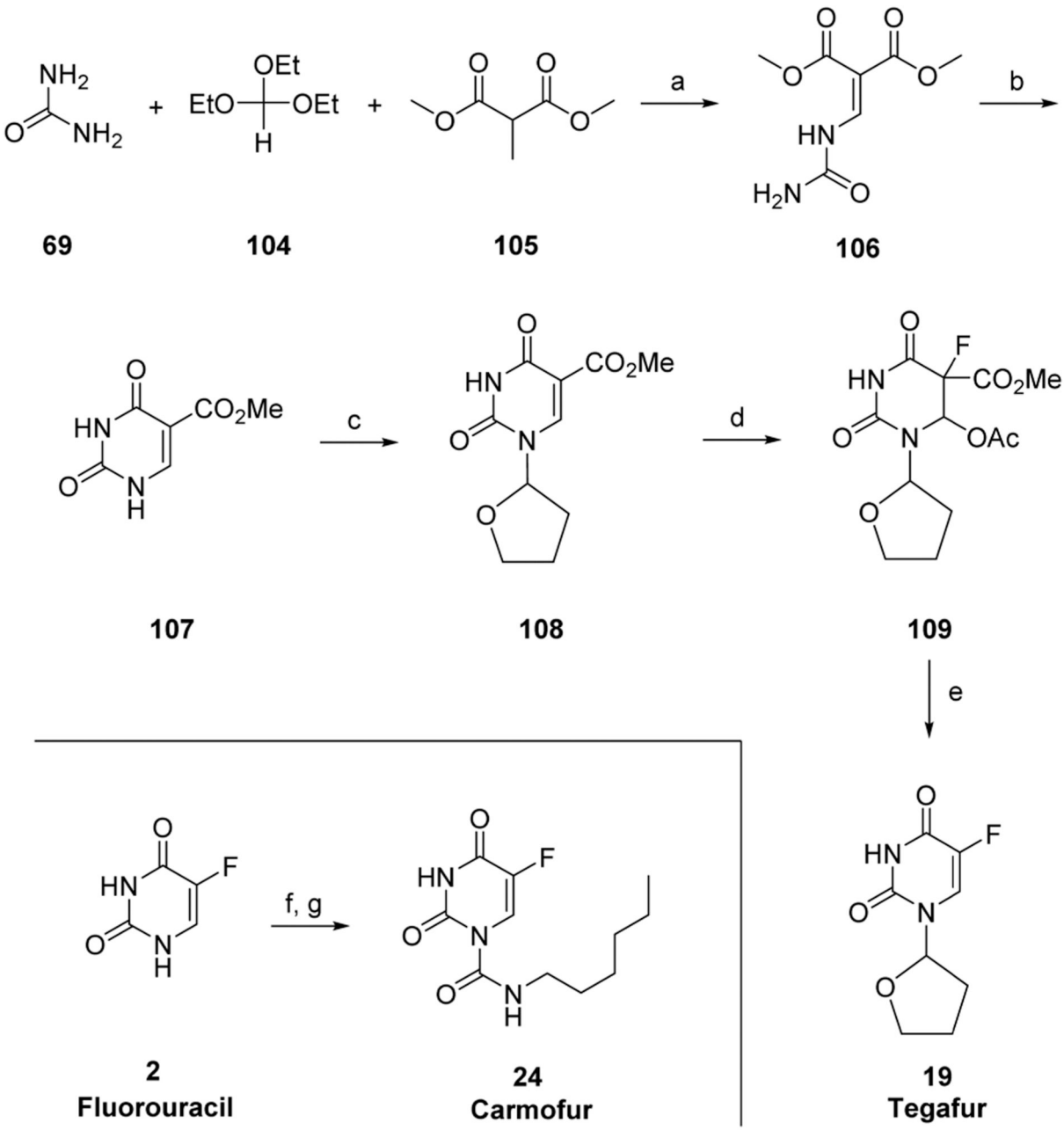
Scheme 11. Synthesis of Floxuridine 5, 5-Fluoro-2′-deoxycytidine 38, and Tetrahydrouridine 39[a]

[a]Reagents and conditions: (a) Ac_2O, DMAP, rt, 24 h, 88% (for **95a**); Ac_2O, pyr, rt, 4.5 h, 99% (for **95b**); (b) CF_3OF, CCl_3F, $CHCl_3$, −30 °C, evaporation, then Et_3N, MeOH, H_2O, rt, (55% for **5**),(69% for **38**); (c) Rh/Al, H_2 (g), H_2O, overnight; (d) H_2O, pH 6, 92% (over two steps); (e) Rh/Al, H_2 (g), NaOH, H_2O, 24 h, 45% of **99**, 35% of **39**; (f) $NaBH_4$, H_2O, freezer, overnight.



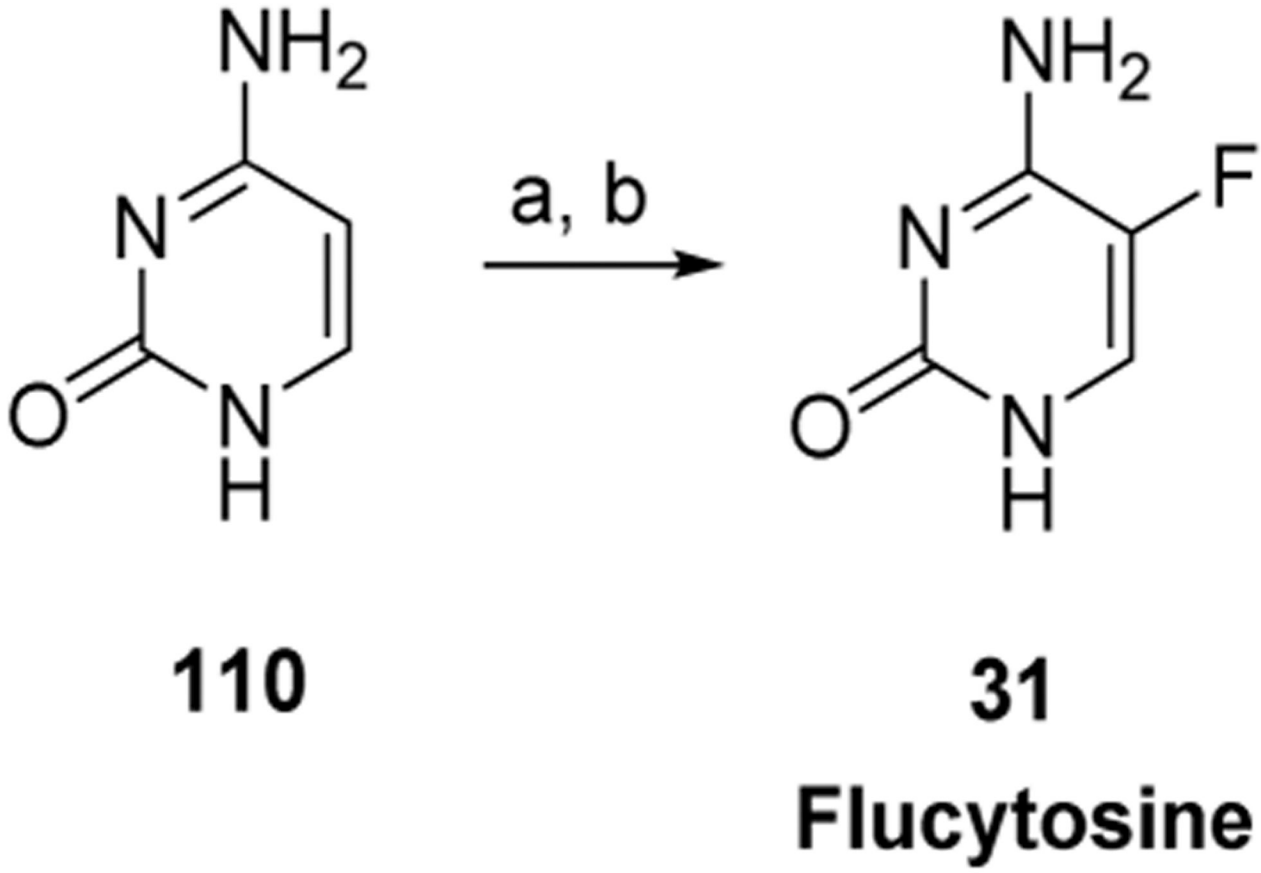
Scheme 12. Synthesis of Floxuridine 5[a]

[a]Reagents and conditions: (a) AcCl, MeOH, 25 °C, 45 min, then pyr; (b) pyr, DMAP, 4-ClBzCl, 0 °C, 1 h then 25 °C, 12 h; (c) HOAc, HCl (g), 0 °C, 7–10 min, 82% (α-anomer crystallizes from the solvent); (d) *p*-nitrophenol, $CHCl_3$, 30 °C, 12 h, 92%; (e) NH_3/MeOH, 30 °C, 16 h, 81%.



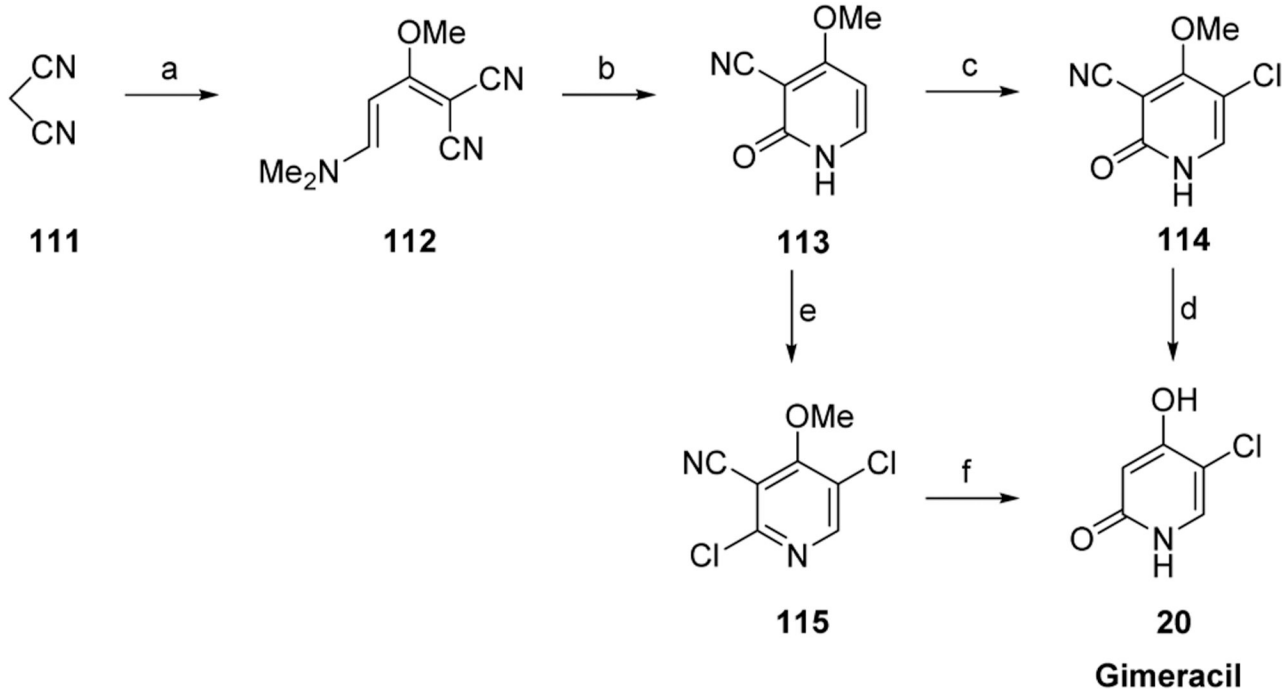
Scheme 13. Synthesis of Tegafur 19 and Carmofur 24[a]

[a]Reagents and conditions: (a) 130 °C, 4 h, 69%; (b) NaOMe, MeOH, reflux, 10 min, 84%; (c) 2,3-dihydrofuran, pyr, 180 °C; (d) F_2, AcOH, rt; (e) 1 N NaOH, rt, 1 h, 62% (over two steps); (f) aq HCHO, 55 °C, 6 h, 82%; (g) $KBrO_3$, 80 °C, then $H_2N(CH_2)_5CH_3$, DCC, MeCN, 0 °C to rt, 6 h, 40%.



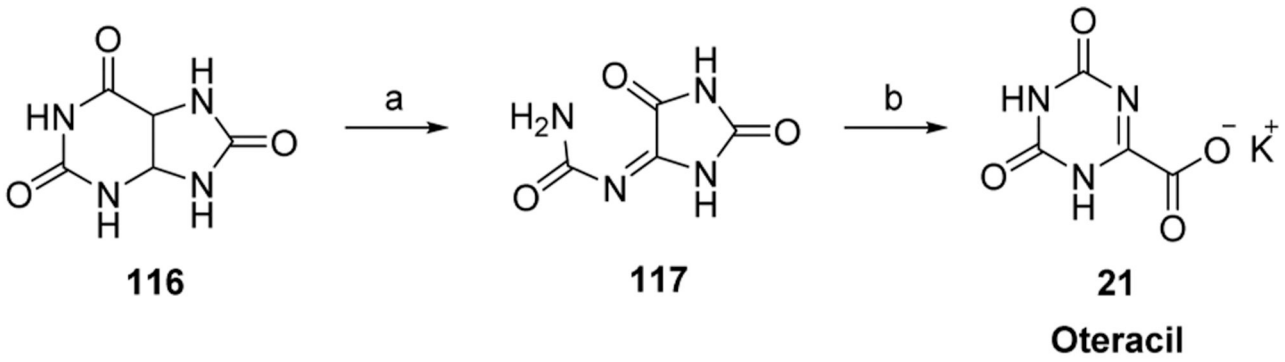
Scheme 14. Synthesis of Flucytosine 31[a]

[a]Reagents and conditions: (a) CF_3OF, CCl_3F, MeOH, −78 °C to rt; (b) Et_3N, MeOH, H_2O, rt, 8 h, 85% (over two steps).



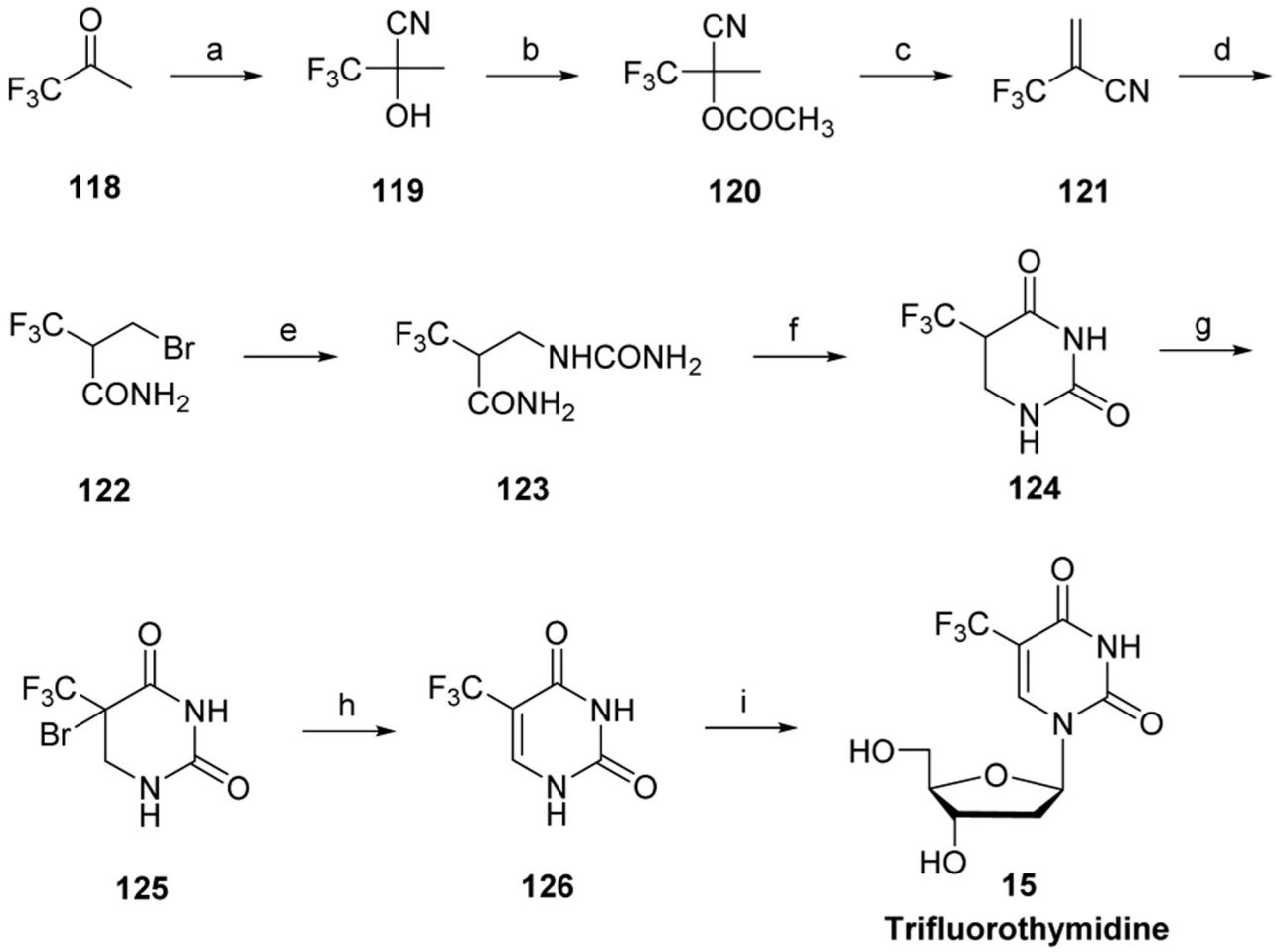
Scheme 15. Synthesis of Gimeracil 20[a]

[a]Reagents and conditions: (a) $CH_3C(OCH_3)_3$, MeOH, then $(CH_3)_2NHCH(OCH_3)_2$, reflux, 92%; (b) aq AcOH, 130 °C, 2 h, 95%; (c) SO_2Cl_2, HOAc, 50 °C, 0.5 h, 91%; (d) 40% H_2SO_4, 130 °C, 4 h, 91%; (e) SO_2Cl_2, HOAc, 50 °C, 45 min, 86%; (f) 75% H_2 SO_4, 140 °C, 3 h, then NaOH, then pH 4–4.5, 89%.



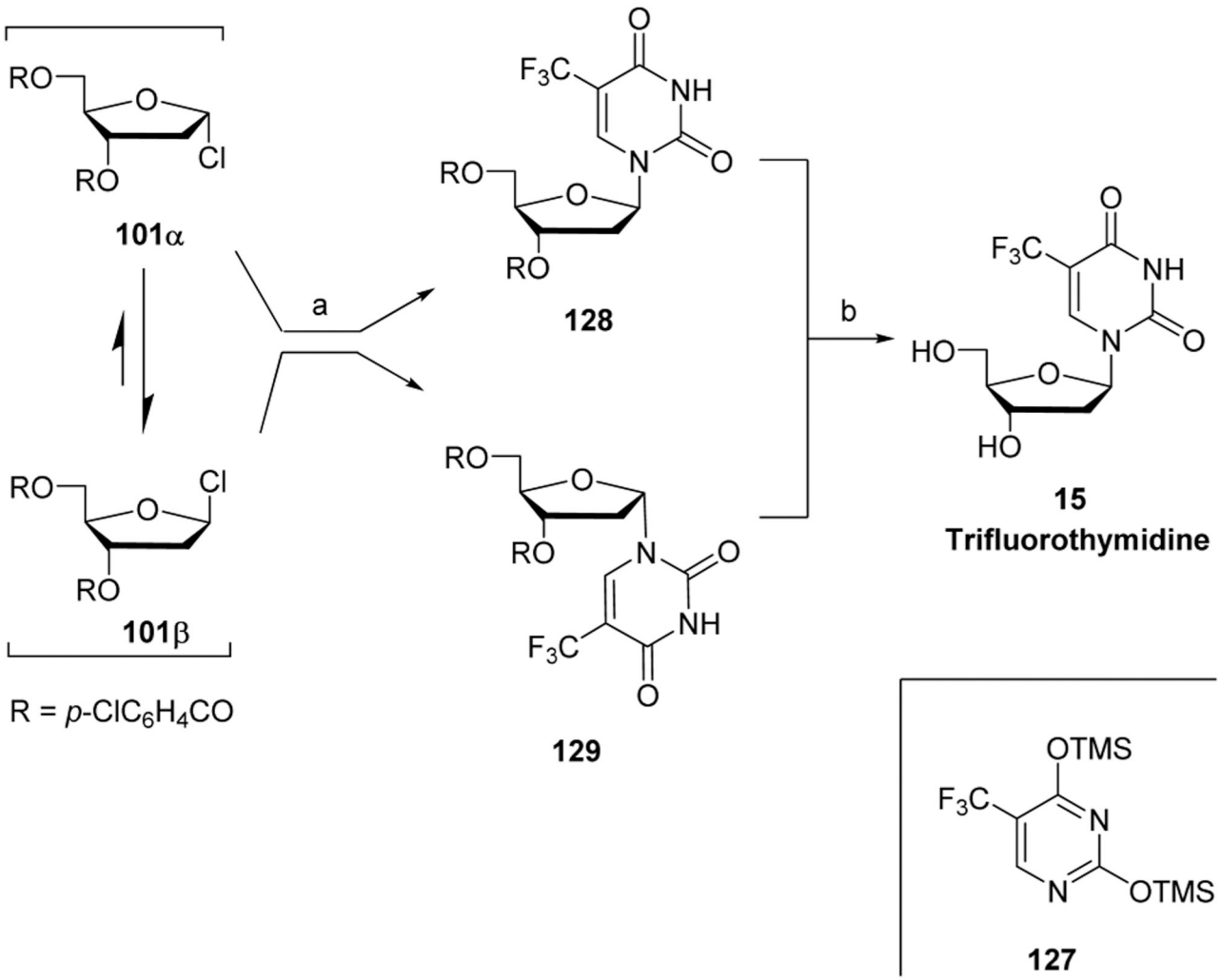
Scheme 16. Synthesis of Oteracil 21[a]

[a]Reagents and conditions: (a) LiOH, I_2, H_2O, 5 °C, 5 min, then AcOH, 75%; (b) aq KOH, 20 min, rt, 82%.



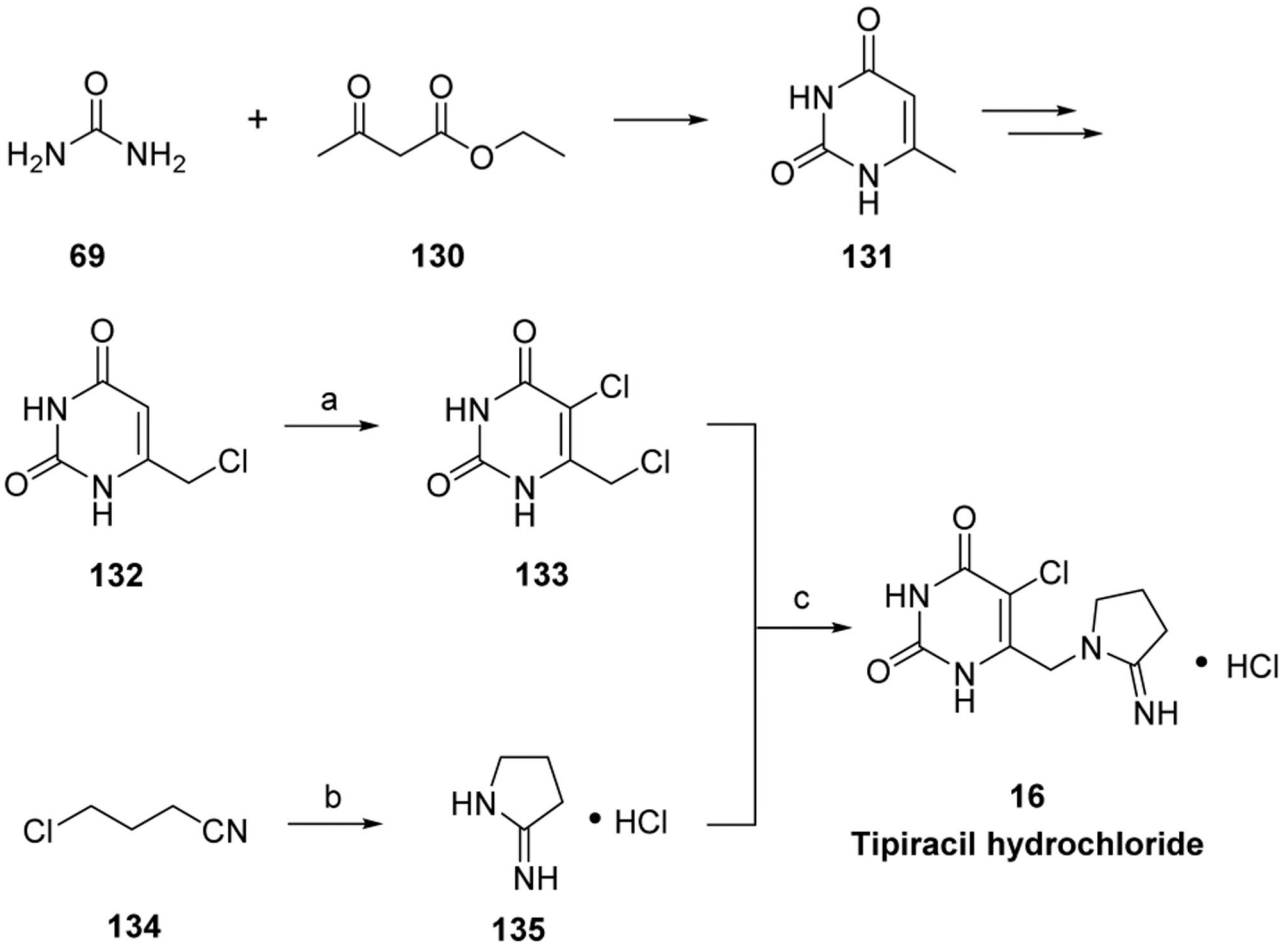
Scheme 17. Synthesis of Trifluorothymidine 15[a]

[a]Reagents and conditions: (a) NaCN, H_2SO_4, H_2O, 10 °C, 3 h, 99%; (b) Ac_2O, concd H_2SO_4, reflux, 1 h, 78%; (c) 500 °C, 4 h, 64%; (d) HBr (g), MeOH, 0 °C, 36 h, 82%; (e) $(NH_2)_2CO$, H_2O, 100 °C, 30 min, 28%; (f) aq HCl, reflux, 1 h, 58%; (g) Br_2/AcOH, reflux, 3 h, 85%; (h) DMF, 140 °C, 75 min, 80%; (i) thymidine, bactotryptone, NaCl, *Escherichia coli* B, 0.067 M phosphate buffer (pH 6.7), 37 °C, 3.5 h, 14%.



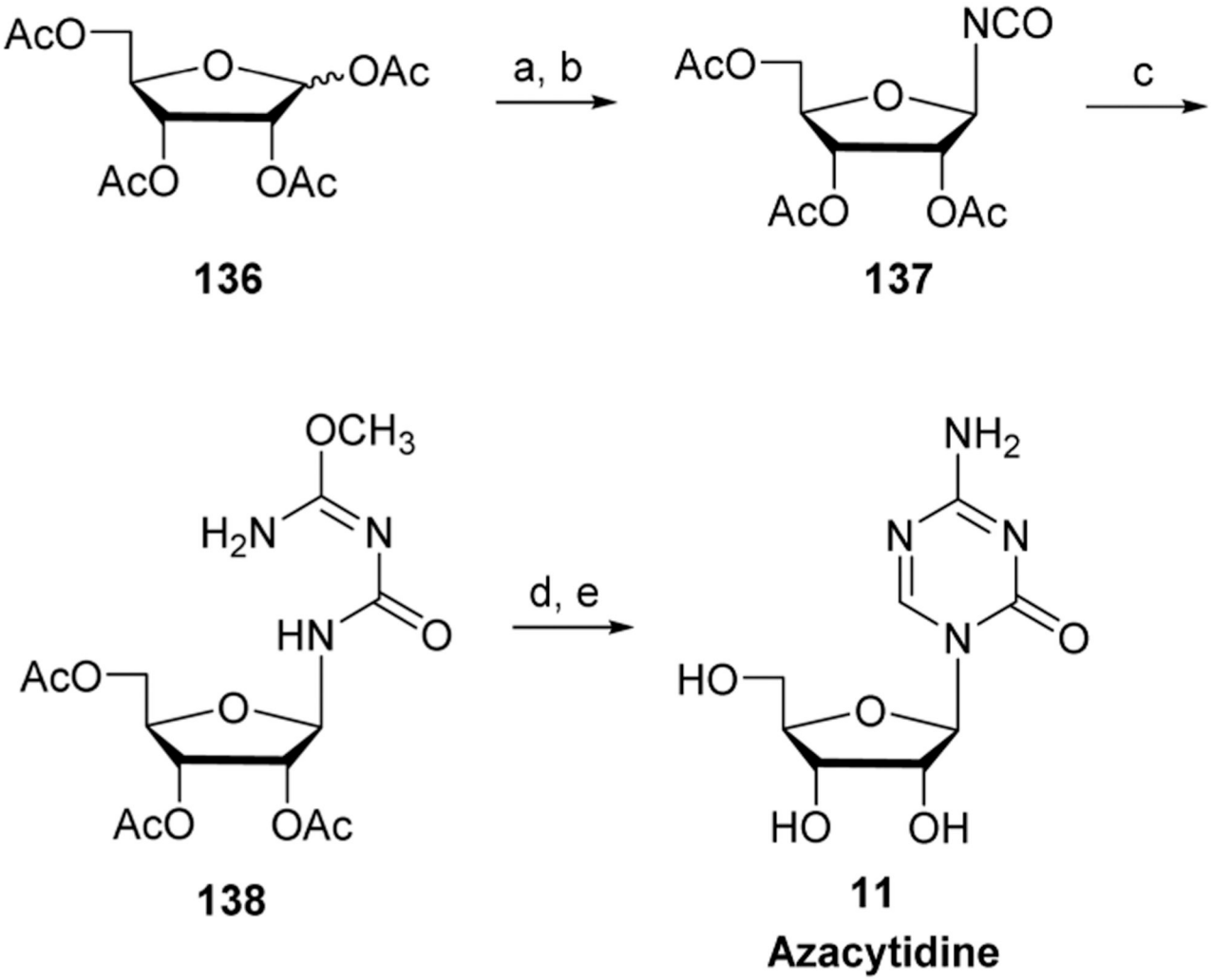
Scheme 18. Synthesis of Trifluorothymidine 15[a]

[a]Reagents and conditions: (a) AcCl, MeOH, 25 °C then pyr; (b) pyr, DMAP, 4-ClBzCl, 0 °C, 1 h, then 25 °C, 12 h; (c) HOAc, HCl (g), 0 °C, 7–10 min, 82% (over three steps); (d) 127, anisole, 50 °C, 3.5 h, 85% (β/α = 85.7:14.3); (e) NaOMe, MeOH, 4 °C, 3.5 h, then AcOBu, 97% (β/α = 99.92:0.08).



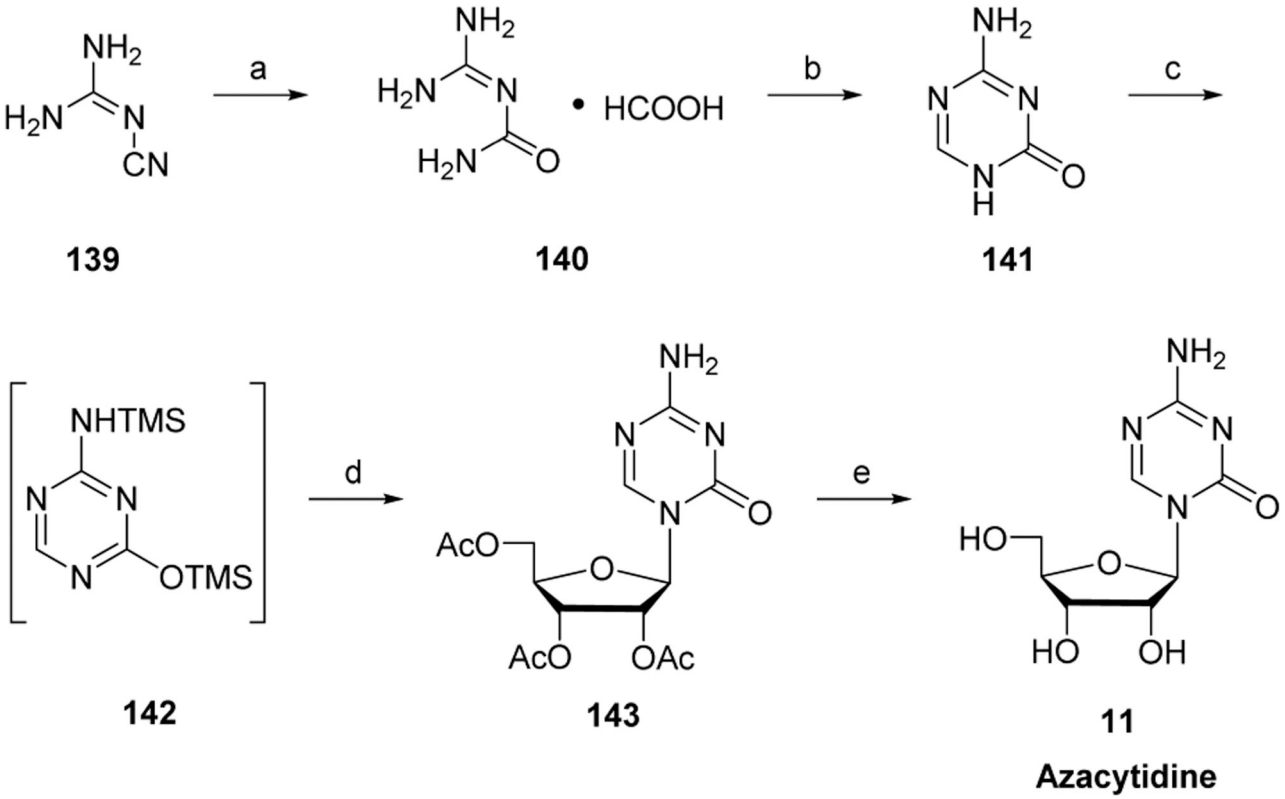
Scheme 19. Synthesis of Tipiracil Hydrochloride 16[a]

[a]Reagents and conditions: (a) SO_2Cl_2, AcOH, 50 °C, 2.5 h, 83%; (b) NH_3/MeOH, 120 °C, 10 h, 83%; (c) NaOEt, DMF, rt, 16 h, then aq HCl, 38%.



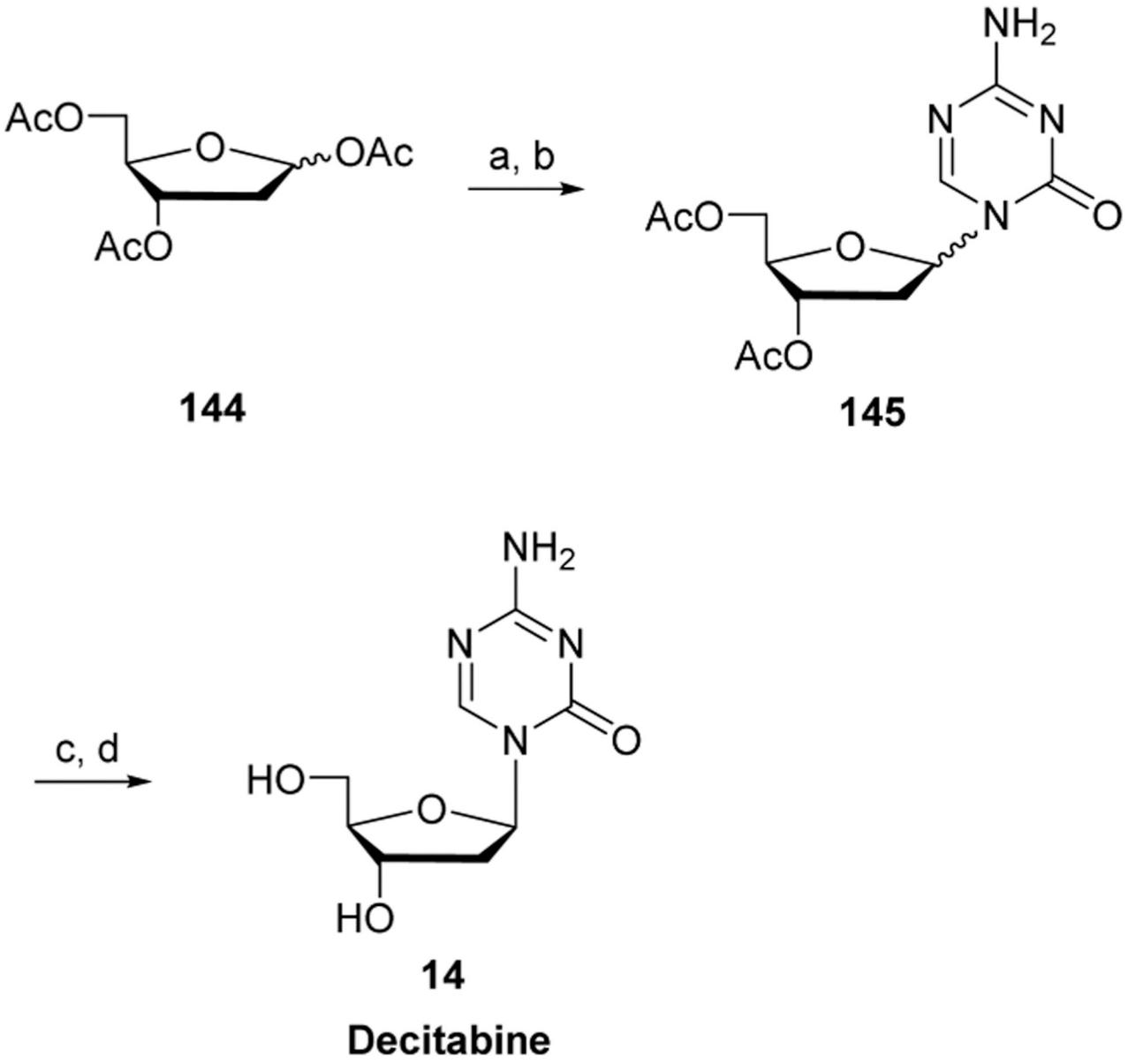
Scheme 20. Synthesis of Azacytidine 11[a]

[a]Reagents and conditions: (a) HCl (g), Ac_2O, Et_2O; (b) AgNCO, 60%; (c) 2-methylisourea, 68%; (d) $HC(OC_2H_5)_3$; (e) NH_3/MeOH, 40%.



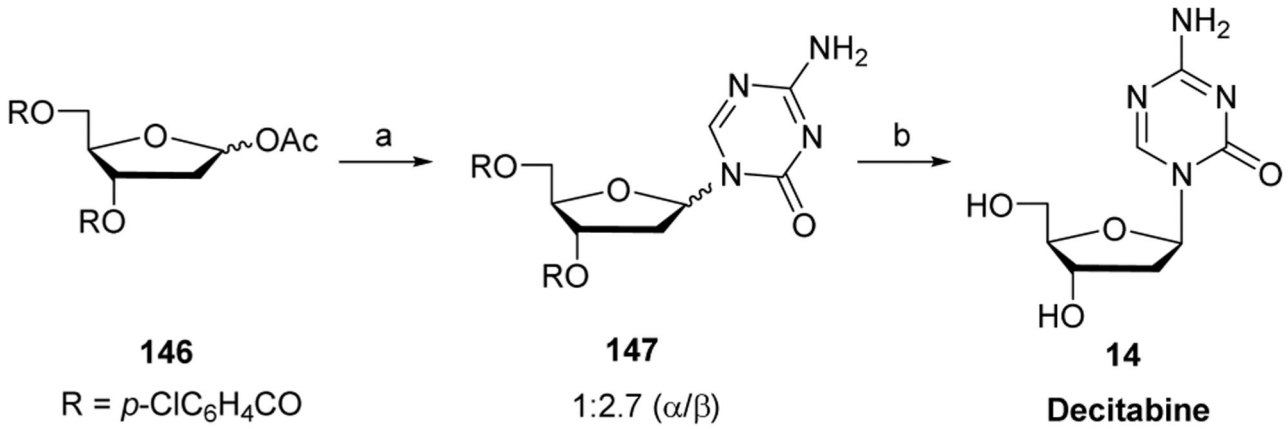
Scheme 21. Synthesis of Azacytidine 11[a]

[a]Reagents and conditions: (a) HCO_2H, 80 °C, 15 min, 85%; (b) $(CH_3)_2NCH(OCH_3)_2$, NaOMe, MeOH, 40–50 °C, 4 h, 90%; (c) HMDS, $(NH_4)_2SO_4$, CH_3CN, reflux, 4–8 h; (d) 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, CF_3SO_3H, EtOAc, 45 °C, 30 min, then HCl, DCM, rt, 45 min; (e) *n*-$BuNH_2$, MeOH, 65 °C, 1 h, 49% (over three steps).



Scheme 22. Early Synthesis of Decitabine 14[a]

[a]Reagents and conditions: (a) AcCl, HCl, Et_2O, 0 °C; (b) bis(trimethylsilyl)-5-azacytosine 142, CH_3CN, 10%; (c) NH_3/EtOH; (d) anomer separation, 7%.



146

R = *p*-ClC$_6$H$_4$CO

147

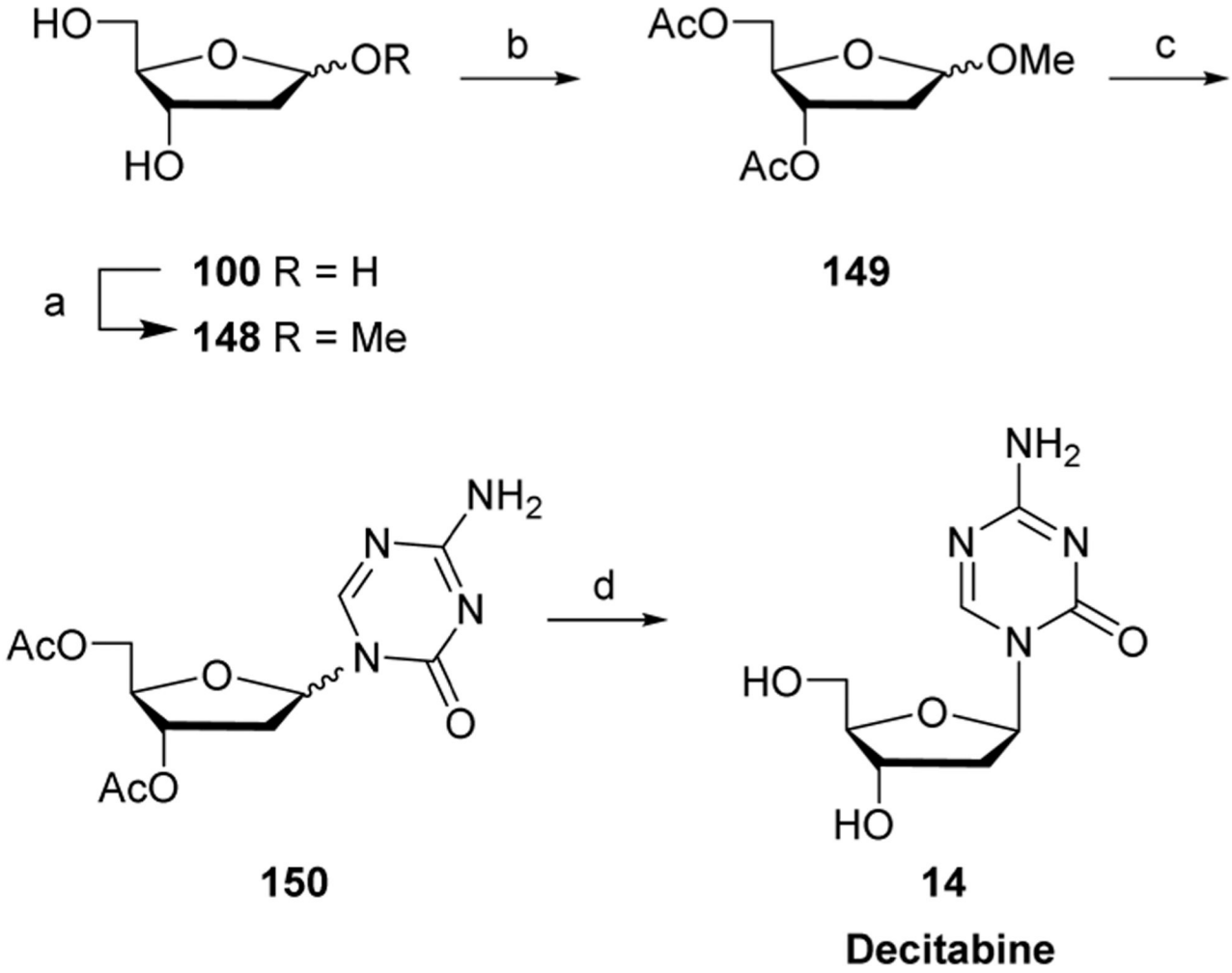
1:2.7 (α/β)

14

Decitabine

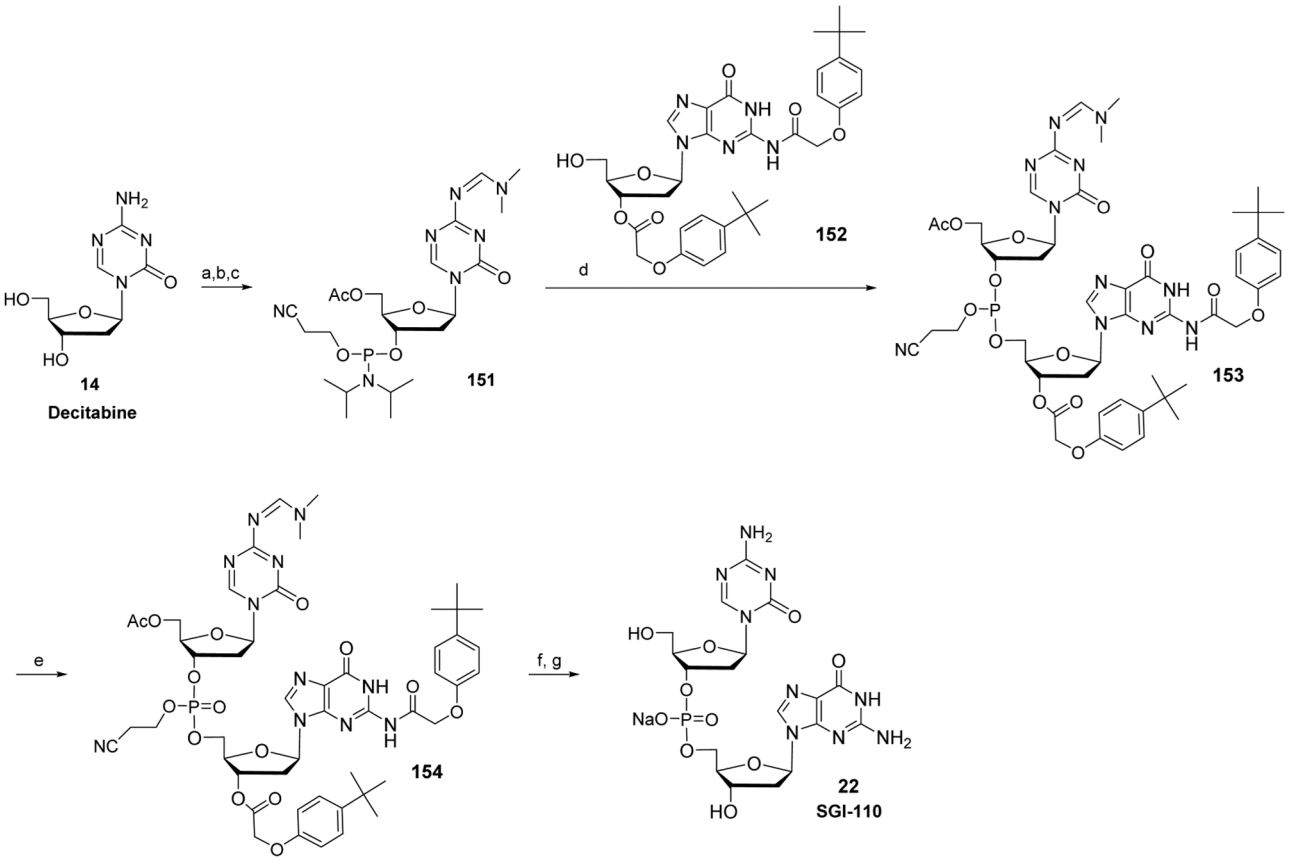
Scheme 23. Kilogram-Scale Synthesis of Decitabine 14[a]

[a]Reagents and conditions: (a) bis(trimethylsilyl)-5-azacytosine **142**, TMSOTf, DCM, cold, 55%; (b) NaOMe/MeOH, 65%.



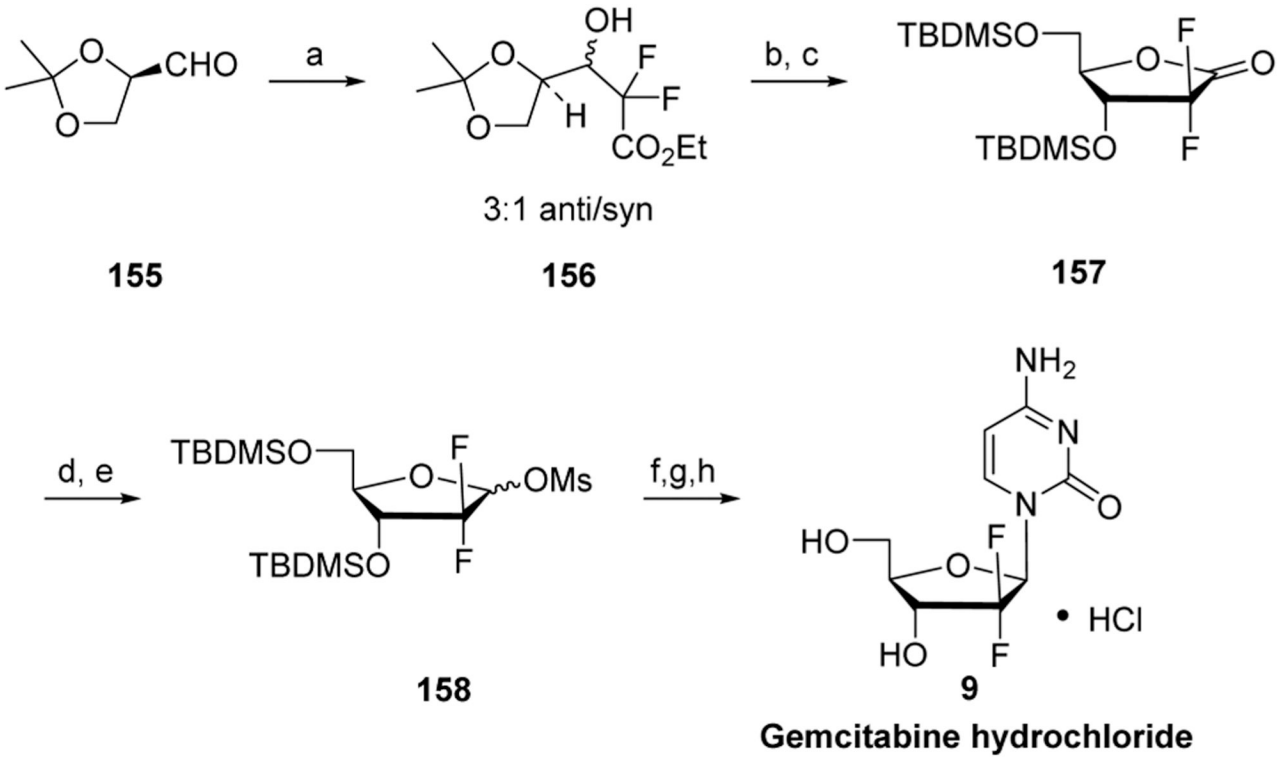
Scheme 24. Synthesis of Decitabine 14[a]

[a]Reagents and conditions: (a) AcCl/MeOH, 99%; (b) AcCl/pyr/DCM, 78%; (c) 5-azacytosine, HMDS, NH_4SO_4, TMSOTf, 60% (β/α = 1:1); (d) NH_3/MeOH, 34%.



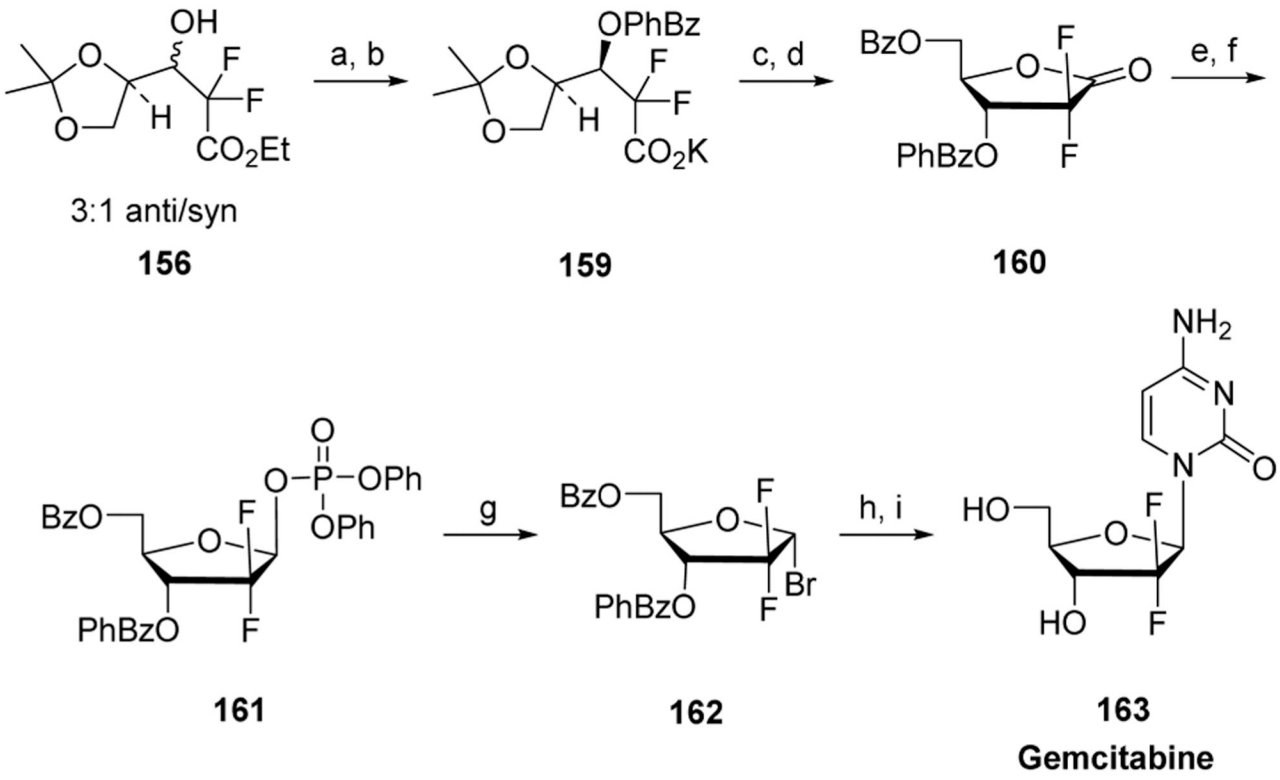
Scheme 25. Synthesis of SGI-110 22[a]

[a]Reagents and conditions: (a) DMF, dimethylacetal; (b) vinyl acetate, Lipozyme RM IM, CH_3CN, dioxane; (c) $[(i\text{Pr})_2\text{N}]\text{POCH}_2\text{CH}_2\text{CN}$, DCM; (d) DCM; (e) *t*BuOOH; (f) NH_3/MeOH; (g) NaOAc, H_2O, EtOH.



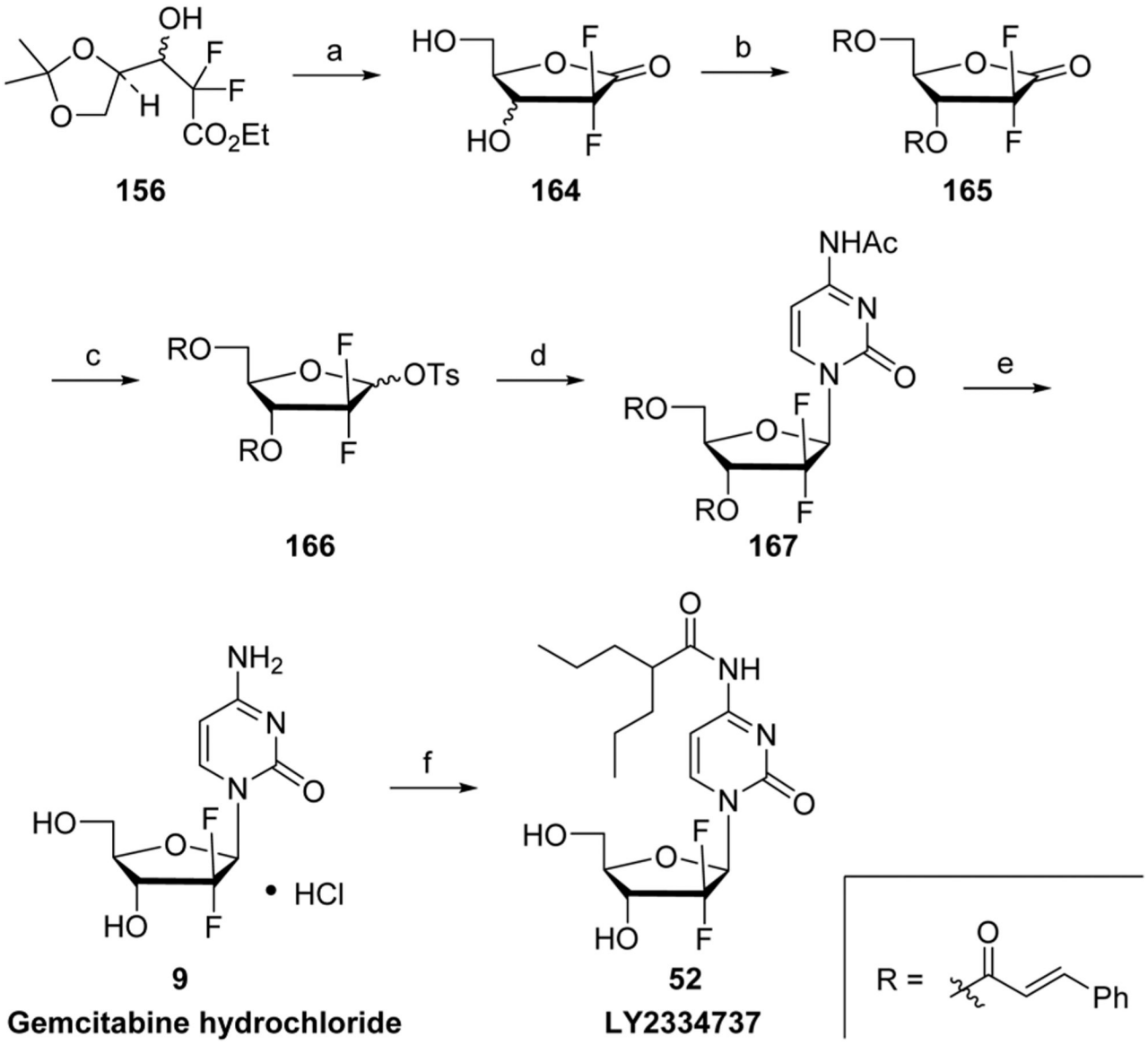
Scheme 26. Synthesis of Gemcitabine Hydrochloride 9[a]

[a]Reagents and conditions: (a) $BrCF_2CO_2Et$, Zn, THF, Et_2O, 87% (3:1 anti/syn); (b) Dowex 50, MeOH, H_2O; (c) TBDMSOTf, lutidine, DCM, 86% (over two steps); (d) DIBAL-H; (e) MsCl, Et_3N, DCM, 71% (over two steps); (f) 2,4-bis(trimethylsilyl)cytosine, TMSOTf, DCE, reflux; (g) AG 50W-X8 resin, MeOH; (h) reverse phase HPLC, 10% (over two steps).



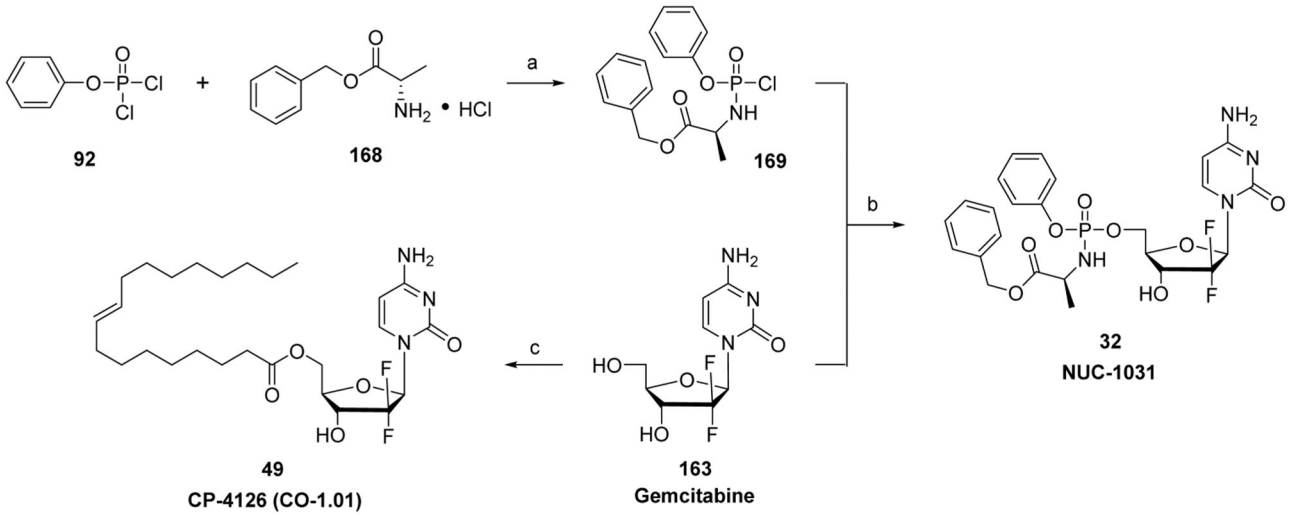
Scheme 27. Synthesis of Gemcitabine 163[a]

[a]Reagents and conditions: (a) PhBzCl, Et_3N, DCM; (b) K_2CO_3, H_2O, THF, MeOH, 67%; (c) HCl, CH_3CN, reflux; (d) BzCl/Pyr, EtOAc, rt, 72%; (e) LiAl(*O*-*t*Bu)$_3$H, THF; (f) ClP(O)(OPh)$_2$, Et_3N, PhMe, rt, 77%; (g) HBr/HOAc, rt, 82%; (h) 2,4-bis(trimethylsilyl)cytosine, octane/heptane, Ph_2O, 140–150 °C; (i) NH_3/MeOH, H_2O, rt, 65%.



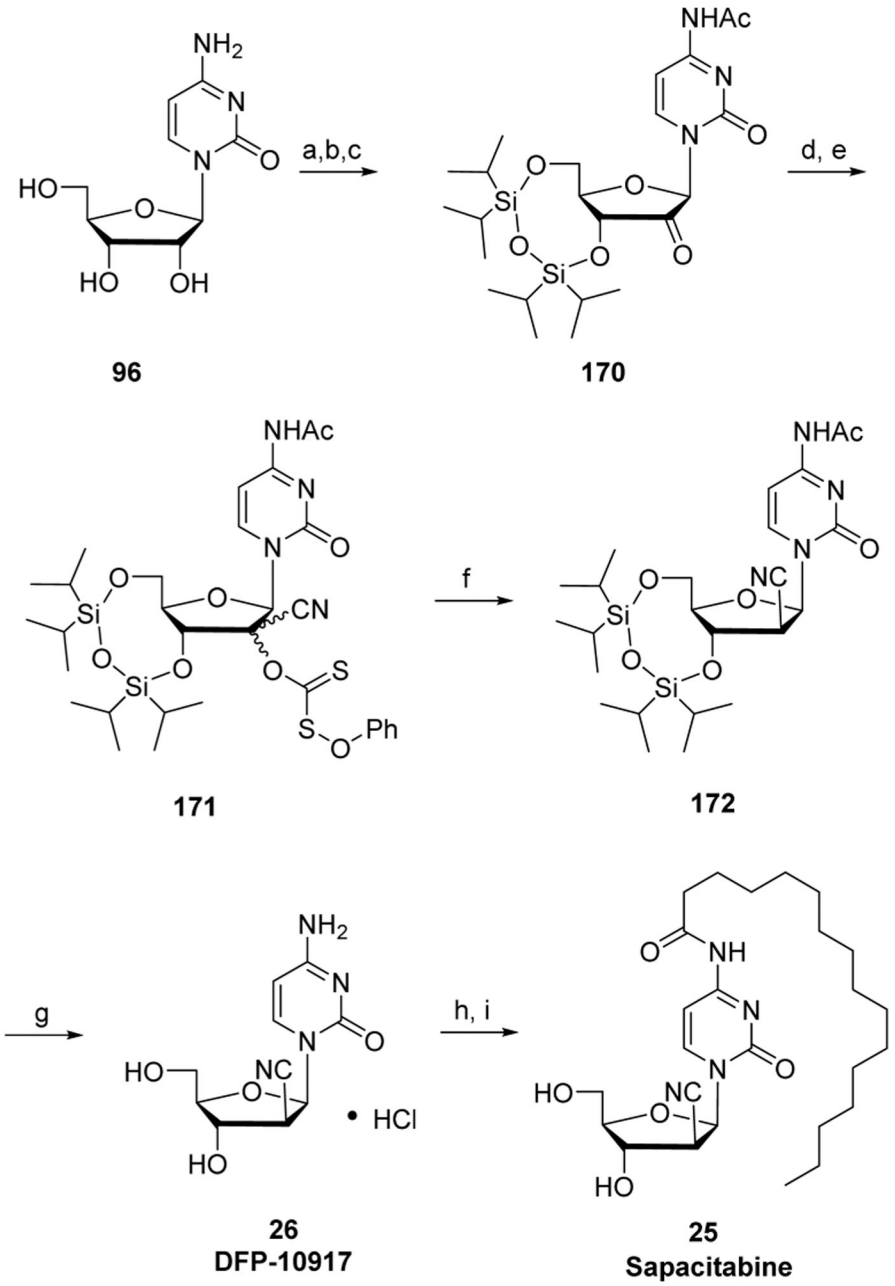
Scheme 28. Synthesis of Gemcitabine Hydrochloride 9 and LY2334737 52[a]

[a]Reagents and conditions: (a) Dowex 50W-X12 resin, MeOH, H_2O, rt, 4 days, 94%; (b) (*E*)-cinnamoyl chloride, pyr, EtOAc, 30 °C, 3 h, 43%; (c) LiAl(*O*-*t*Bu)$_3$H, THF, −10 °C, 2 h, then TsCl, Et_3N, PhMe, −10 °C, 5 h, 62%; (d) *N*-acetyl cytosine, $(TMS)_2NH$, TMSOTf, DCE, reflux, 12 h, then 5% $NaHCO_3$, 47%; (e) NH_3/MeOH, rt, overnight, then HCl, Me_2CO, rt, 12 h, 80%; (f) 2-propylpentanoic acid, EDC, HOBt, NMM, DMF, DMSO, 55 °C, 17 h, 95%.



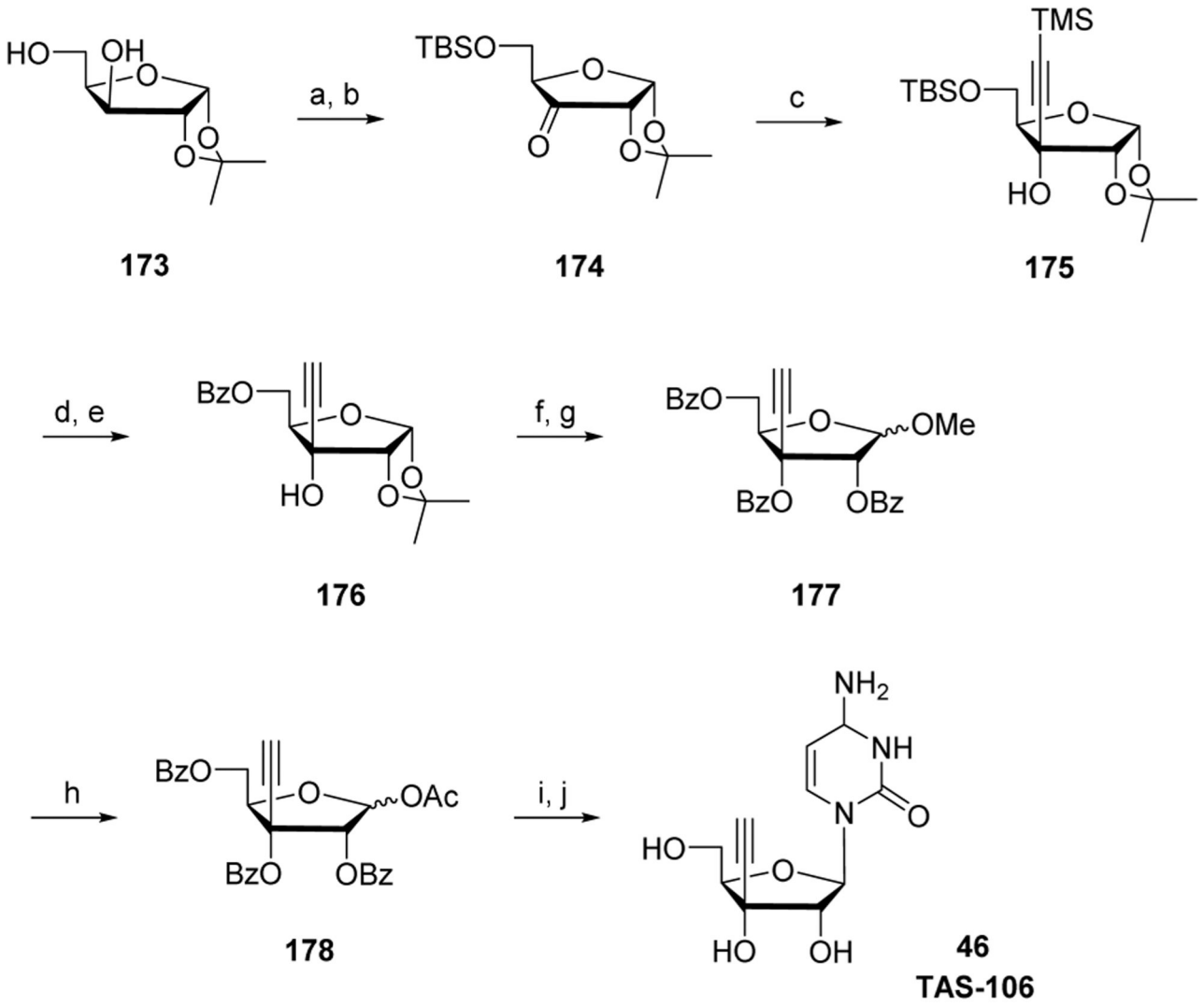
Scheme 29. Synthesis of CP-4126 49 and NUC-1031 32[a]

[a]Reagents and conditions: (a) Et_3N, DCM, −80 °C, 1 h, 98%, (b) NMI, pyr, THF, −80 °C to rt, 2 h, 16%; (c) HCl (g), DMF, elaidic acid chloride, rt, 12 h, 30%.



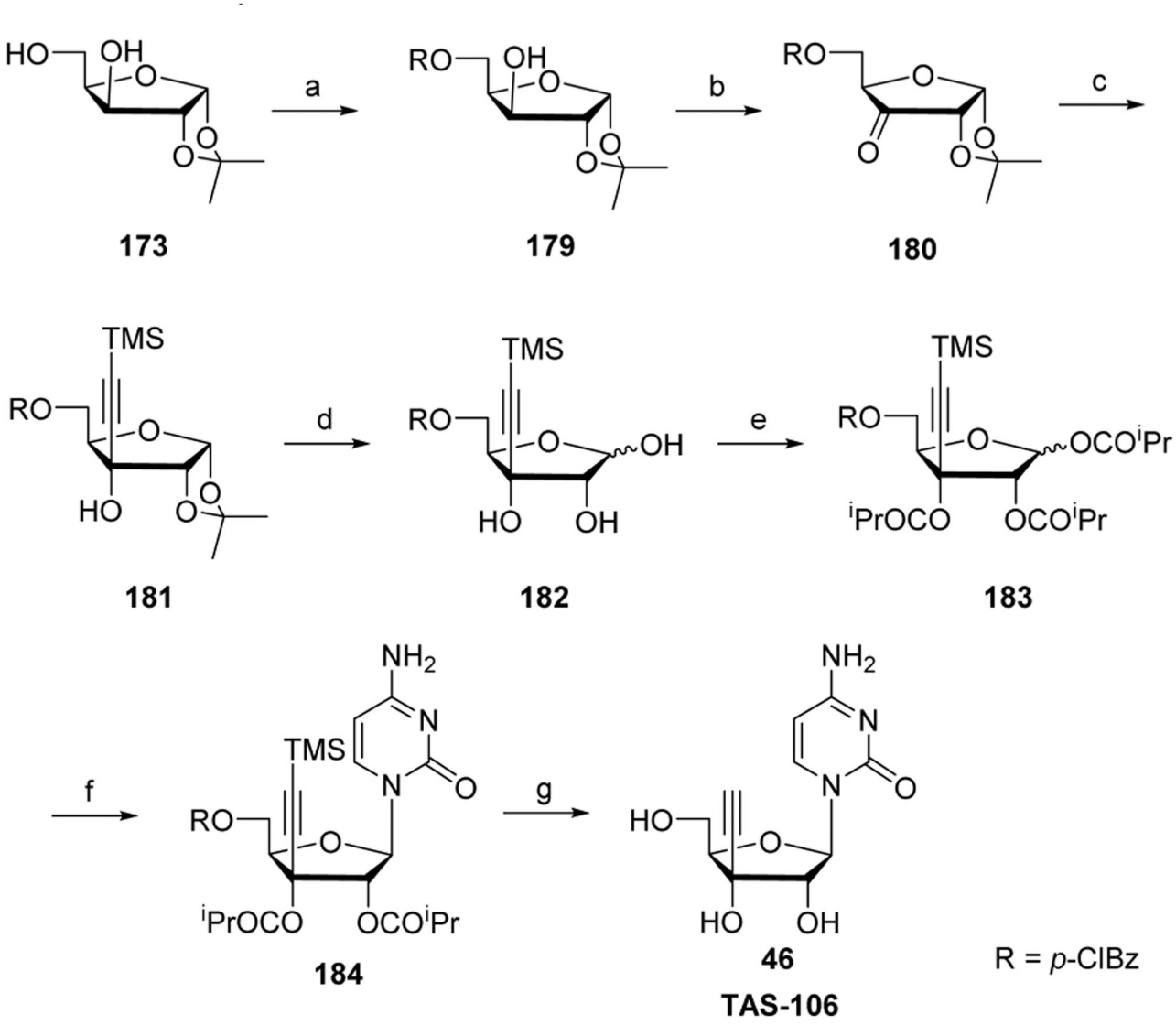
Scheme 30. Discovery Synthesis of DFP-10917 26 and Process Synthesis of Sapacitabine 25[a]

[a]Reagents and conditions: (a) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyr; (b) Ac_2O, EtOH; (c) CrO_3/pyr/Ac_2O, DCM; (d) NaCN, $NaHCO_3$, Et_2O, H_2O; (e) PhOC(S)Cl, DMAP, CH_3CN; (f) Bu_3SnH, AIBN, PhMe (57% over 3 steps); (g) Bu_4NF, CH_3CO_2H, THF then HCl/MeOH, 49% (over two steps); (h) Et_3N, MeOH, DCM, 30 °C, 10 min, 97%; (i) palmitic anhydride, dioxane, H_2O, 85 °C, 89%.



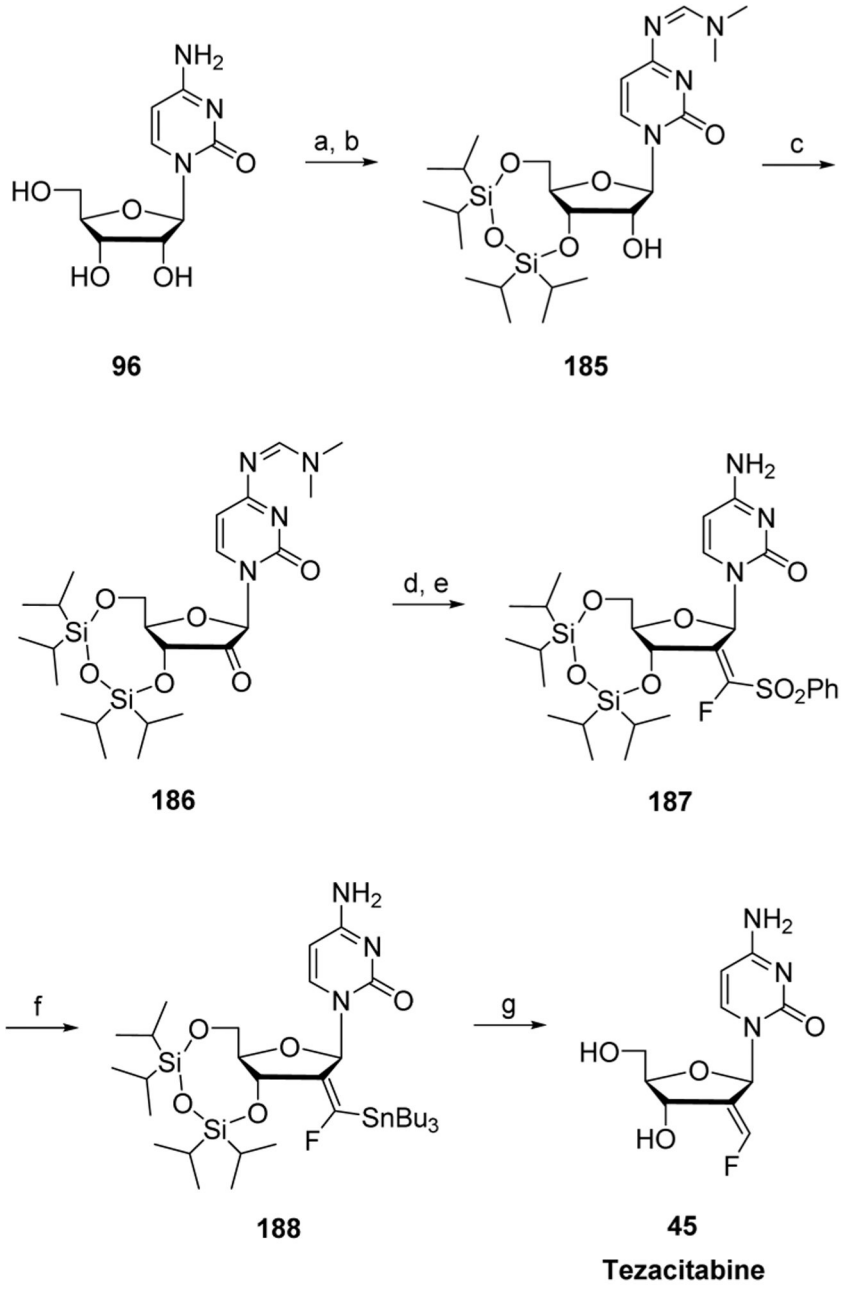
Scheme 31. Synthesis of TAS-106 46[a]

[a]Reagents and conditions: (a) TBDMSCl, pyr, rt, 1.5 h, 100%; (b) CrO_3, Ac_2O, pyr, rt, 1.5 h, 77%; (c) trimethylsilylacetylene, *n*-BuLi, THF, −78 °C, 99%; (d) *n*-Bu_4NF, THF, rt; (e) BzCl, pyr, rt, 93% (over two steps); (f) 20% HCl, MeOH, rt; (g) BzCl, DMAP, pyr, 100 °C, 87% (over two steps); (h) concd H_2SO_4, AcOH, Ac_2O, rt, 93%; (i) 2,4-bis(trimethylsilyl)cytosine, $SnCl_4$, CH_3CN, 0 °C to rt, 18 h, 81%; (j) NH_3/MeOH, rt, 2 days, 90%.



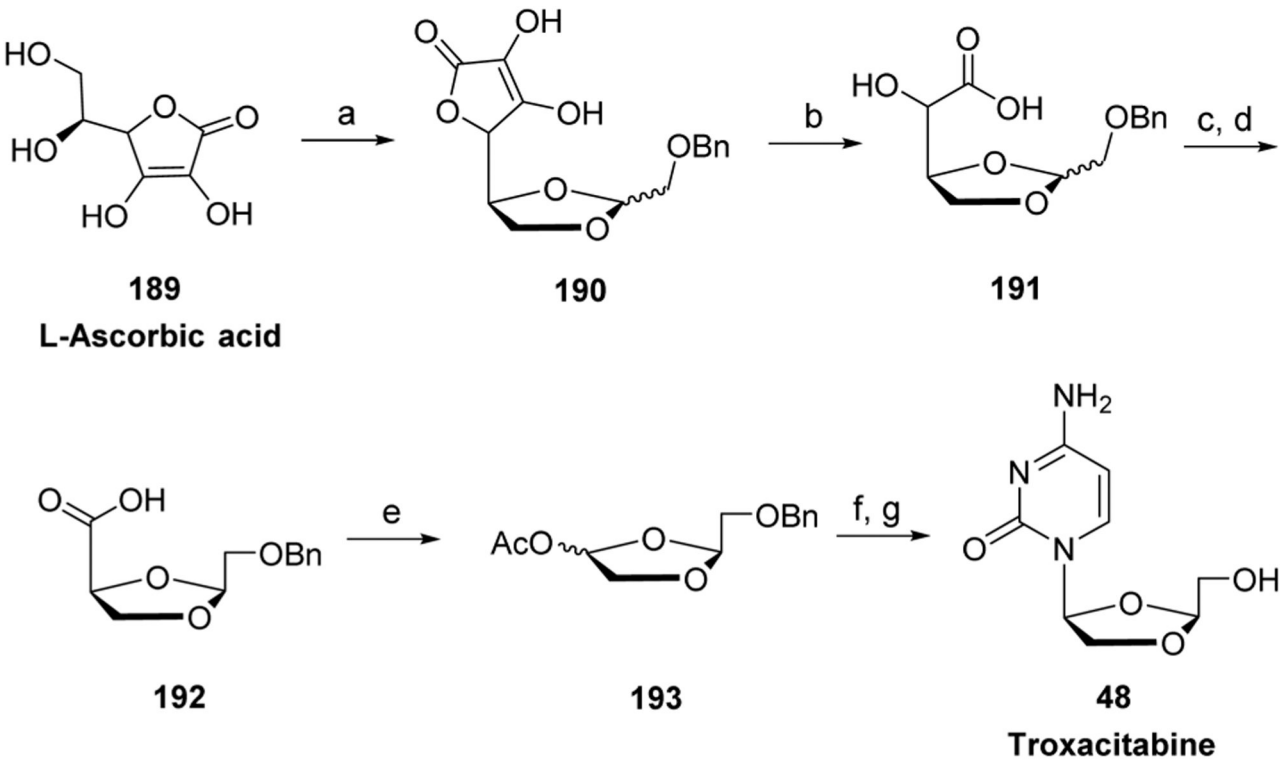
Scheme 32. Synthesis of TAS-106 46[a]

[a]Reagents and conditions: (a) 4-ClBzCl, Et_3N, DCM, 0 °C, 2 h, 74%; (b) TEMPO, DCM $NaClO_4$, $NaHCO_3$, H_2O, 0 °C, 30 min, then 2-propanol, rt, 10 min, 88%; (c) trimethylsilylacetylene, EtMgBr, THF, 4 °C, 30 min, 25%; (d) HCO_2H, H_2O, reflux, 45 min, 97%; (e) *i*-BuCl, Et_3N, DMAP, rt, 5 h, 87%; (f) 2,4-bis(trimethylsilyl)cytosine, $SnCl_4$, MeCN, 30 °C, 3 h, 81%; (g) DBU, MeOH, 30 °C, 3 h, 68%.



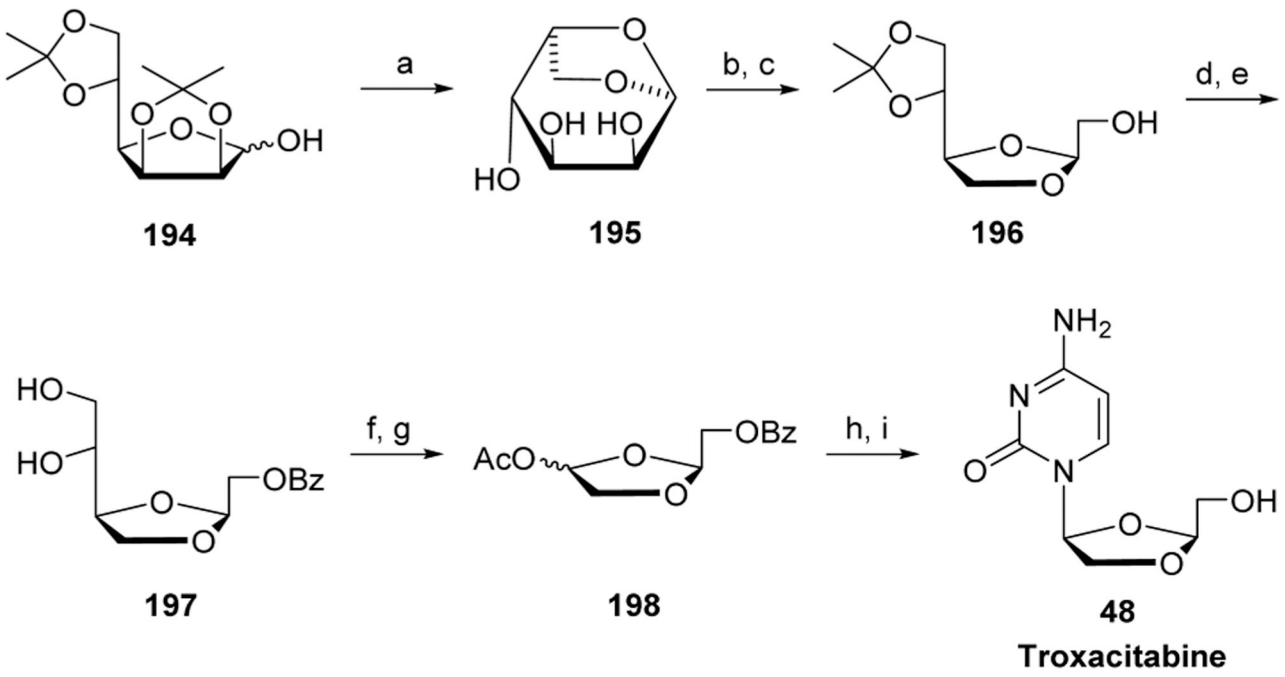
Scheme 33. Synthesis of Tezacitabine 45[a]

[a]Reagents and conditions: (a) TIPSiCl, pyr, 34 °C, 18 h; (b) $(CH_3)_2NCH(OCH_3)_2$, 37 °C, 4 h, 85% (over two steps); (c) $(COCl)_2$, DMSO, DCM, −75 °C, 0.5 h, then Et_3N, −75 °C to rt, 1 h, 90%; (d) $PhSO_2CH_2F$, $ClP(O)(OEt)_2$, 1 M LiHMDS, −60 °C to rt, 3 h; (e) NH_3/MeOH, rt, overnight, 66% (over two steps); (f) Bu_3SnH, AIBN, cyclohexane, reflux, 18 h, 83%; (g) CsF, MeOH, reflux, 24 h, 69%.



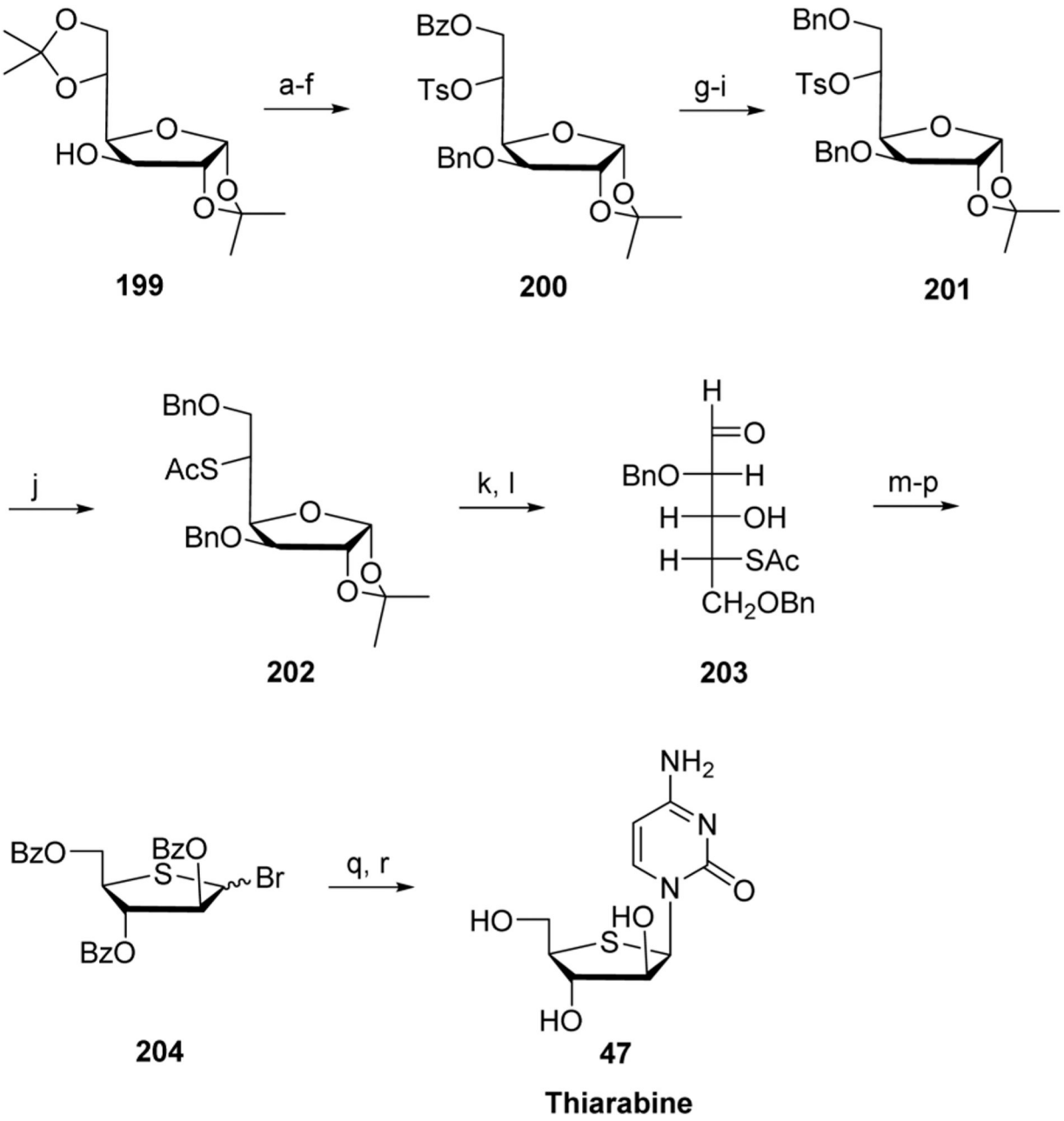
Scheme 34. Synthesis of Troxacitabine 48[a]

[a]Reagents and conditions: (a) $PhCH_2OCH_2CH(OMe)_2$, TsOH, CH_3CN, 95%; (b) H_2O_2, K_2CO_3, EtOH; (c) $RuCl_3$ hydrate, NaOCl, DCE/MeCN/H_2O, $BnEt_3NCI$; (d) H^+, 51% (over three steps); (e) $Pb(OAc)_4$, MeCN/DCM, pyr, 80%; (f) silylated *N*-acetylcytosine, TMSOTf, DCM, rt; (g) deprotection, 30%.



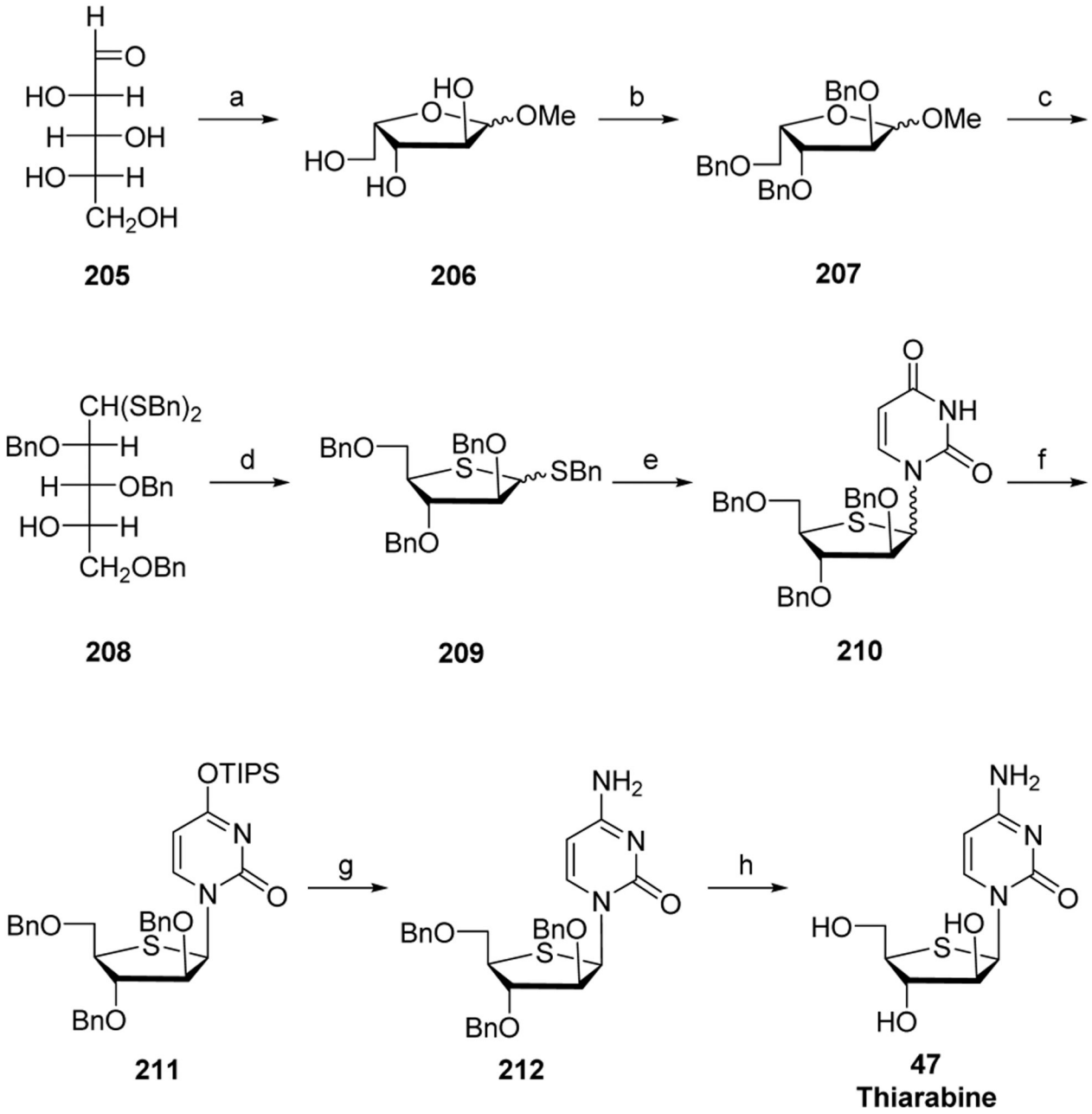
Scheme 35. Synthesis of Troxacitabine 48[a]

[a]Reagents and conditions: (a) aq HCl, reflux, 20 h, 60%; (b) $NaIO_4$, MeOH, H_2O, then $NaBH_4$; (c) TsOH, Me_2CO, 6 h, 62%; (d) BzCl, pyr, DCM, rt, 2 h; (e) TsOH, MeOH, rt, 2 h, 83% (over two steps); (f) $NaIO_4$, $RuO_2{\cdot}H_2O$, CH_3CN:CCl_4:H_2O (1:1:1.5 v/v), rt, 5 h; (g) $Pb(OAc)_4$, pyr, THF, rt, 45 min, 63% (over two steps); (h) N^4-Bzcytosine, HMDS, NH_4SO_4, DCE, reflux, 2.5 h, then TMSOTf, DCE, rt, 1.5 h; (i) NH_3/MeOH, 0 °C, rt, 72 h, 38% (over two steps).



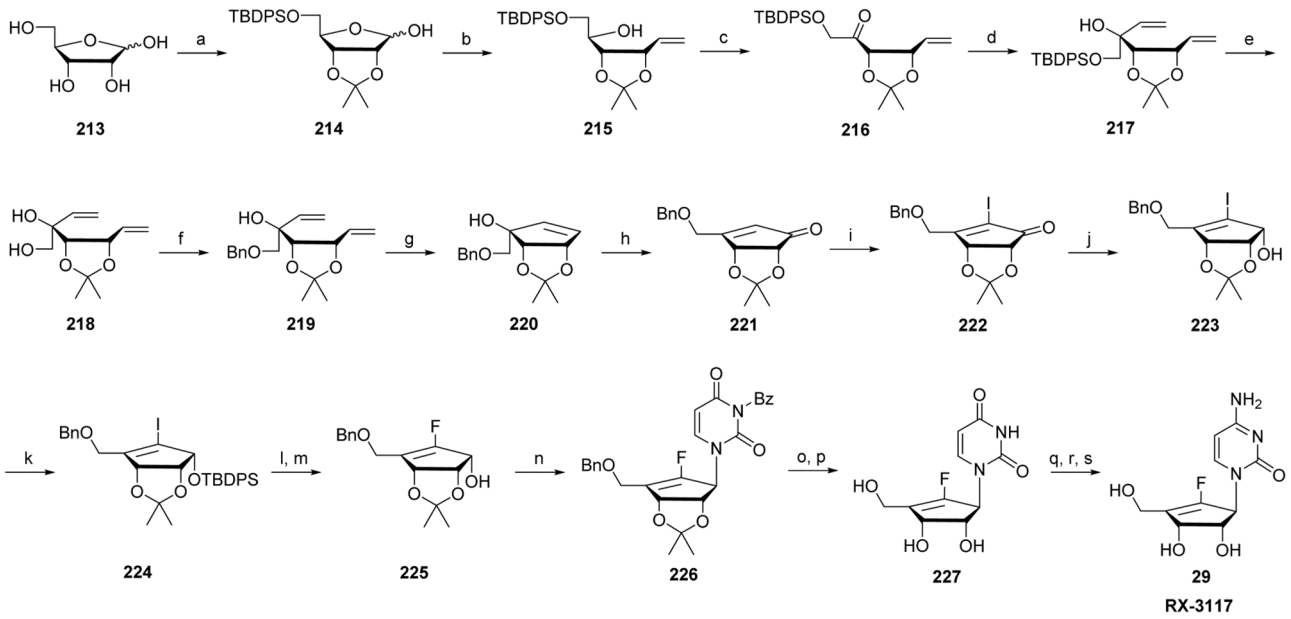
Scheme 36. Synthesis of Thiarabine 47[a]

[a]Reagents and conditions: (a) Na, ether, reflux, 16 h, then BnBr, 70 °C, 16 h; (b) Ac_2O, H_2O, 40 °C, 16 h; (c) AcCl, pyr, 25 °C, 16 h, 78% (over three steps); (d) Na, MeOH, 16 h; (e) BzCl, pyr, −15–25 °C, 20 h; (f) TsCl, pyr, $CHCl_3$, 40 °C, 36 h, 92% (over three steps); (g) NaOMe, $CHCl_3$, MeOH, −15 to 0 °C, 2 h, 87%; (h) NaOBn, BnOH, 40 °C; (i) TsCl; (j) $KSC(O)CH_3$, DMF, 115 °C, 73%; (k) HOAc, H_2O, 70 °C, 36 h, 78%; (l) $NaIO_4$, EtOH, H_2O, 30 °C, 0.5 h, 97%; (m) aq HCl, MeOH, reflux, 3 h, 41%; (n) Na, NH_3, ~100%; (o) BnCl; (p) HBr/HOAc; (q) bis(trimethylsilyl)-*N*-acetylcytosine, 130 °C, 3 h, 20%; (r) NH_3/MeOH, 0 °C, 2 days, 89%.



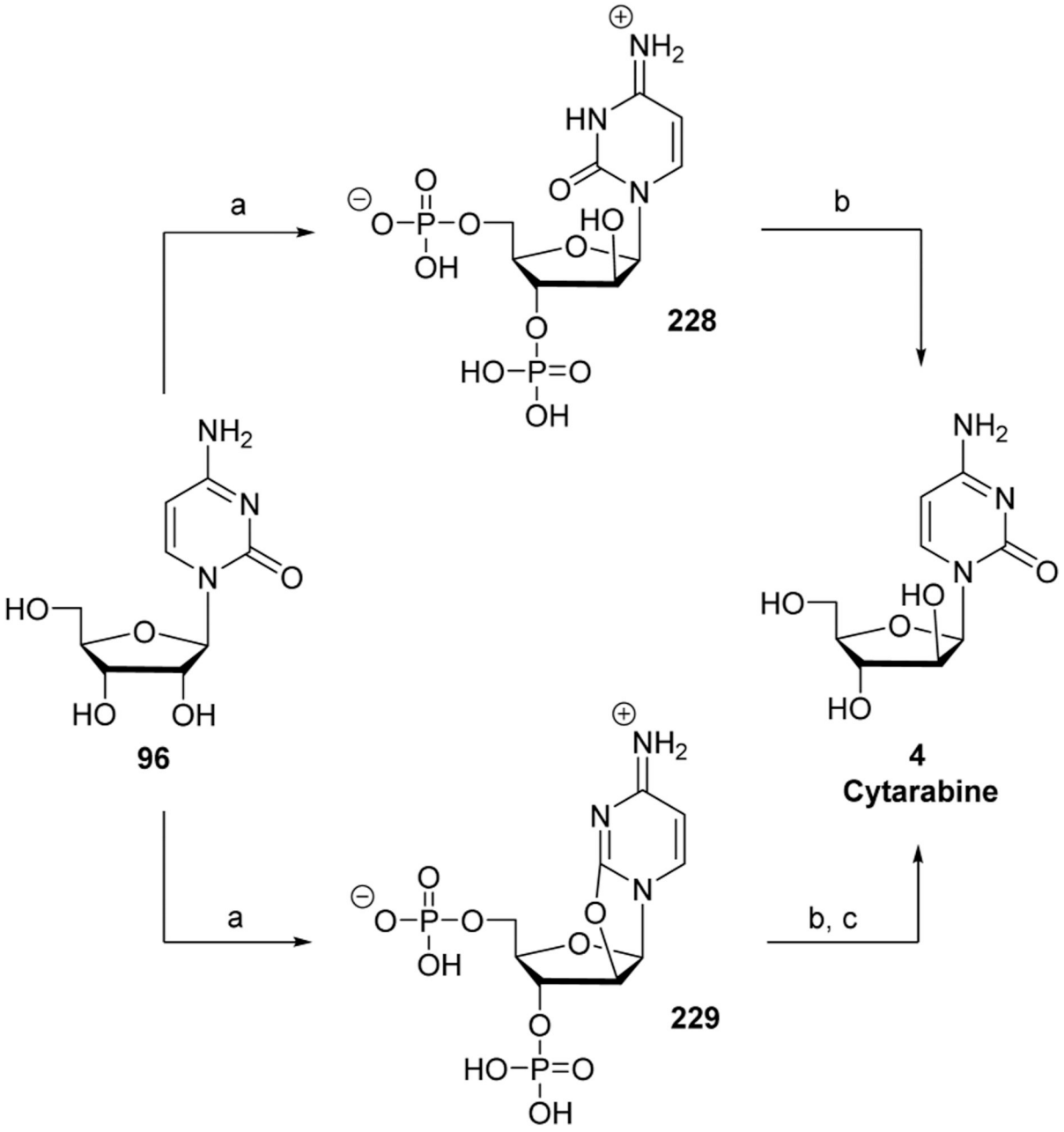
Scheme 37. Synthesis of Thiarabine 47[a]

[a]Reagents and conditions: (a) HCl, MeOH, rt, 5 h, 95%; (b) BnBr, NaH, TBAI, THF, rt, 3 days, 87%; (c) BnSH, $SnCl_4$, DCM, rt, overnight, 57%; (d) Ph_3P, imidazole, I_2, PhMe, CH_3CN, 90 °C, 24 h, 83%; (e) uracil, BSA, NBS, MeCN, 50–55 °C, 18 h, column purification (α:β = 1:1.15), 36%; (f) TPSCl, Et_3N, DMAP, 5 °C to rt, overnight; (g) NH_4OH, MeCN, rt, overnight, 50%.



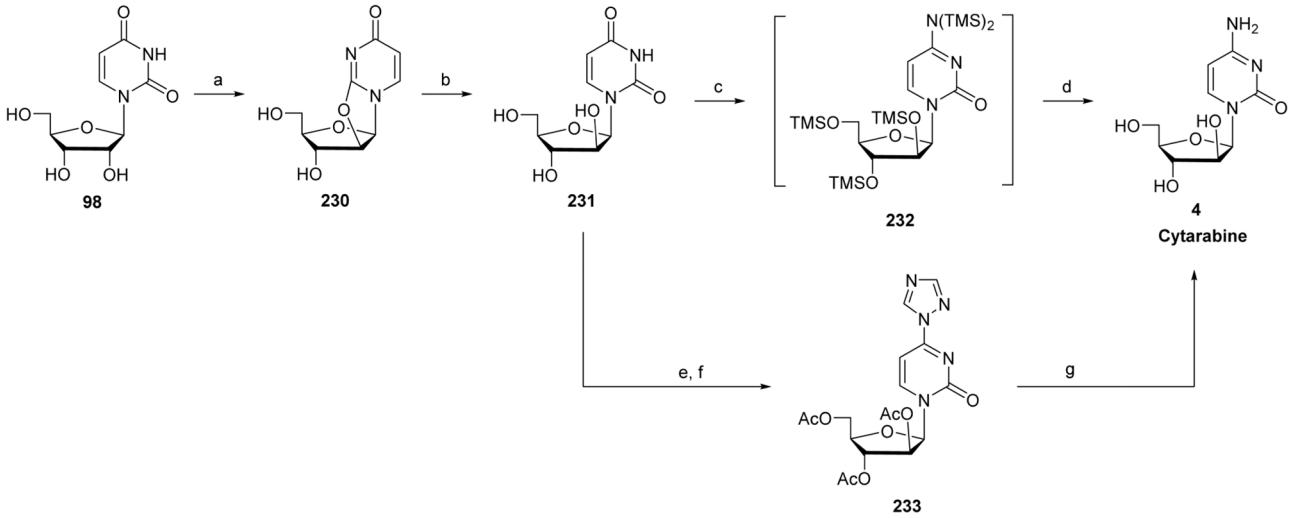
Scheme 38. Synthesis of RX-3117 29[a]

[a]Reagents and conditions: (a) concd H_2SO_4, Me_2CO, then TBDPSCl, imidazole, 99%; (b) Ph_3PCH_3Br, *t*-BuOK, THF, 93%; (c) $(COCl)_2$, DMSO, DCM, −78 °C, 1 h, then Et_3N, rt, 96%; (d) CH_2=CHMgBr, THF, −78 °C, 84%; (e) *n*-Bu_4NF, THF, 97%; (f) $Bu_2Sn(O)$, PhMe, 15 h, then TBAI, BnBr, 50 °C, 16 h, 76%; (g) Grubbs' catalyst (2nd generation), PhMe, 80 °C, 90%; (h) PDC, DMF, rt, 18 h, 59%; (i) I_2, pyr, THF, 55%; (j) $NaBH_4$, $CeCl_3$, MeOH, 93%; (k) TBDPSCl, imidazole, DMF, 97%; (l) NFSI, *n*-BuLi, THF, −78 °C; (m) *n*-Bu_4NF, THF, 63% (over two steps); (n) N^3-benzoyluracil, DEAD, Ph_3P, THF, 87%; (o) NH_3/MeOH; (p) BBr_3, DCM, −78 °C, 50%; (q) Ac_2O, pyr, then $POCl_3$, Et_3N, 1,2,4-triazole; (r) NH_4OH, dioxane; (s) NH_3/MeOH, 40% (over three steps).



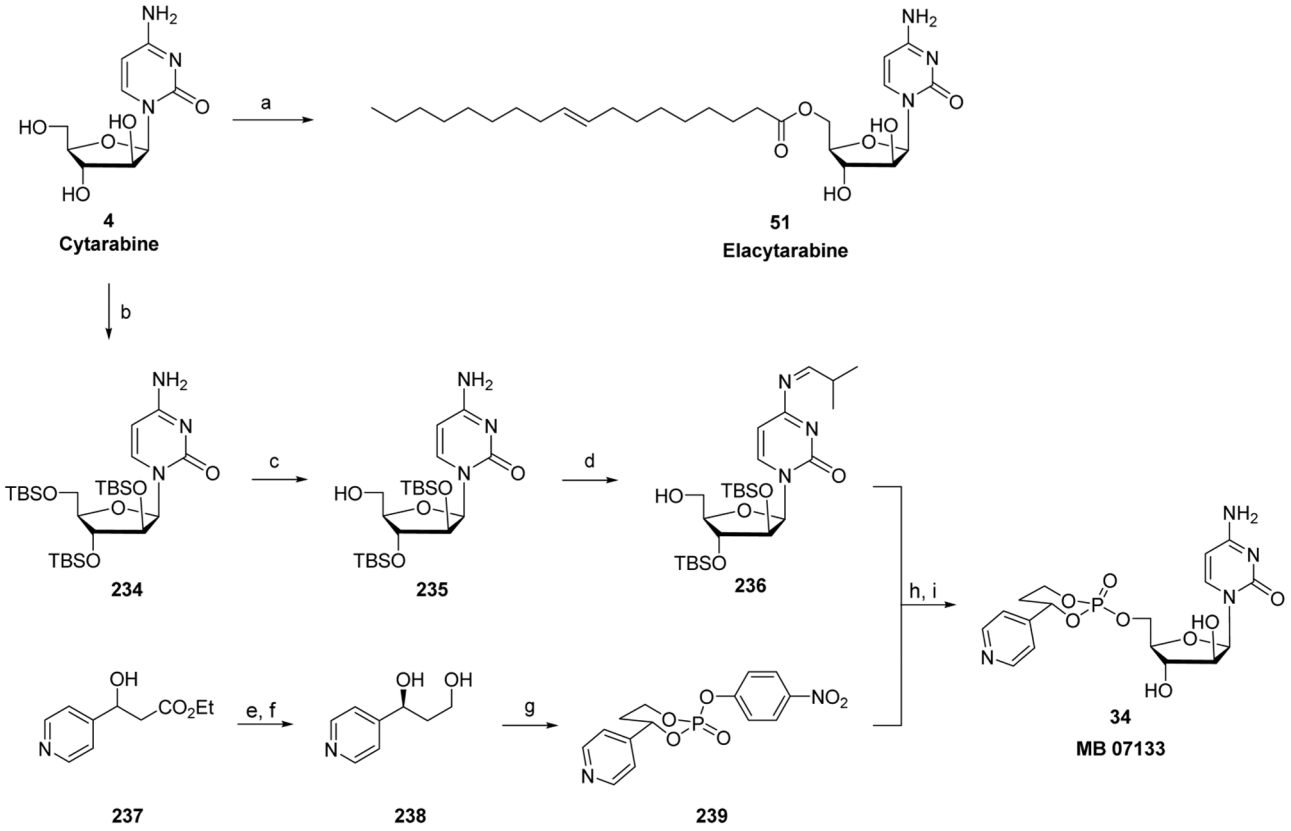
Scheme 39. Synthesis of Cytarabine 4[a]

[a]Reagents and conditions: (a) polyphosphoric acid; (b) prostatic phosphatase; (c) OH^-.



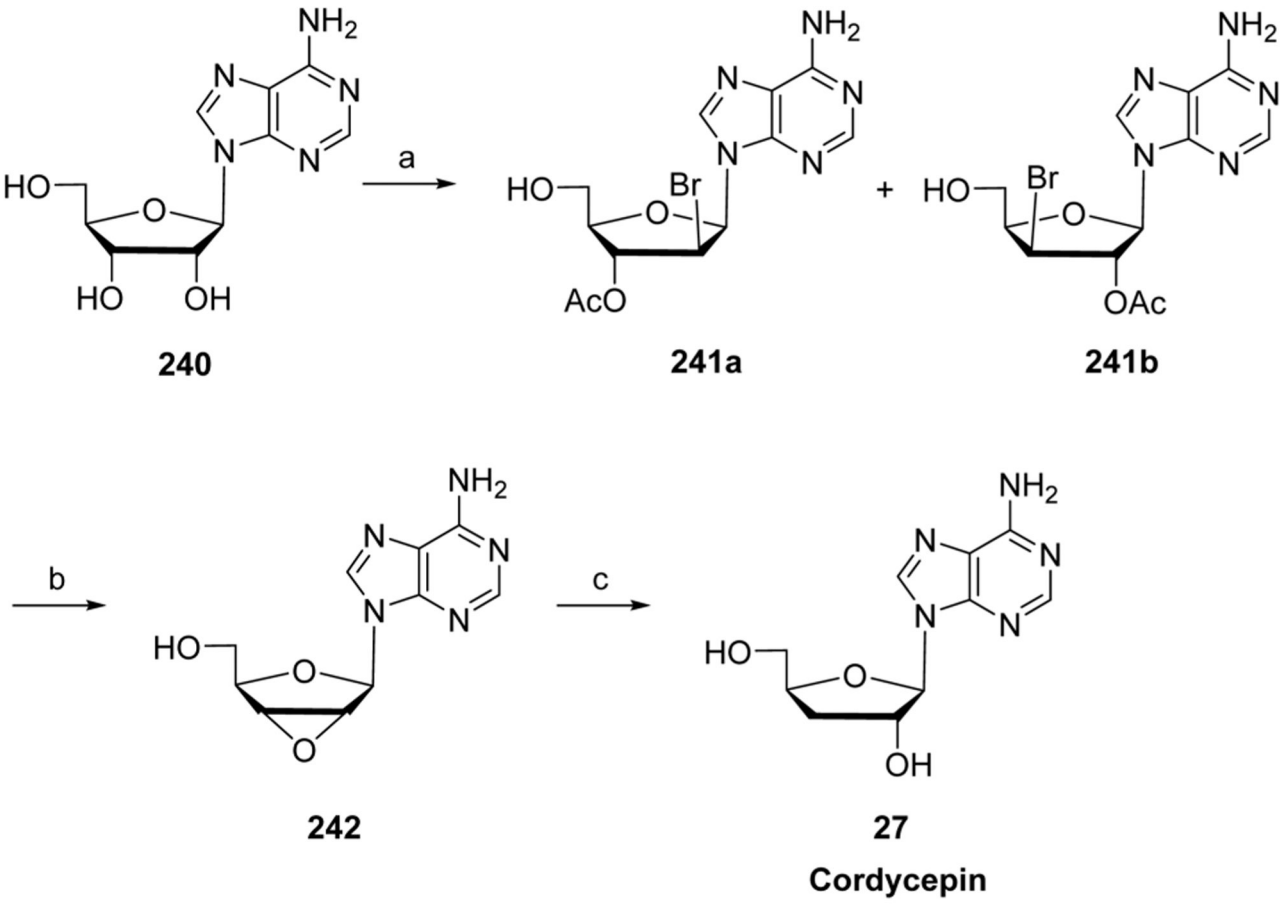
Scheme 40. Synthesis of Cytarabine 4[a]

[a]Reagents and conditions: (a) $(PhCO)_2O$, DMF, 100 °C, then K_2CO_3, 137 °C, 1.5 h, 78%; (b) aq HCl, 80 °C, 2 h, 68%; (c) HMDS, high pressure, 150 °C, 80 h; (d) MeOH, 5 °C, 1 h, 87% (over two steps); (e) Ac_2O, pyr, rt, 3 h, 91%; (f) $POCl_3$, 1H-1,2,4-triazole, Et_3N, $CHCl_3$, <8 °C then rt, overnight, 84%; (g) NH_4OH, NH_3, dioxane, H_2O, rt, overnight, 70%.



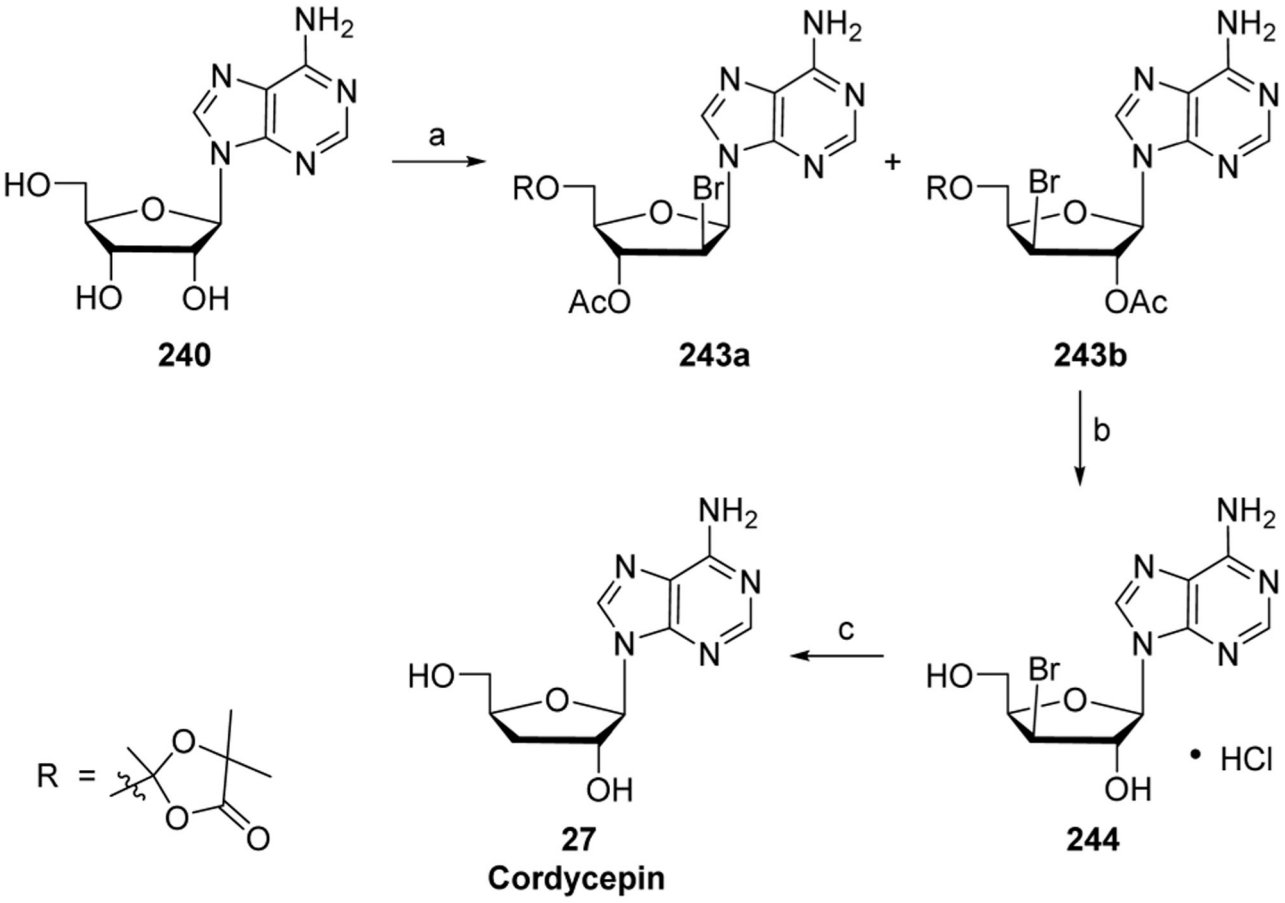
Scheme 41. Synthesis of Elacytarabine 51 and MB 07133 34[a]

[a]Reagents and conditions: (a) oleic anhydride, DMF, *Mucor miehei* lipase (MML), 60 °C, 48 h, 88%; (b) TBSCl, imidazole, DMF, rt, overnight, 85%; (c) 50% TFA, THF, −10 °C, 18 h, 65%; (d) DMF-dimethyl acetal, pyr, rt, 16 h, 90%;(e) EDC, DMAP, L-*N*,*N*-dimethyl-phenylalanine, DCM, rt, 16 h, 83%; (f) $LiAlH_4$, ether, −20 °C, 1 h, 66%; (g) 4-nitrophenyl phosphorodichloridate, THF, pyr, 0 °C to rt, 3.5 h, then sodium 4-nitrophenoxide, 40 °C, 4 h, 63%; (h) *t*-BuMgCl, THF, rt, 16 h, 78%; (i) 70% TFA, 60 °C, 16 h, 83%.



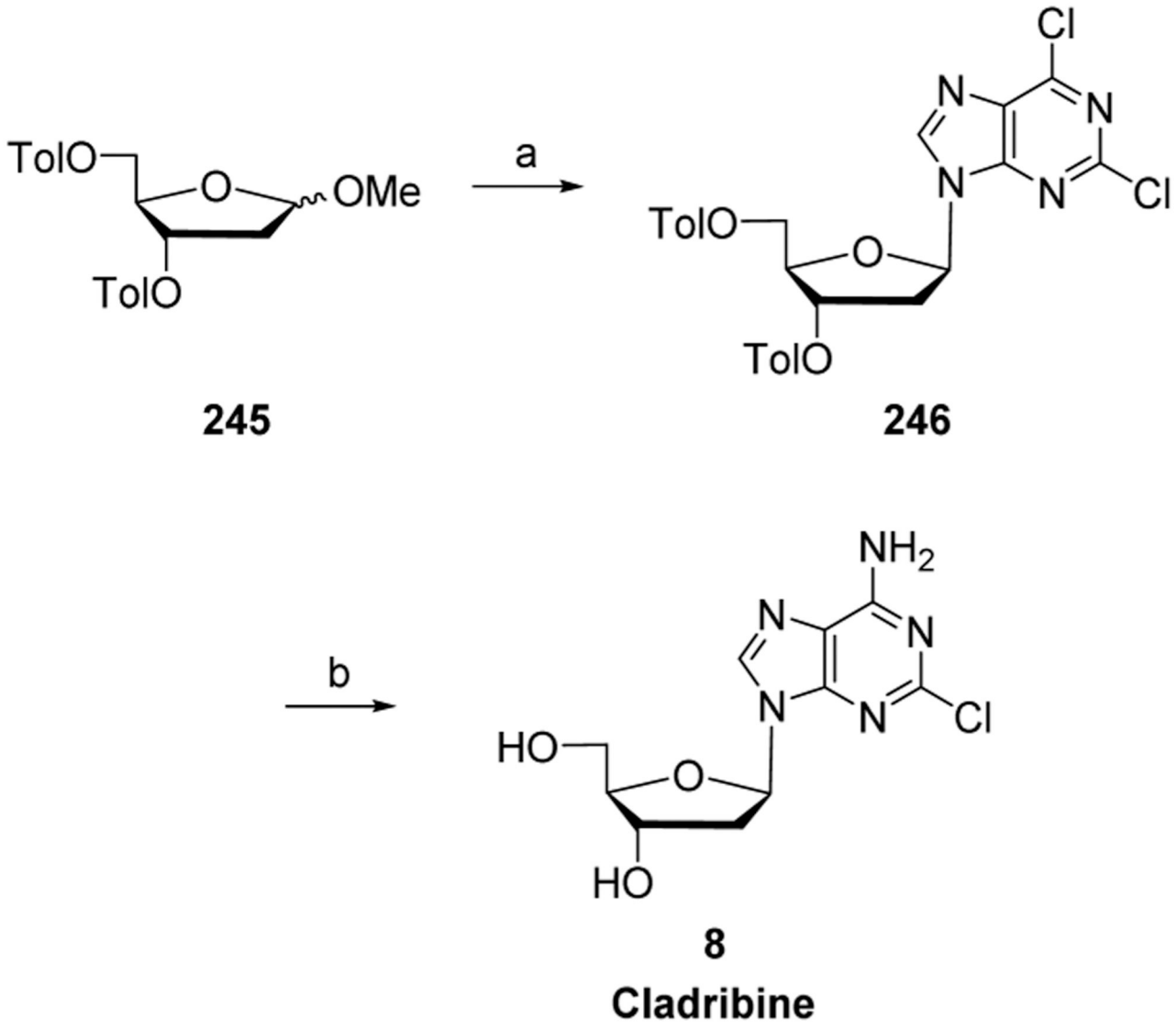
Scheme 42. Synthesis of Cordycepin 27[a]

[a]Reagents and conditions: (a) 2-acetoxyisobutyryl bromide, CH_3CN, H_2O, rt, 1 h; (b) Amberlite IRA-400 (OH^-) resin, MeOH, 92% (over two steps); (c) 1 M $LiEt_3BH$, THF, Me_2SO, 0 °C to rt, overnight, then 5% $HOAc/H_2O$, Et_3B, 98%.



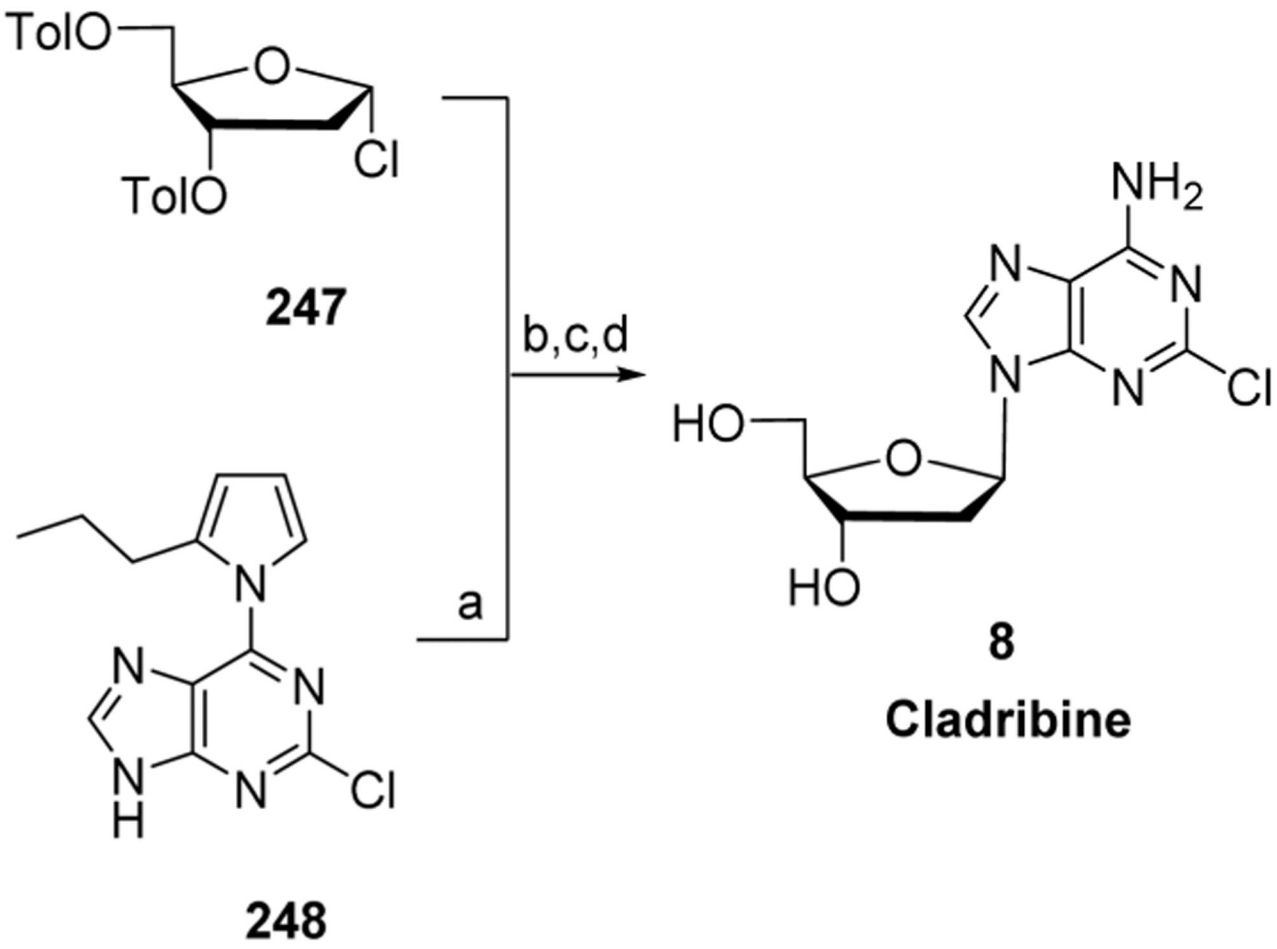
Scheme 43. Synthesis of Cordycepin 27[a]

[a]Reagents and conditions: (a) 2-acetoxyisobutyryl bromide, CH_3CN, EtOAc, <35 °C, 6 h, 52% for **243b**; (b) AcBr, MeOH, concd HCl, 20 °C, rt, 2–4 days, 70%; (c) AcONa, EtOH, H_2O then Pd/C, H_2 (50 psi), rt to 50 °C, 60%.



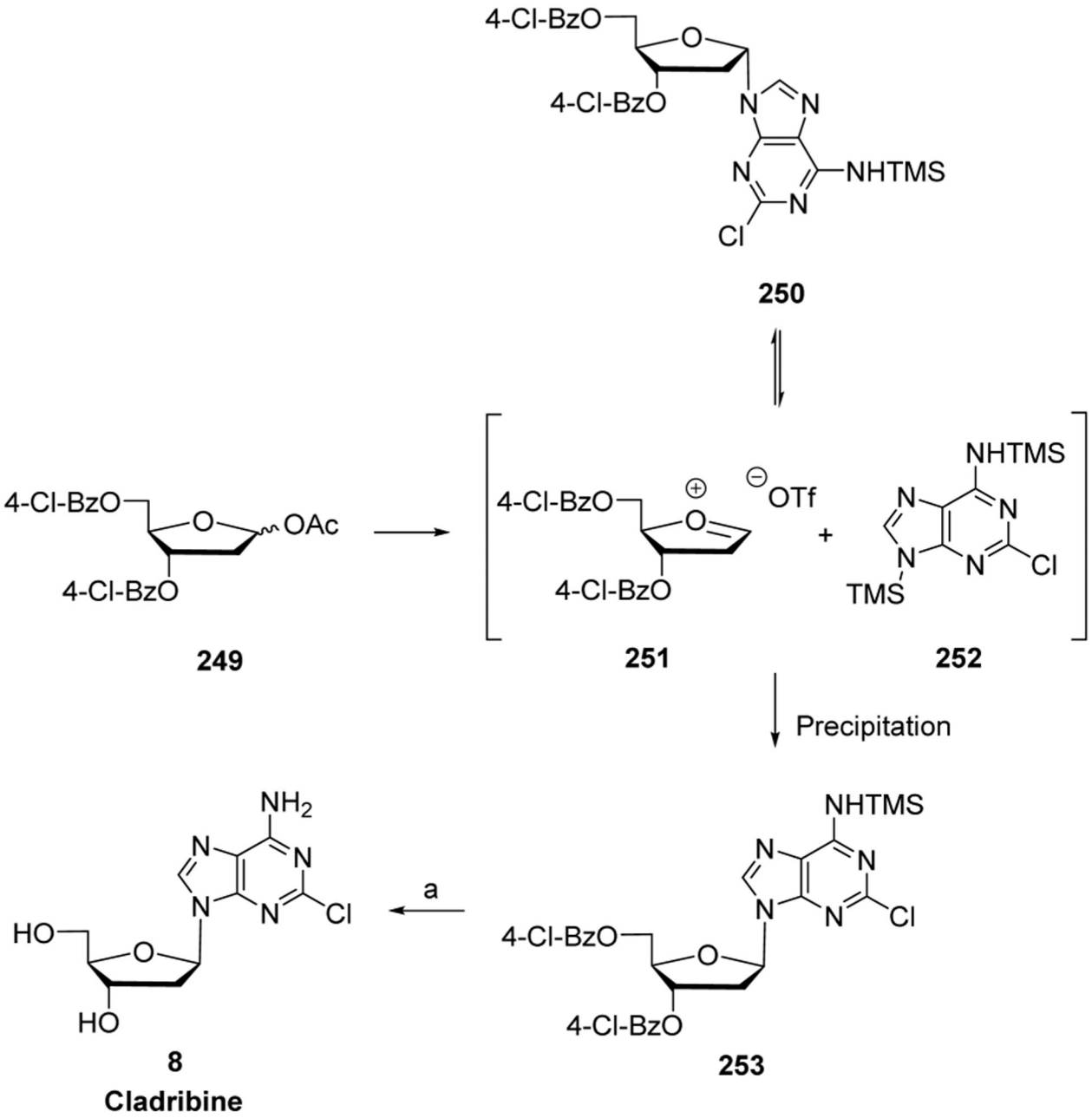
Scheme 44. Synthesis of Cladribine 8[a]

[a]Reagents and conditions: (a) 2,6-dichloropurine, melt, then dichloroacetic acid, and fusion under vacuum; (b) NH_3/NaOMe.



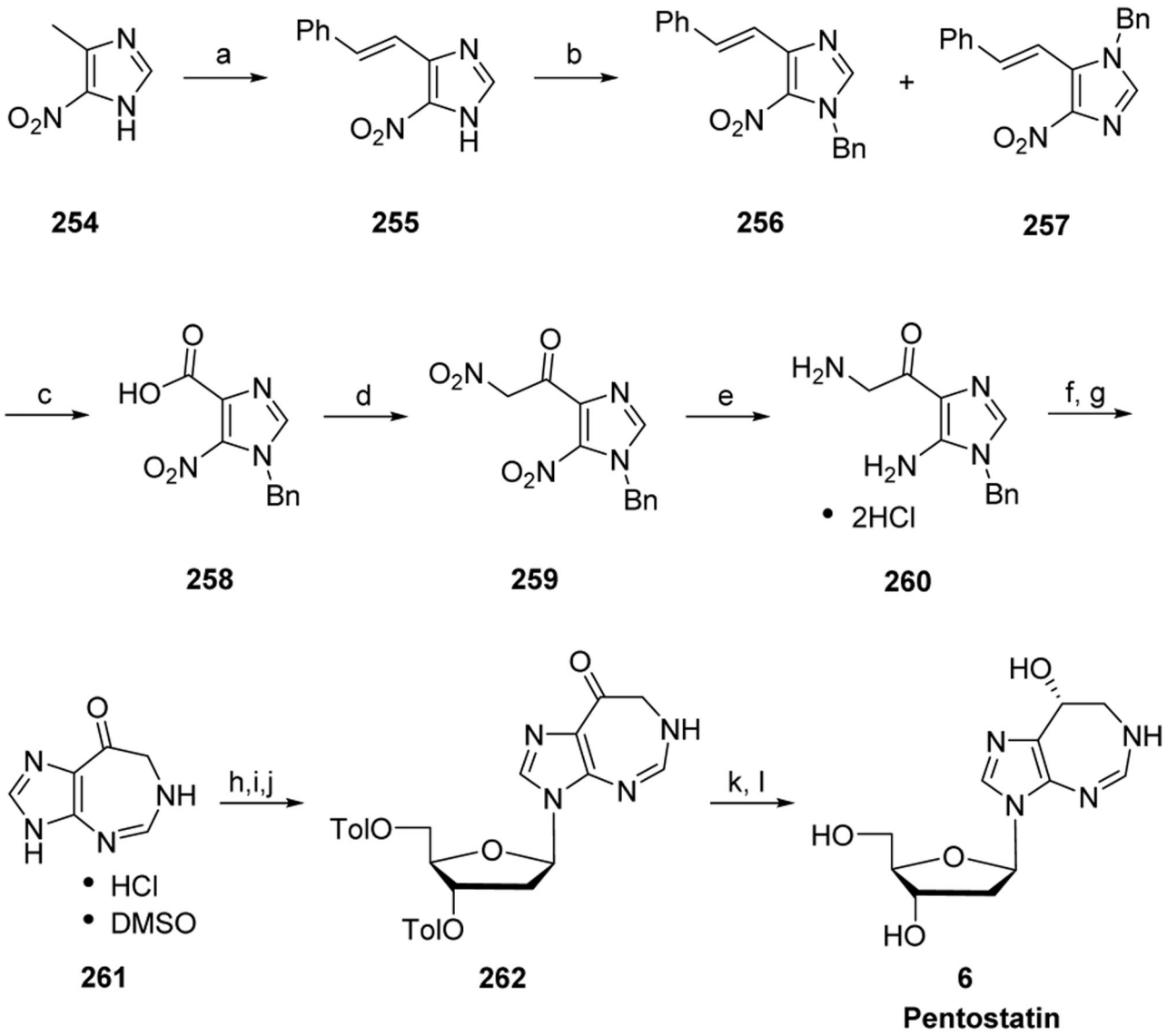
Scheme 45. Synthesis of Cladribine 8[a]

[a]Reagents and conditions: (a) NaH, CH_3CN, rt, 8 h; (b) then **247**, 0 °C to rt, 22 h, 100% (over two steps); (c) BnI, CH_3CN, 60 °C, 1.5 h; (d) NH_3/MeOH, 60 °C, 11 h, >90% (over two steps).



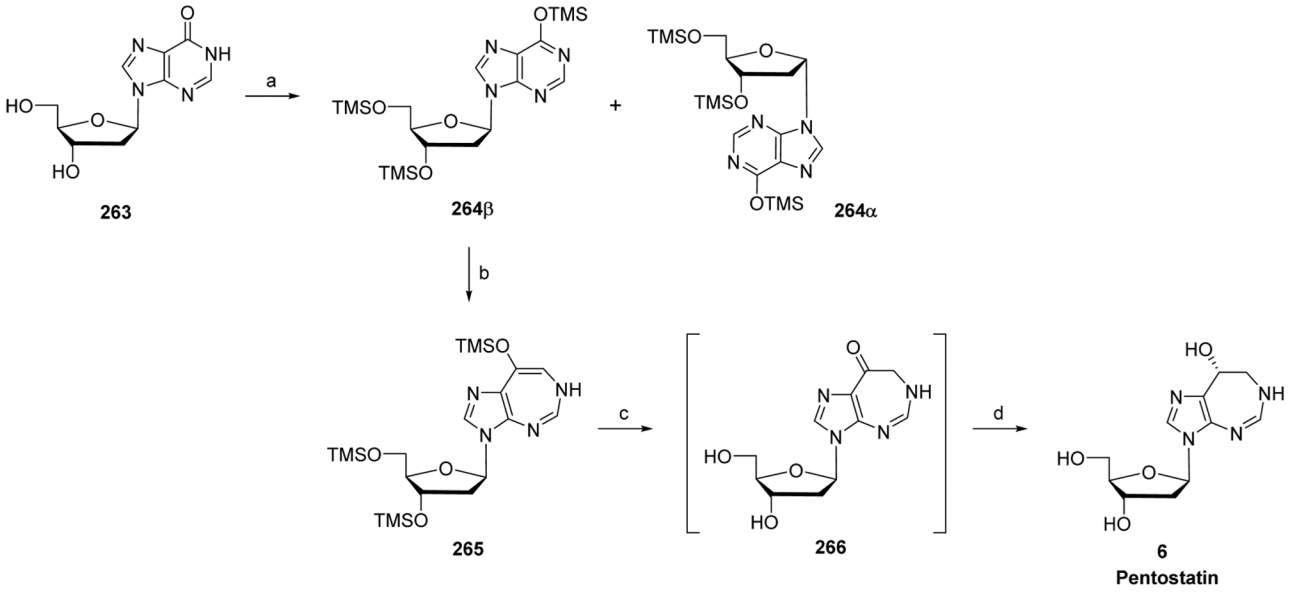
Scheme 46. Synthesis of Cladribine 8[a]

[a]Reagents and conditions: (a) 25% NaOMe/MeOH.



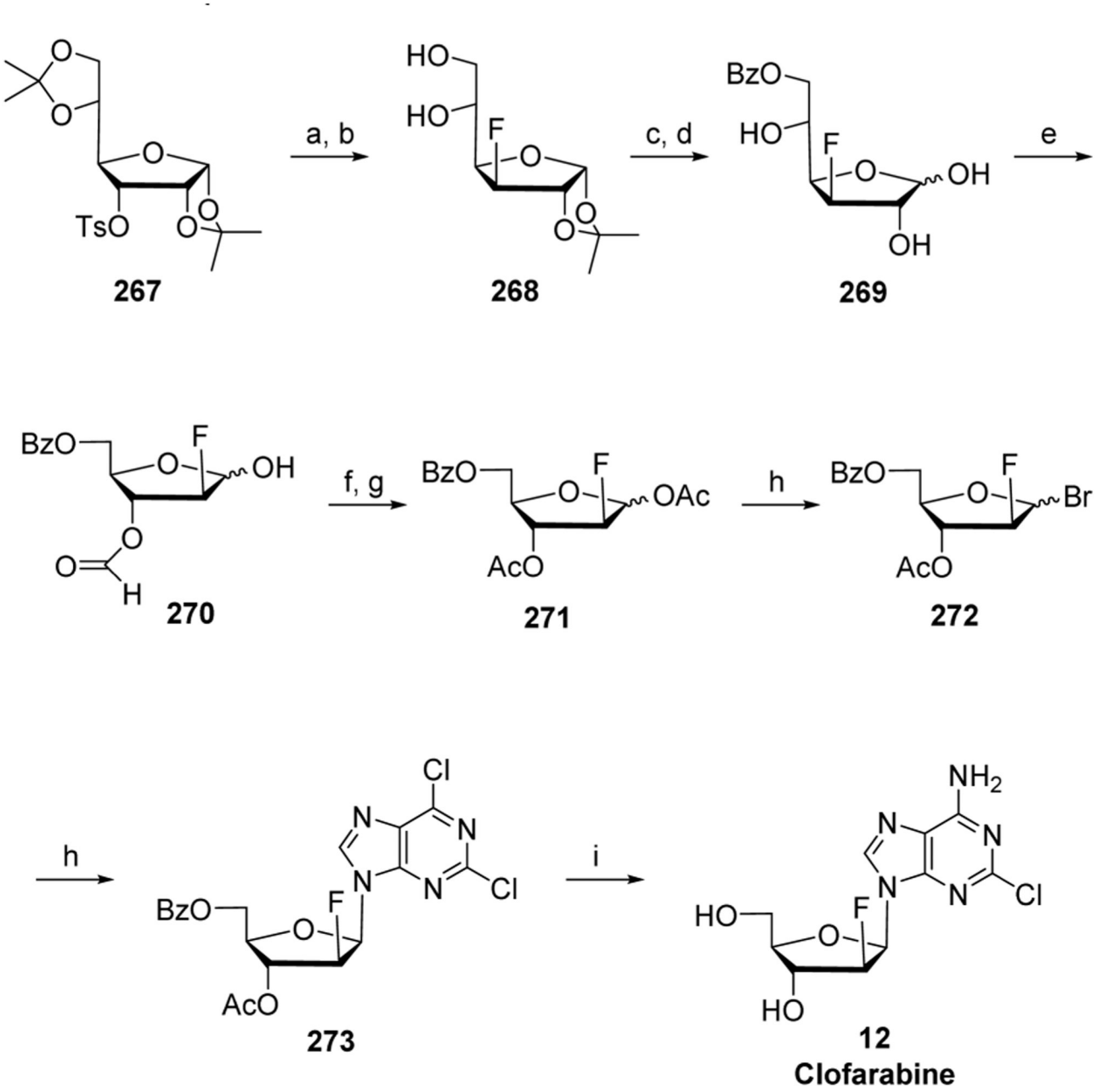
Scheme 47. Synthesis of Pentostatin 6[a]

[a]Reagents and conditions: (a) PhCHO, piperidine, 95 °C, 21 h, 70%; (b) BnCl, K_2CO_3, DMF, 75 °C, 6 h, 96%; (c) O_3, DCM, −78 °C, 8 h, then HCO_2H, H_2O_2, 0 °C to rt, overnight, 67%; (d) CDI, THF, reflux, 1 h, then *t*-BuOK, CH_3NO_2, THF, 0 °C, 45 min, 68%; (e) $SnCl_2$, concd HCl, 60 °C, 2.5 h, then H_2S, rt, 75%; (f) H_2, Pd/C, MeOH, H_2O, rt, 16 h, 96%; (g) $HC(OEt)_3$, Me_2SO, 65 °C, 15 min, 81%; (h) *N,N*-bis(trimethylsilyl)trifluoroacetamide, pyr, CH_3CN, rt, overnight; (i) 2-deoxy-3,4-di-*O*-*p*-toluoyl-D-pentofuranosyl chloride, $SnCl_4$, DCE, CH_3CN, −50 °C, 45 min; (j) $NaHCO_3$, H_2O, rt, 0.5 h, 23% (over three steps); (k) NaOMe, MeOH, rt, 1 h; (l) $NaBH_4$, H_2O, MeOH, 25 °C, 30 min, 26% (over two steps).



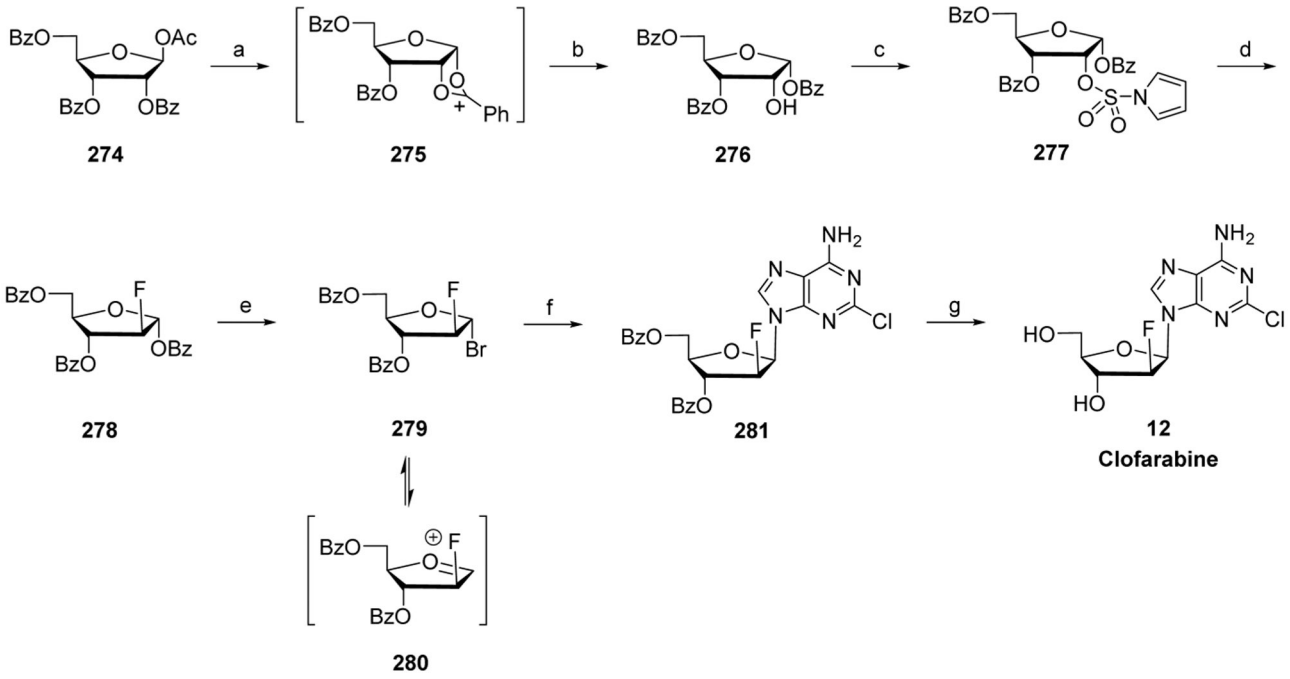
Scheme 48. Synthesis of Pentostatin 6[a]

[a]Reagents and conditions: (a) $(Me_3Si)_2NC(O)CF_3$, pyr, MeCN, 12 h; (b) $BF_3{\cdot}Et_2O$, DCM, 0 °C, 30 min; (c) TBAF, THF, 0 °C, 2 h; (d) $NaBH_4$, MeOH, H_2O, rt, 1 h.



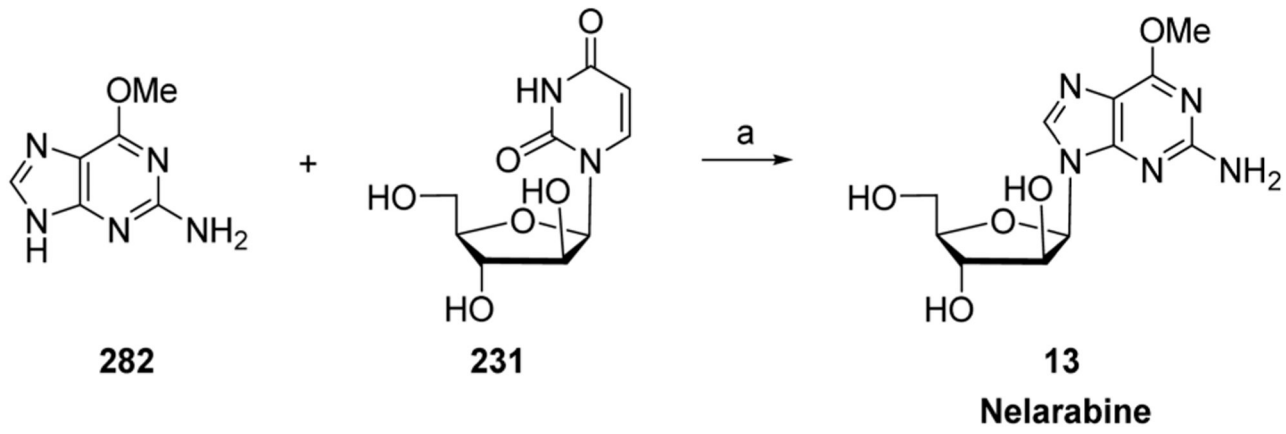
Scheme 49. Synthesis of Clofarabine 12[a]

[a]Reagents and conditions: (a) KF, acetamide, 210 °C, 62%; (b) MeOH–0.7% H_2SO_4 (1:1 v/v); (c) BzCl in DCM, pyr, −15 °C, 80% (over two steps); (d) Amberlite IR-120 (H^+), H_2O/dioxane, 80 °C, 78%; (e) KIO_4, H_2O; (f) NaOMe, MeOH; (g) Ac_2O, pyr, 80% (over three steps); (h) HBr/HOAc, DCM; (i) 2,6-dichloropurine, DCE, 100 °C, 32% (β-anomer); then NH_3/EtOH, steel bomb, 3 days, solvent switch to MeCN/H_2O, LiOH, 42%.



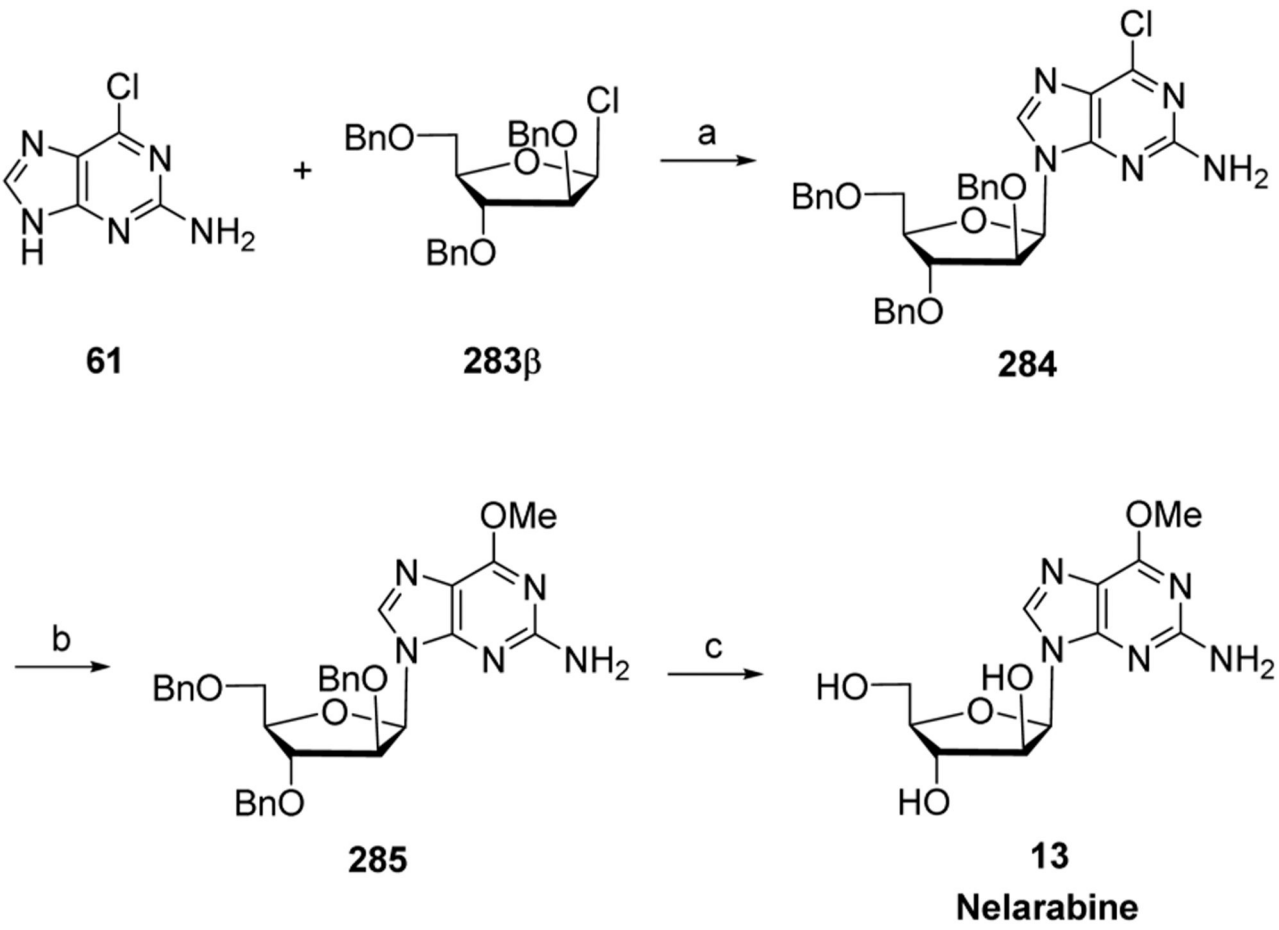
Scheme 50. Synthesis of Clofarabine 12[a]

[a]Reagents and conditions: (a) 0.5 M HBr, DCM, 0 °C; (b) H_2O, 81% (over two steps); (c) NaH, DMF, 0 °C, 0.5 h, then *N,N*′-sulfuryldiimidazole, −40 °C to rt, 2 h, 85%; (d) KHF_2, 2,3-butanediol, HF (50% in H_2O), 160 °C, 1 h, 63%; (e) HBr/HOAc, DCM, rt, 16 h, 98%; (f) 2-chloroadenine, CH_3CN, *t*-amyl alcohol, *tert*-BuOK, CaH_2, 50 °C, 40 min, then **279**, DCE, 50 °C, 19 h, 50% (β/α = 80:1); (g) NaOMe, MeOH, 33 °C, 7 h, 64%.



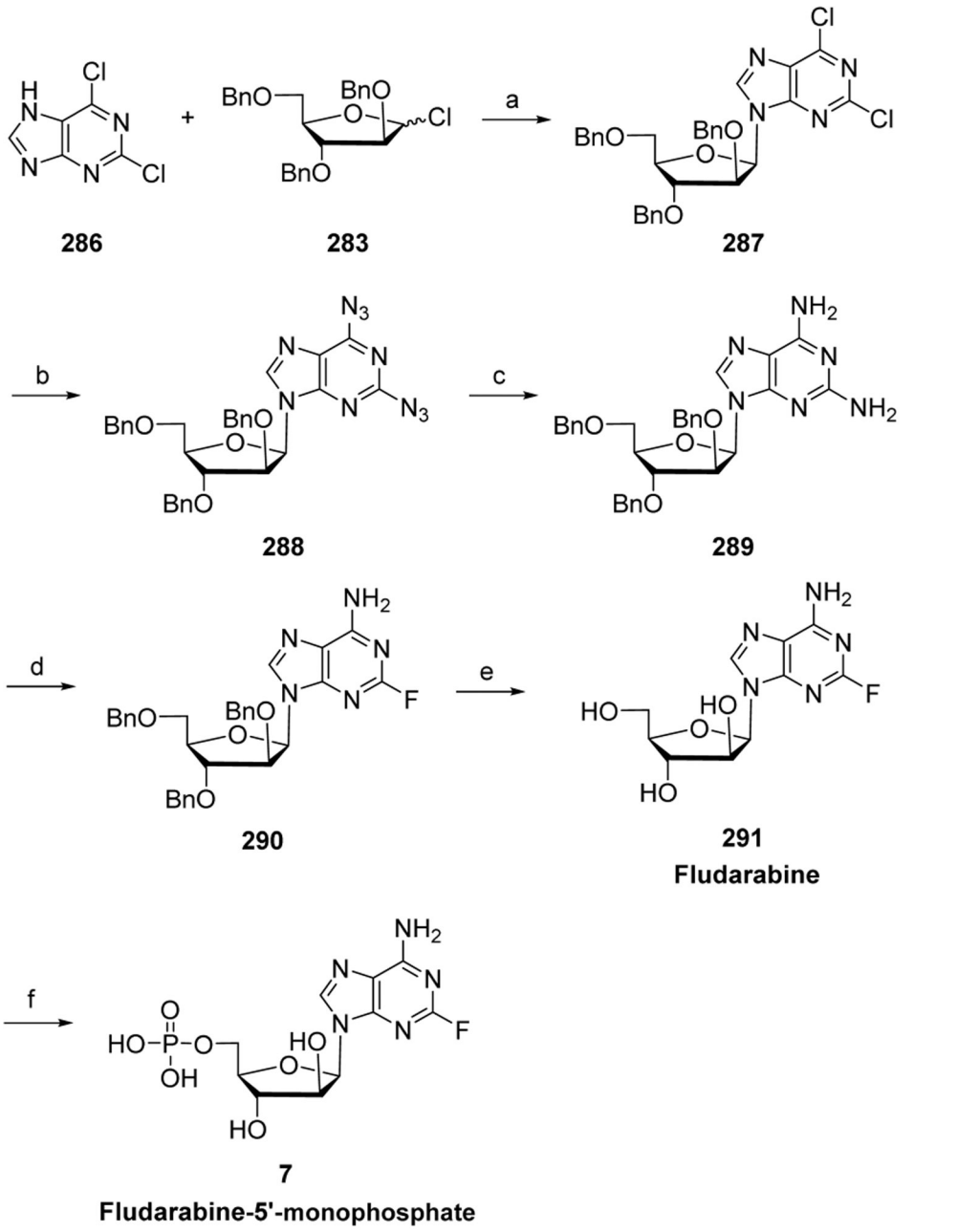
Scheme 51. Synthesis of Nelarabine 13[a]

[a]Reagents and conditions: (a) 10 mM K_3PO_4, *n*-PrOH, H_2O, pH 6.75, uridine phosphorylase, purine nucleoside phosphorylase, 37 °C, 26 days, 17%.



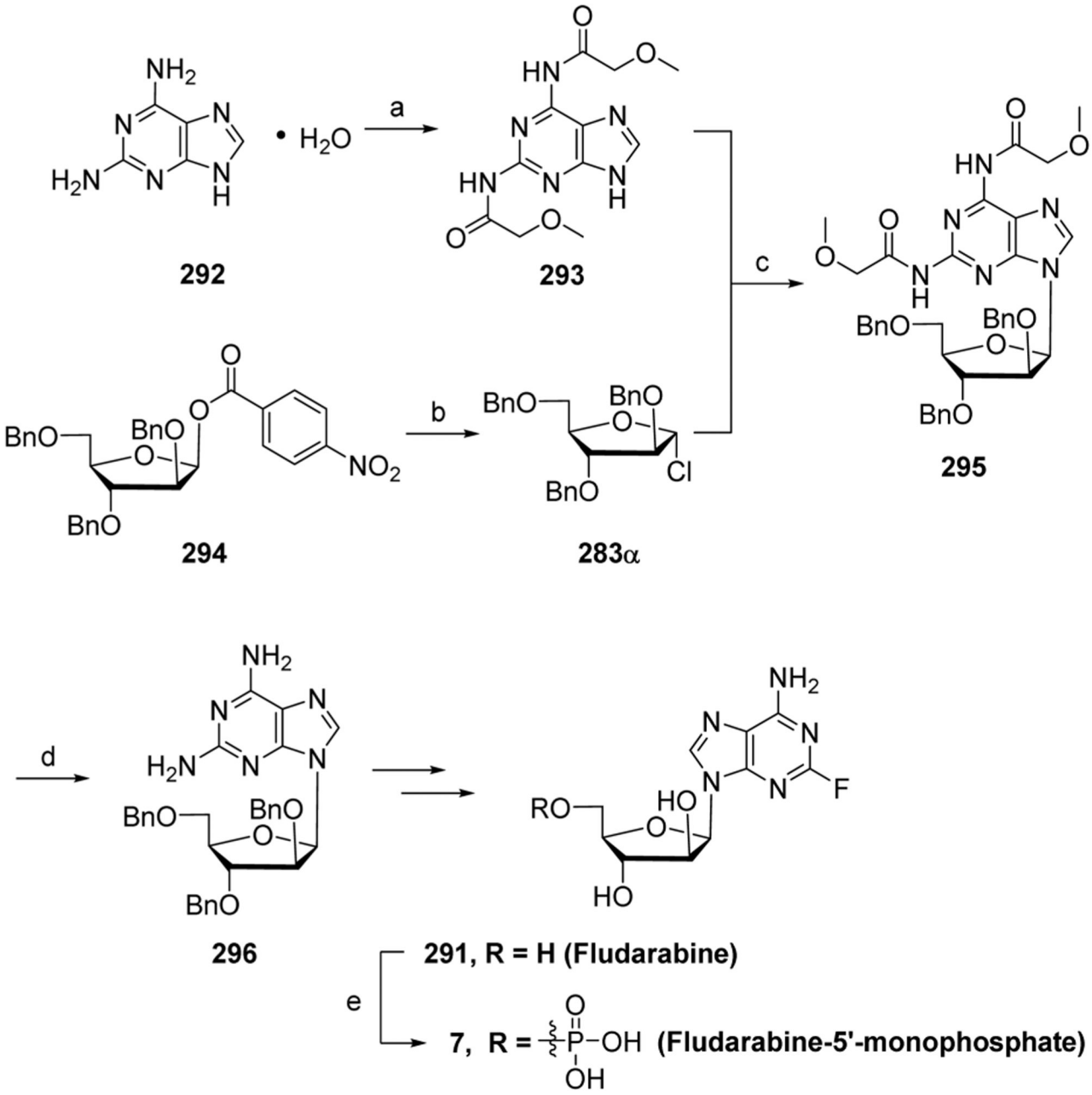
Scheme 52. Synthesis of Nelarabine 13[a]

[a]Reagents and conditions: (a) NaH, MeCN; (b) MeONa, MeOH; (c) Pd/C, $HCOONH_4$, MeOH, reflux, 90 min, 91%.



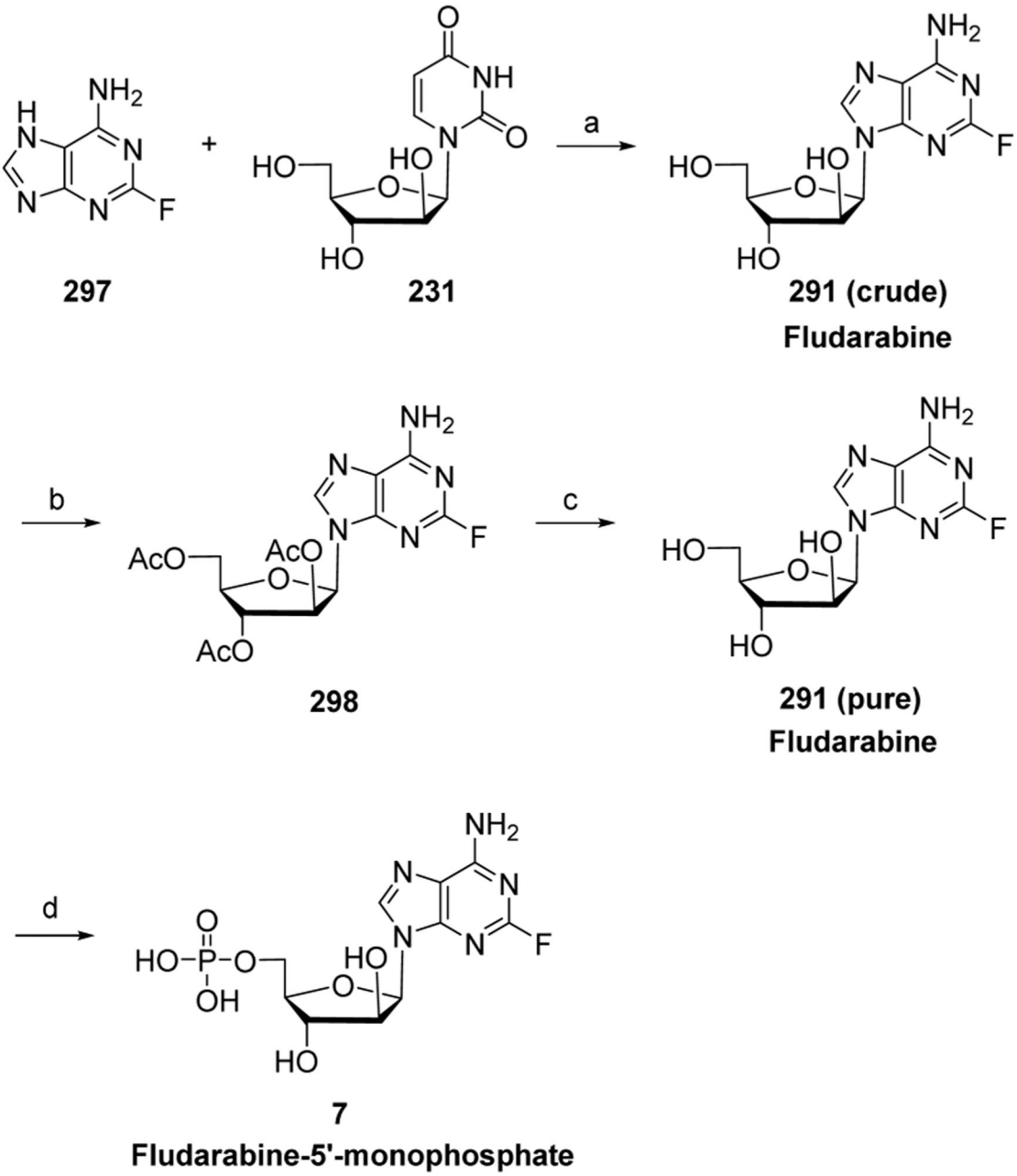
Scheme 53. Synthesis of Fludarabine 291 and Fludarabine-5′-monophosphate 7[a]

[a]Reagents and conditions: (a) $Hg(CN)_2$, $CaSO_4$, $MeNO_2$, reflux, 3 h, 11%; (b) NaN_3, EtOH, H_2O, 1 h, 98%; (c) Pd/C, H_2, EtOH, rt, 6 h, 75%; (d) HBF_4, $NaNO_2$, $CHCl_3$, −10 °C, 40 min, 36%; (e) Na, NH_3, 34%; (f) $POCl_3$, $PO(OEt)_3$, 0 °C, 3.5 h, 96%.



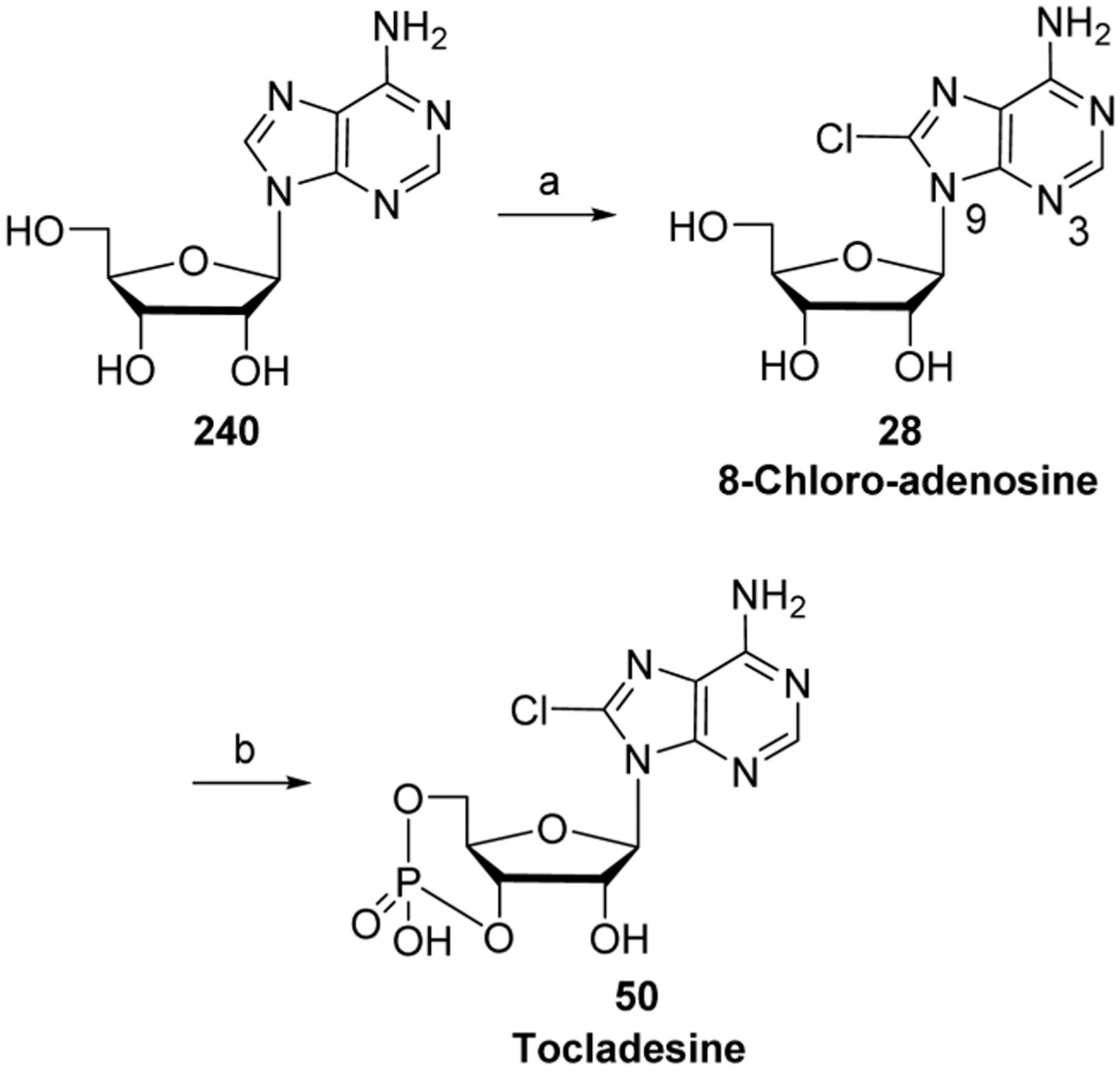
Scheme 54. Synthesis of Fludarabine 291 and Fludarabine-5′-monophosphate 7[a]

[a]Reagents and conditions: (a) methoxyacetic anhydride, pyr, 88 °C, 1 h, then methyl ethyl ketone, overnight, 90%; (b) HCl (g), DCM, 4–6 °C, 2 h, quantitative; (c) DIPEA, DCE, reflux, overnight, quantitative; (d) NaOMe, MeOH, 68%; (e) $POCl_3$, TMP, 0 °C, 18 h, 56%.



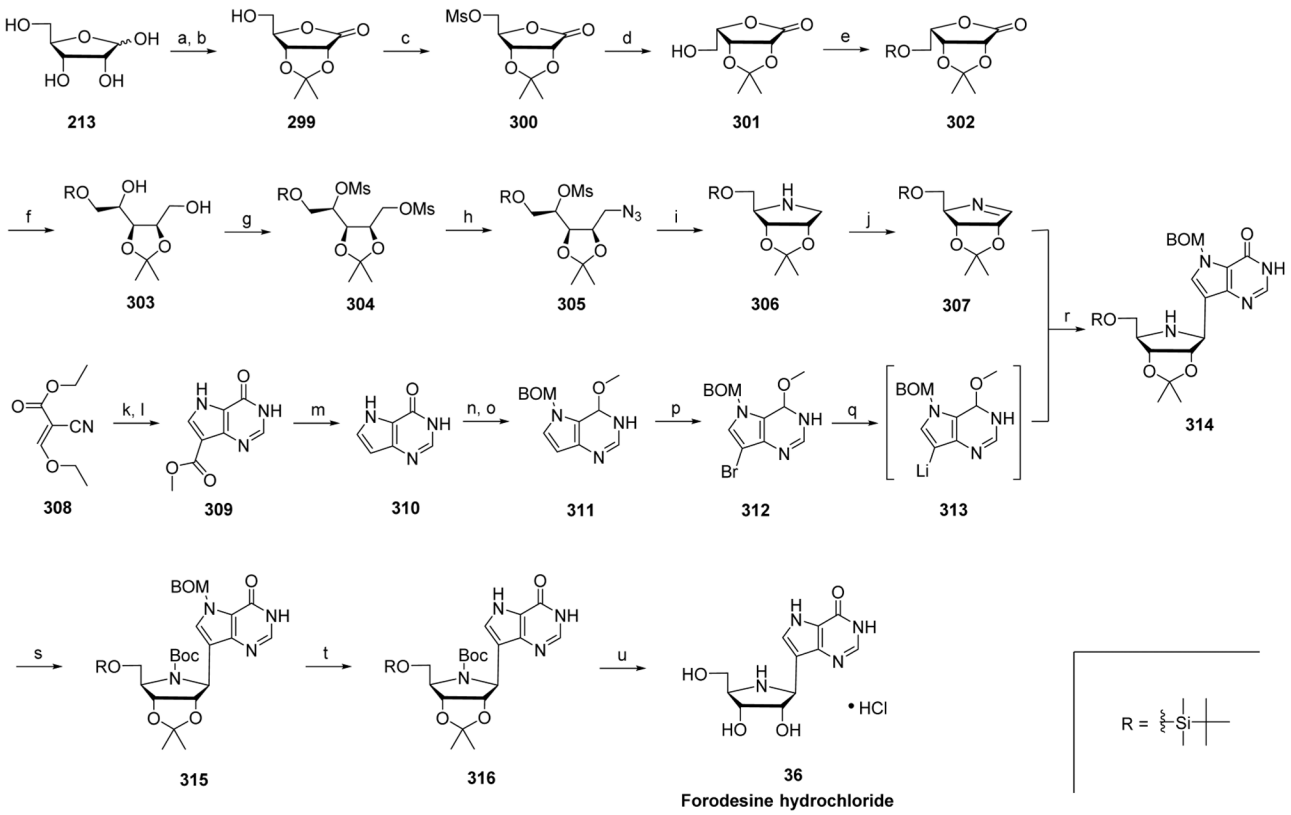
Scheme 55. Synthesis of Fludarabine 291 and Fludarabine-5′-monophosphate 7[a]

[a]Reagents and conditions: (a) *Enterobacter aerogenes*, K_2HPO_4, H_2O, 60 °C, 24 h, 35%; (b) Ac_2O, 95 °C, 9 h, 87%; (c) NH_4OH, H_2O, MeOH, rt, 19 h, 74%.



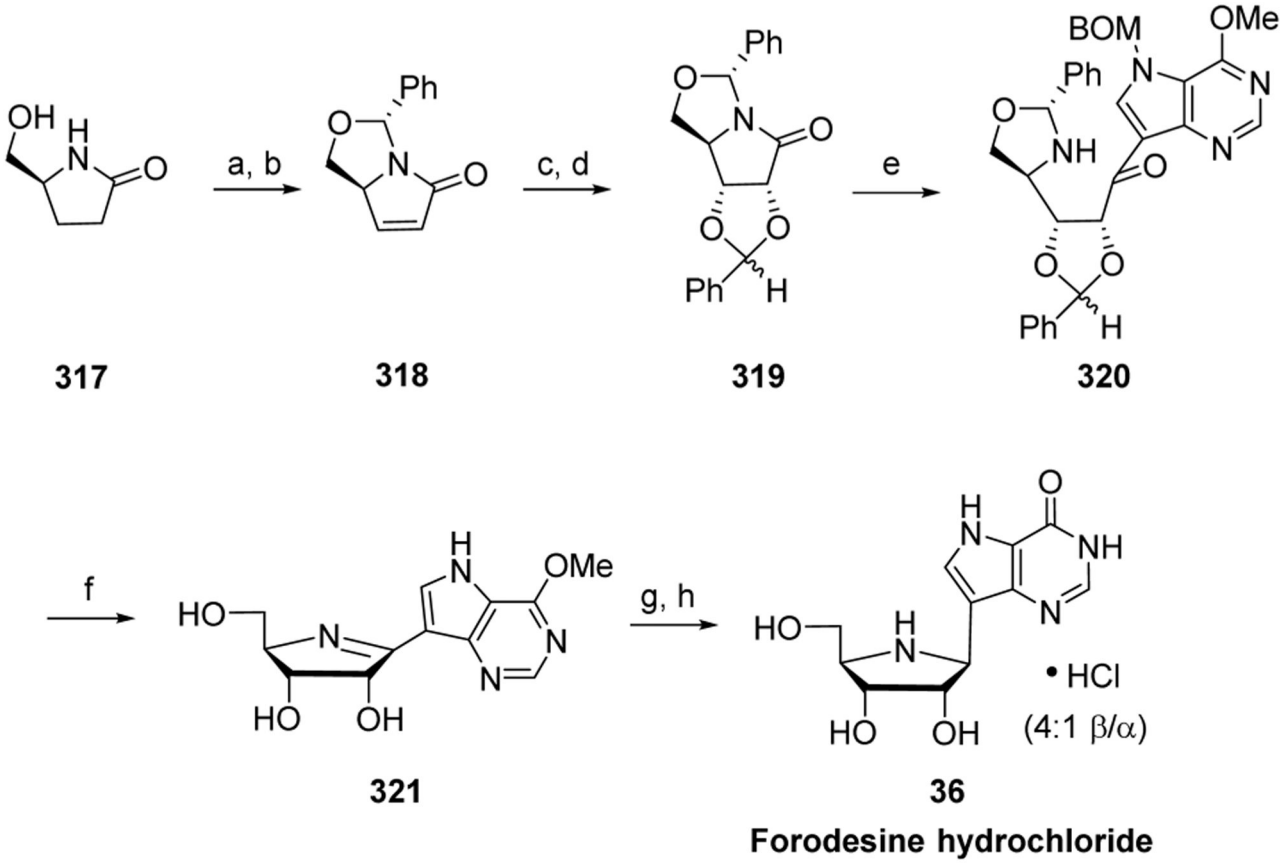
Scheme 56. Synthesis of 8-Chloroadenosine 28 and Tocladesine 50[a]

[a]Reagents and conditions: (a) AcCl, *m*-CPBA, DMF, rt, 20 min, 40%; (b) $POCl_3$, $PO(OEt)_3$, 0 °C, 6 h, then aq NaOH, 2 h, 23%.



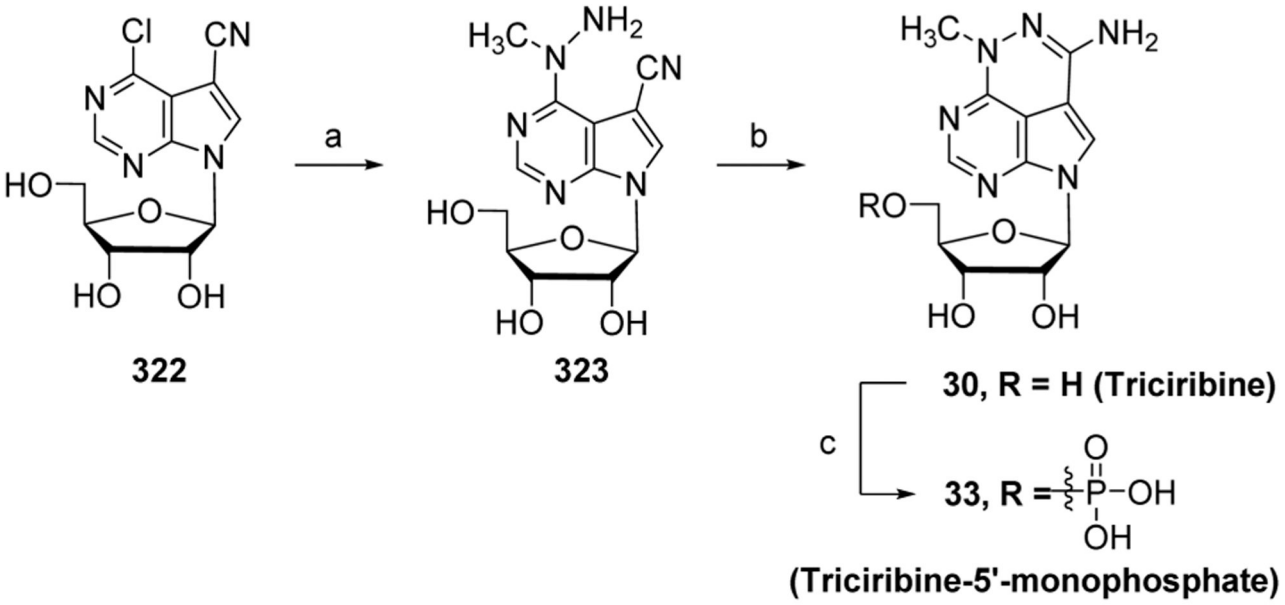
Scheme 57. Synthesis of Forodesine Hydrochloride 36[a]

[a]Reagents and conditions: (a) Br_2, K_2CO_3, H_2O, 5 °C to rt, overnight; (b) concd H_2SO_4, Me_2CO, reflux, 4 h, then 30 °C, 8 h, 55% (over two steps); (c) MsCl, Et_3N, DCM, −20 °C, 1 h, then rt, 8 h; (d) aq KOH, <30 °C, 4 h, 59% (over two steps); (e) TBDMSCl, imidazole, DMF, 20 °C, 4 h, 91%; (f) $LiBH_4$, THF, −30 °C to rt, 24 h, 75%; (g) $(MeSO_2)_2O$, pyr, 0 °C to rt, 18 h, 88%; (h) NaN_3, DMF, 100 °C, 2 h, 42%; (i) H_2, Pd, NaOAc, dioxane, 20 °C, 72 h, 94%; (j) NCS, pentane, rt, 1 h, then lithium tetramethylpiperidide (LiTMP), THF, −78 °C, 85%; (k) $H_2NCH(CO_2C_2H_5)_2$, NaOMe, MeOH, reflux, 4 h, 68%; (l) formamidine acetate, C_2H_5OH, reflux, 27 h, 61%; (m) aq KOH, reflux, 40 h, 74%; (n) $POCl_3$, reflux, 2 h; (o) BOMCl, NaH, THF, 0 °C to rt, 1 h, then NaH, MeOH, 1 h; (p) NBS, DCM, 52% (over three steps); (q) *n*-BuLi, −78 °C; (r) 307, −78 °C to 0 °C; (s) $(Boc)_2O$, DCM, rt, 1 h, 76% (over three steps); (t) H_2, Pd/C, 18 h, then NH_4OH, H_2O, 1 h; (u) concd HCl, MeOH, reflux, 1.5 h, 85% (over two steps).



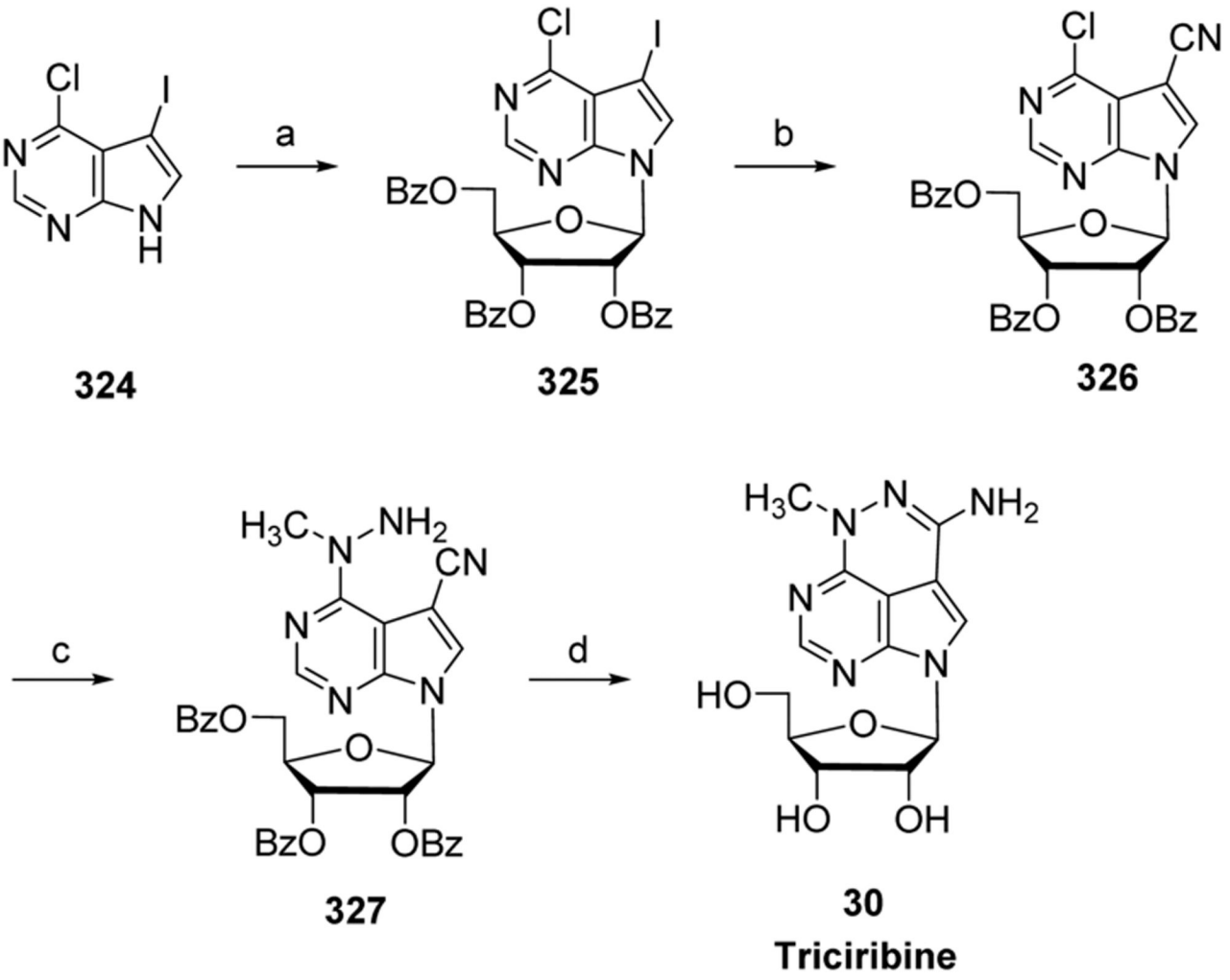
Scheme 58. Synthesis of Forodesine Hydrochloride 36[a]

[a]Reagents and conditions: (a) PhCHO, TsOH; (b) $PhSO_2Me$, 59%; (c) OsO_4, Me CO; (d) DMP/H^+ 2, 63%; (e) lithiated 9-deazahypoxanthine, anisole, Et_2O, −20 °C, 55%; (f) BBr_3, DCM; (g) $BH_3{\cdot}Me_2S$, DMF; (h) $MeOH/H^+$, 90%.



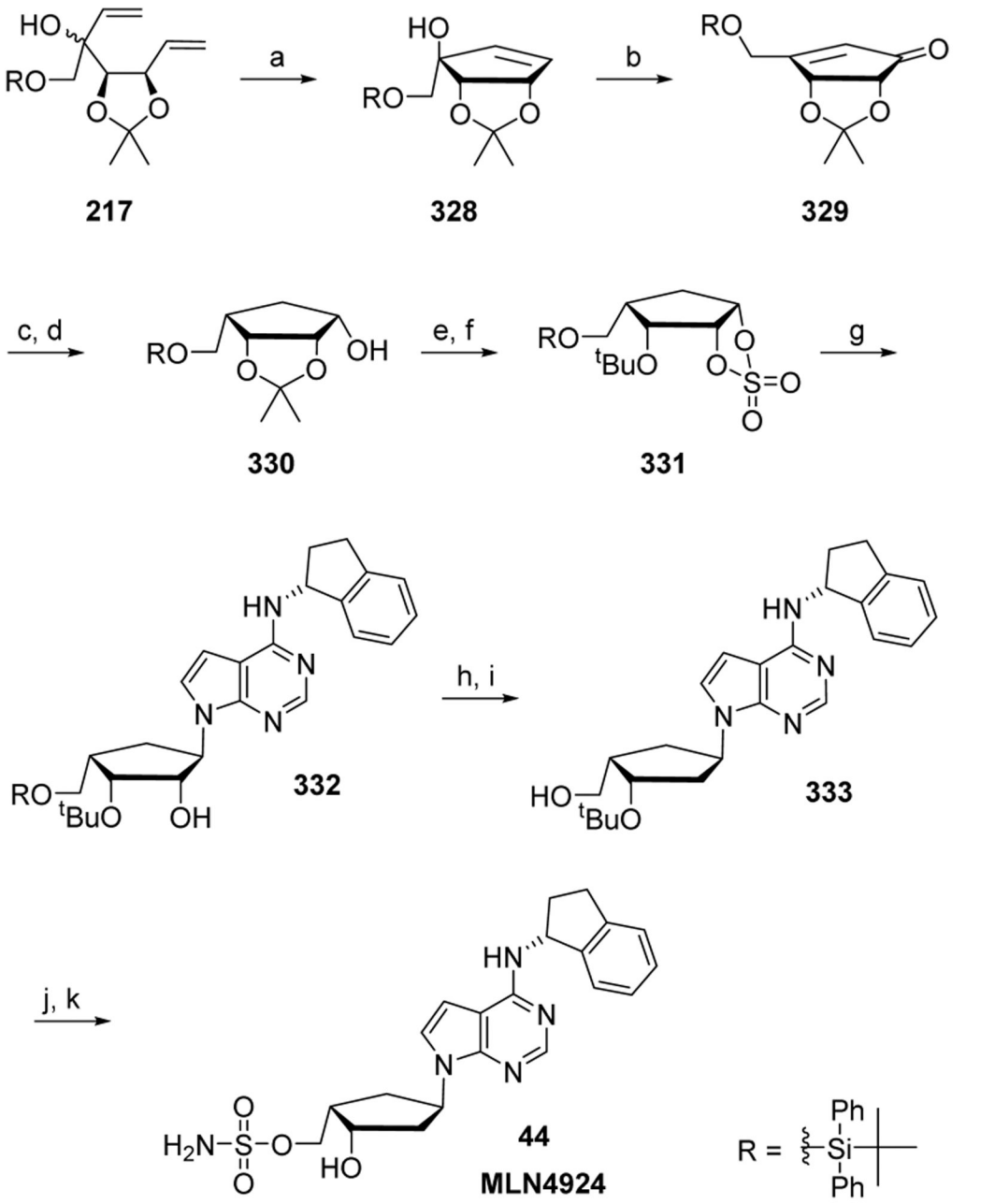
Scheme 59. Early Synthesis of Triciribine 30 and Triciribine-5′-monophosphate 33[a]

[a]Reagents and conditions: (a) CH_3NHNH_2, EtOH, rt, 3 min; (b) H_2O, reflux, 16 h; (c) $POCl_3$, $PO(OMe)_3$, 0 °C, 16 h, 21%;



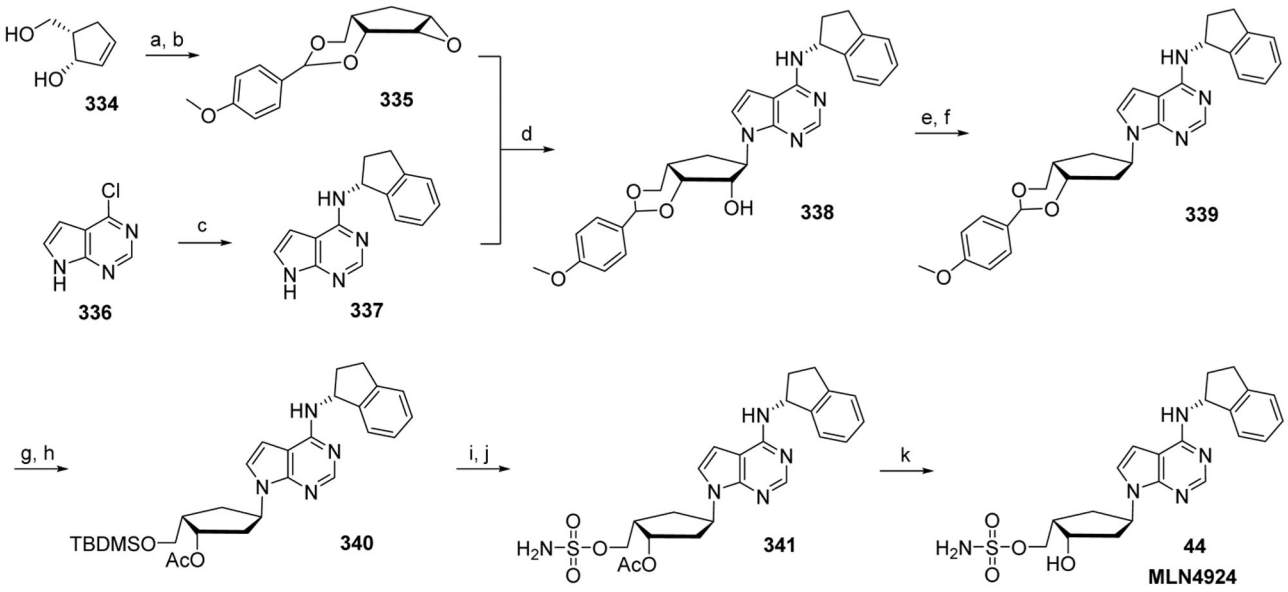
Scheme 60. Synthesis of Triciribine 30[a]

[a]Reagents and conditions: (a) BSA, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, TMSOTf, CH_3CN, 80 °C, 80%; (b) Bu_3SnCN, $Pd(PPh_3)_4$, DMF, 95 °C, 6 h, 72%; (c) H_2NNHCH_3, C_2H_5OH, $CHCl_3$, rt, 3.5 h, 92%; (d) NaOMe, MeOH, rt, 1 h, then reflux, 18 h, 74%.



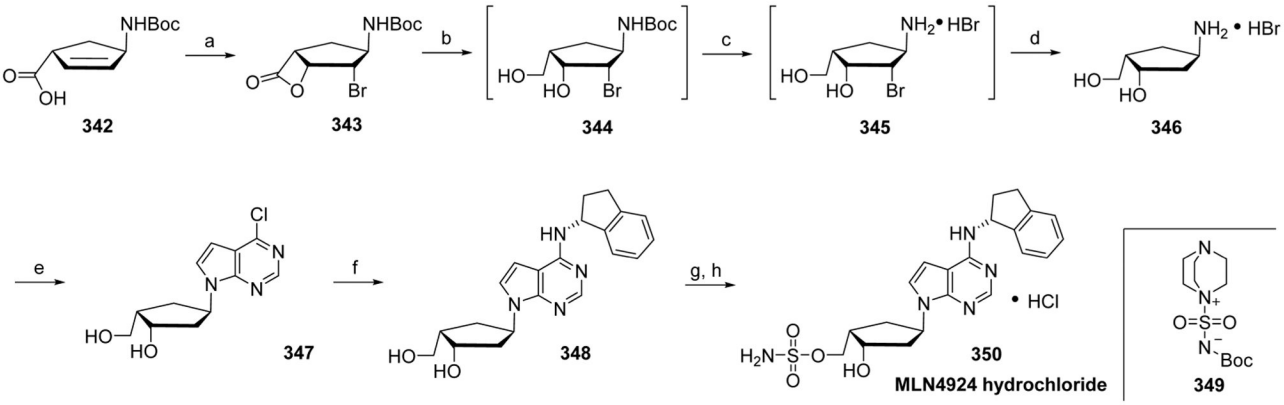
Scheme 61. Synthesis of MLN4924 44[a]

[a]Reagents and conditions: (a) Grubbs' catalyst (2nd generation), DCM, rt, 2 days, 95%; (b) PDC, DMF, rt, 2 days, 84%; (c) H_2, Pd/C, MeOH, rt, overnight, 100%; (d) $NaBH_4$, $CeCl_3{\cdot}H_2O$, MeOH, 0 °C to rt, 0.5 h, 98%; (e) Me_3Al, DCM, 0 °C to rt, 2 days, 62%; (f) $SOCl_2$, Et_3N, 0 °C, 10 min, then $RuCl_3{\cdot}3H_2O$, $NaIO_4$, CCl_4, CH_3CN, H_2O, 87%; (g) N^6-indanyl-7-deazaadenine, NaH, 18-crown-6, THF, 80 °C, overnight, then concd HCl, 80 °C, 2 h, 65%; (h) PhOC(S)Cl, DMAP, DCM, rt, overnight, then Bu_3SnH, AIBN, PhMe, 110 °C, 1 h, 82%; (i) HF-pyridine, THF, pyr, rt, 1 h, 99%; (j) NH_2SO_2Cl, Et_3N, CH_3CN, 0 °C to rt, 1 h, 92%; (k) TFA, rt, 2 h, 90%.



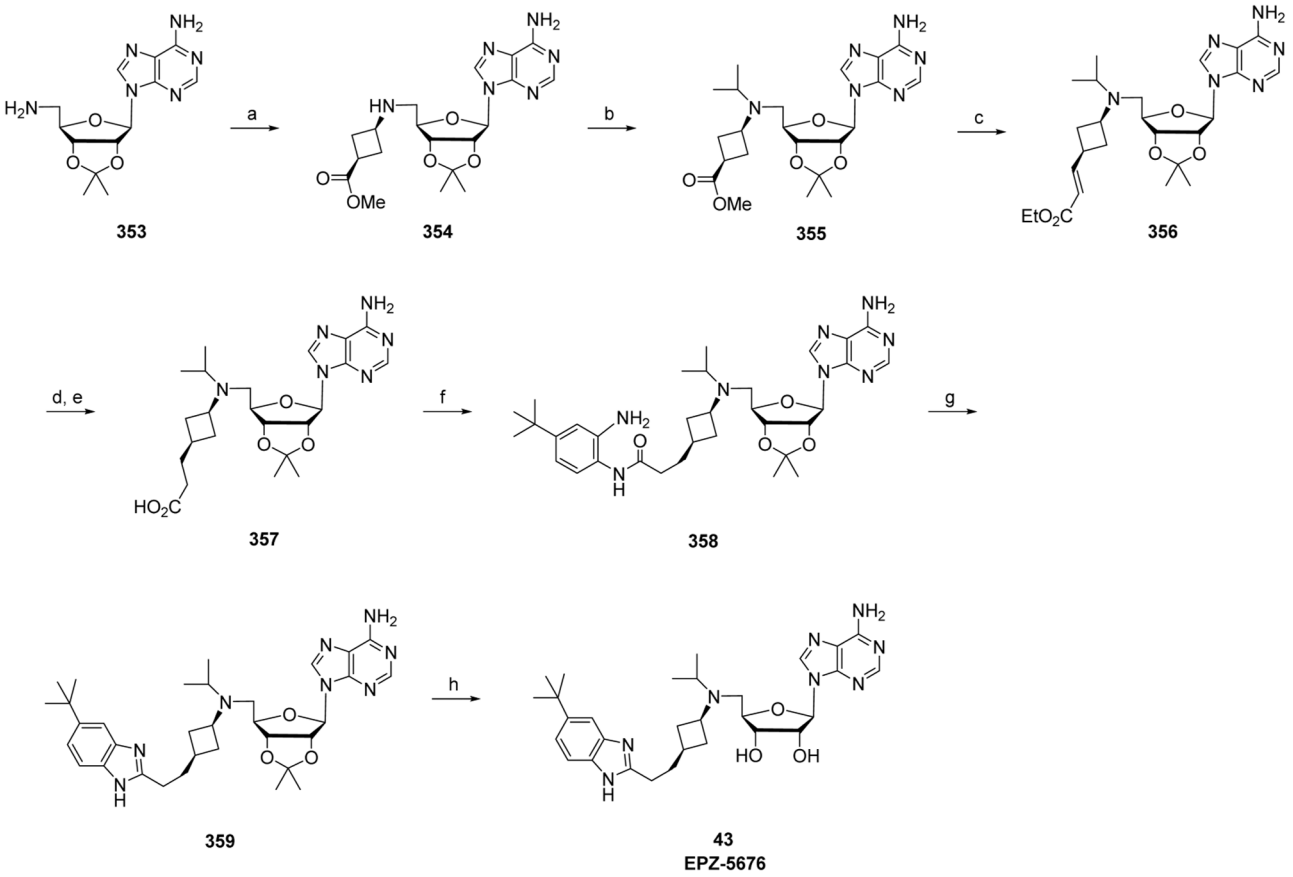
Scheme 62. Synthesis of MLN4924 44[a]

[a]Reagents and conditions: (a) *m*-CPBA, DCM, 4 h, 76%; (b) 1-(dimethoxymethyl)-4-methoxybenzene, PPTS, rt, overnight, 78%; (c) (*S*)-(+)-1-aminoindan, DIPEA, 1-butanol, reflux, 60 h, 80%; (d) NaH, DMF, 110 °C, 2 h, 69%; (e) DMAP, PhOC(S)Cl, DCM, rt, 1 h, 99%; (f) AIBN, Bu_3SnH, PhMe, reflux, 30 min, 79%; (g) AcOH, THF, H_2O, rt, 60 h, 98%; (h) 1*H*-imidazole, DMAP, TBDMSCl, DMF, rt, 2 h, then DMAP, Ac_2O, pyr, rt, overnight, 86%; (i) HF-pyridine, THF, pyr, rt, 1 h, 80%; (j) chlorosulfonamide, Et_3N, MeCN, rt, 1 h, 94%; (k) NH_3/MeOH, rt, 5 days, 90%.



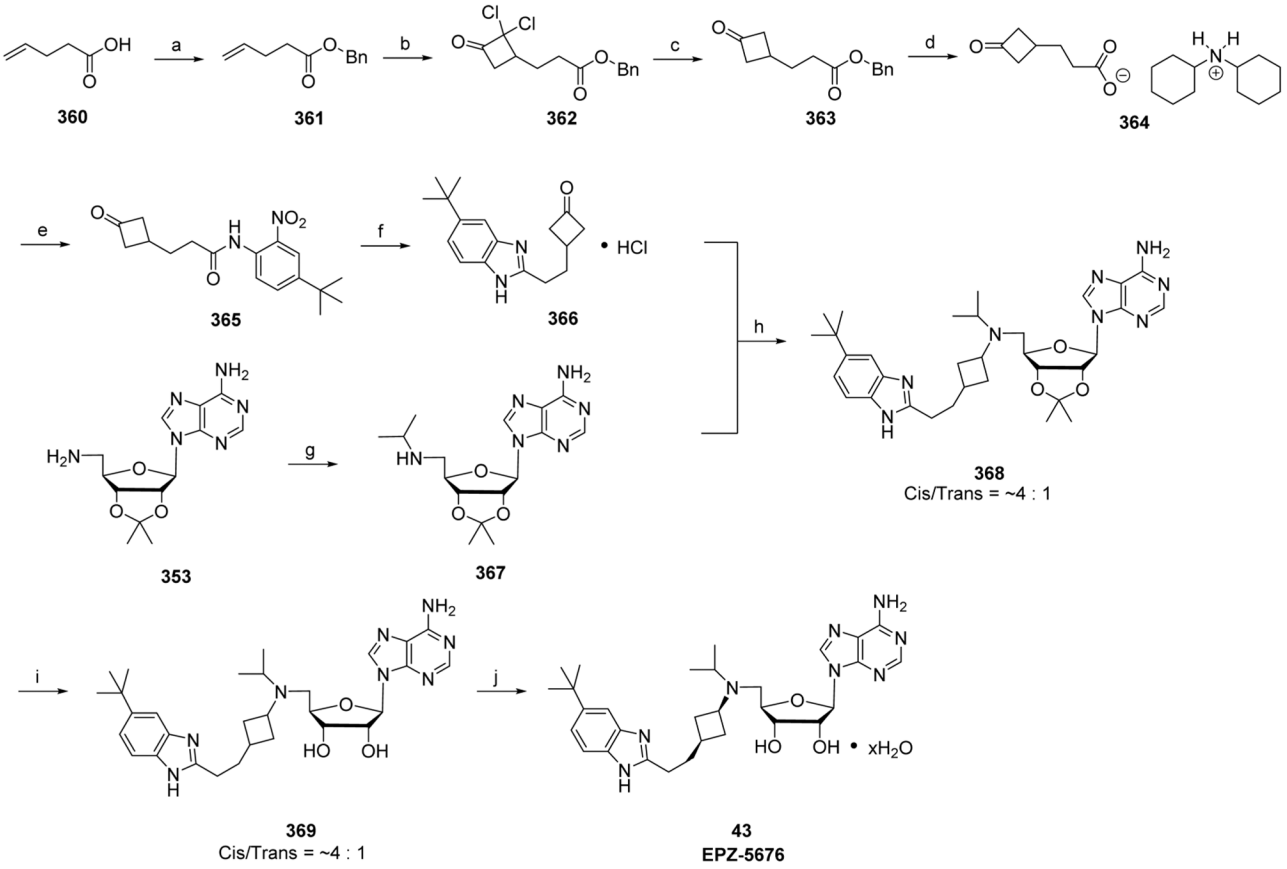
Scheme 63. Synthesis of MLN4924 Hydrochloride 350[a]

[a]Reagents and conditions: (a) Br_2, py, DME, H_2O, 0 °C, 68%; (b) $LiBH_4$, THF, H_2O, −5 °C; (c) 8.84 M HBr, *i*-PrOH, 55 °C; (d) H_2, Pd/C, DIPEA, MeOH, 25 °C, 80% (over three steps); (e) 2-(4,6-dichloropyrimidin-5-yl)acetaldehyde, Et_3N, *i*-PrOH, 75 °C, 78%; (f) (*S*)-(+)-1-aminoindane hydrochloride, DIPEA, 2-butanol, 130 °C, 81%; (g) 349, MeCN, 53 °C, 5 h, then HCl, 59%; (h) 1.25 M HCl, EtOH, crystallization, 93%.



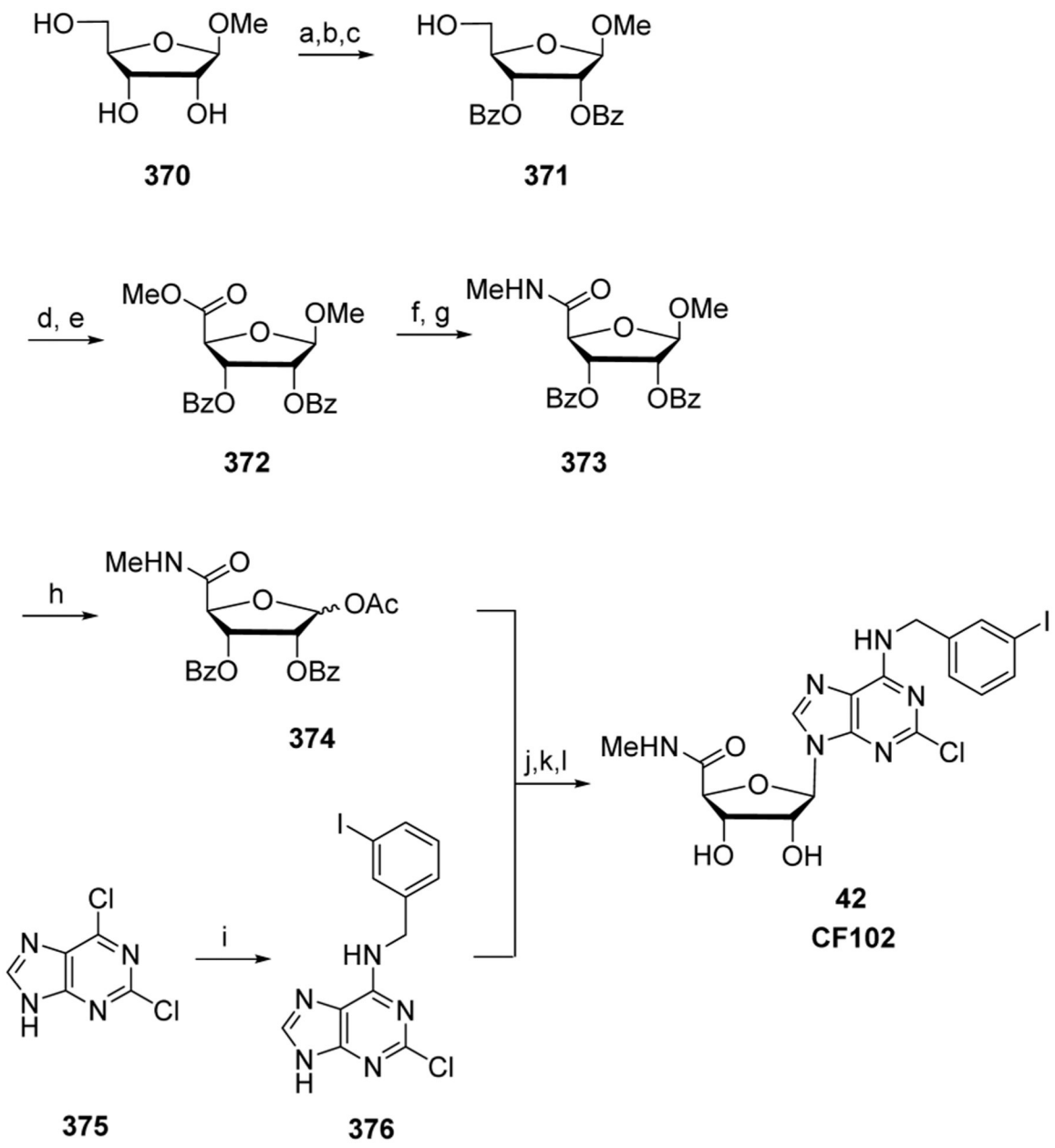
Scheme 64. Synthesis of EPZ-5676 43[a]

[a]Reagents and conditions: (a) methyl 3-oxocyclobutanecarboxylate, Ti(*O*-*i*Pr)$_4$, MeOH, 45 °C, 2 h, then NaCNBH$_4$, rt, overnight, 41%; (b) 2-iodopropane, K$_2$CO$_3$, CH$_3$CN, 95 °C, overnight, 86%; (c) DIBAL-H, DCM, −78 °C to rt, then ethyl 2-(diethoxyphosphoryl)acetate, DBU, LiCl, rt, 1 h, 83%; (d) Pd/C, MeOH, rt, overnight, 78%; (e) LiOH·H$_2$O, THF, MeOH, rt, overnight, 28%; (f) 4-(*tert*-butyl)benzene-1,2-diamine, EDC, HOBt, Et$_3$N, rt, overnight, 50%; (g) AcOH, 65 °C, overnight, 98%; (h) HCl/MeOH, rt, 2 h, then HPLC, 51%.



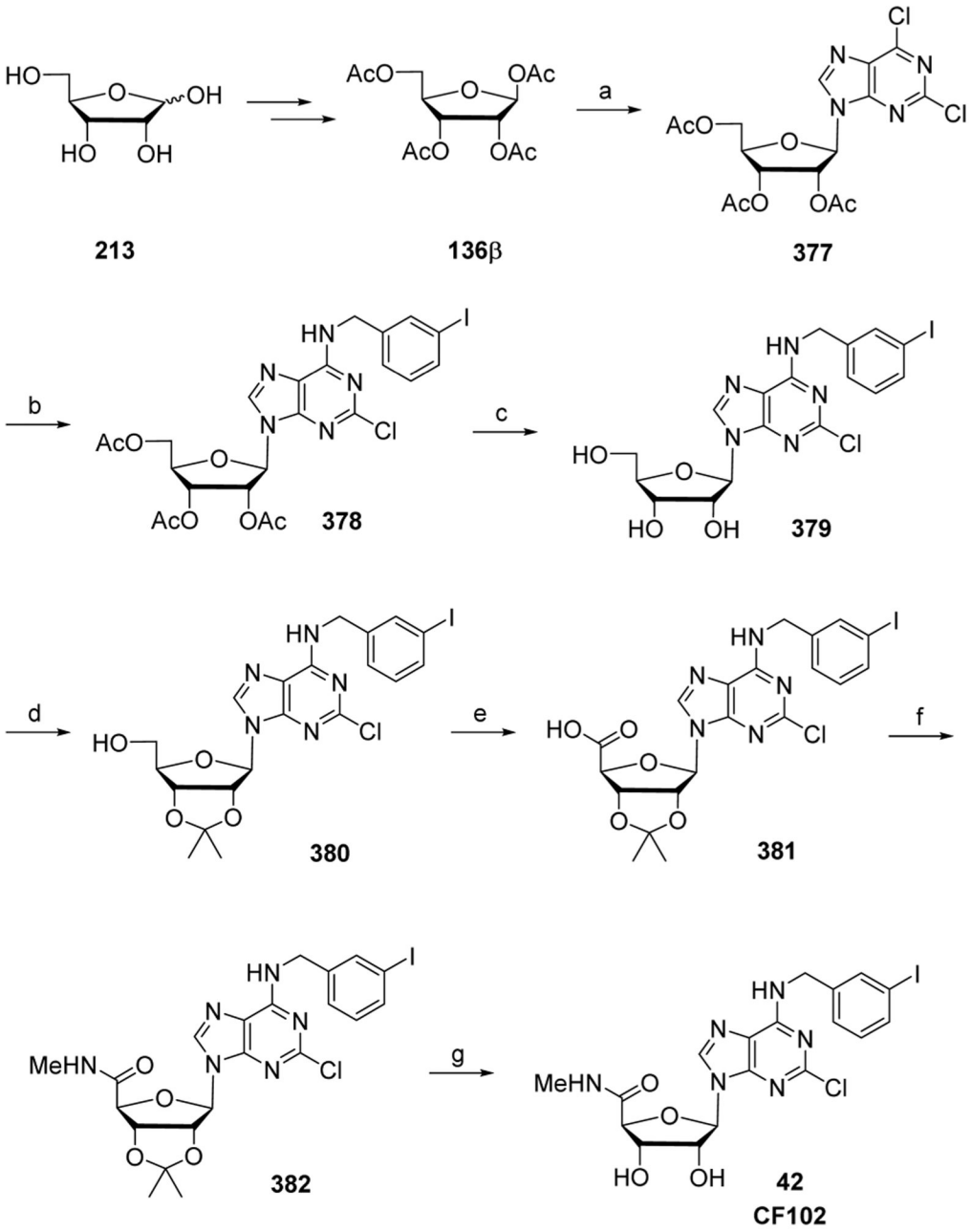
Scheme 65. Synthesis of EPZ-5676 43[a]

[a]Reagents and conditions: (a) BnBr, K_2CO_3, TBAI, Me_2CO, rt, 2 d, 92%; (b) Zn(Cu), $ClCOCCl_3$, $CH_3OCH_2CH_2OCH_3$, Et_2O, 50 °C, 3 days, 96%; (c) Zn, AcOH, 50 °C, 2 h, 93%; (d) H_2, Pd/C, $(CH_3)_2CHCOOCH_3$, PhMe, rt, 20 h, then DCHA, 20 °C, 18 h, 85%; (e) dioxane, DMF, $(COCl)_2$, 20 °C, 18 h, then 4-*tert*-butyl-2-nitroaniline, dioxane, 20–40 °C, 5 h, then 20 °C, 18 h, 89%; (f) Fe, AcOH, 75 °C then rt, overnight, 95%; (g) STAB, Me_2CO, MeOH, AcOH, 20 °C, 2 h, 95%; (h) STAB, MeCN, 55 °C, 16 h, 83%; (i) HCl, MeOH, 45 °C, 9 h then rt, overnight, 93%; (i) $MeCN/H_2O$, crystallization, 65%.



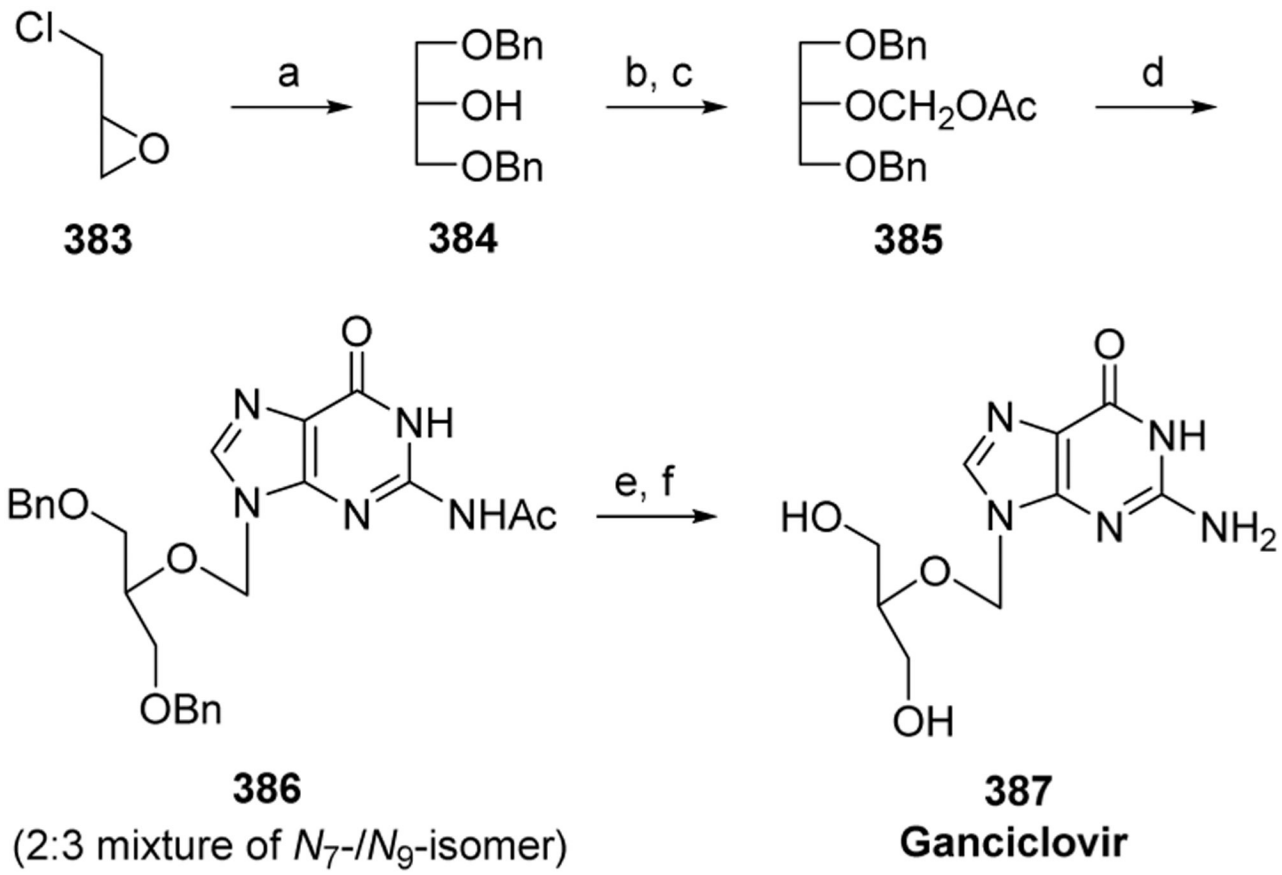
Scheme 66. Synthesis of CF102 42[a]

[a]Reagents and conditions: (a) TBDPSCl, DMAP, DMF, rt, 18 h, 55%;(b) BzCl, py-DCM, 0 °C to rt, 17 h, 99%; (c) *n*-Bu_4NF, THF, rt, 2 h, 89%; (d) RuO_2, $NaIO_4$, $CHCl_3$, CH_3CN, H_2O, rt, 2.5 h, 90%; (e) EDC, DMAP, MeOH rt, 3 h, 72%; (f) $MeNH_2$, THF, 50 °C, 15 h; (g) BzCl, py-DCM, rt, 3 h, 72% (over two steps); (h) Ac_2O, H_2SO_4, AcOH, rt, 15 h, 34%; (i) 3-iodobenzylamine·HCl, Et_3N, EtOH, rt, 5 d, 60%; (j) HMDS, $(NH_4)_2SO_4$, reflux, 4 h; (k) TMSOTf, DCE, reflux, 62 h, 33%; (l) NH_3/MeOH, rt, 16 h, 69%.



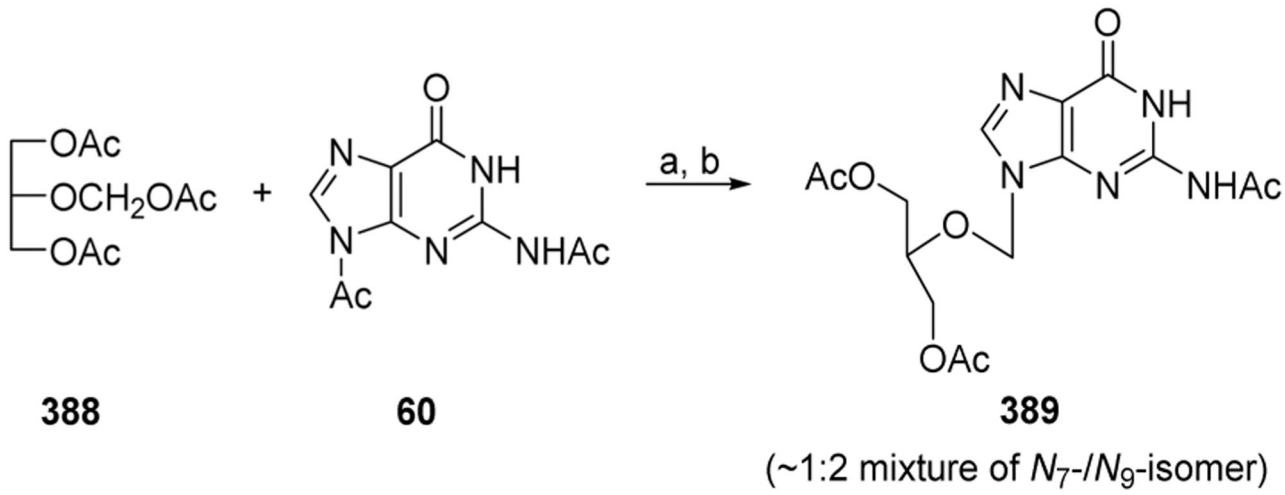
Scheme 67. Synthesis of CF102 42[a]

[a]Reagents and conditions: (a) BSA, TMSOTf, CH_3CN, 2,6-dichloropurine, 50 °C, 18 h, 88%; (b) 3-iodobenzyl amine, Et_3N, EtOH, rt, 2 d, 85%; (c) NaOMe, MeOH, DCM, rt, 1 h, 92%; (d) 2,2-dimethoxypropane, TsOH, DMF, rt, 12 h, 95%; (e) PDC, DMF, rt, 18 h, 70%; (f) $MeNH_2$·HCl, HOBt, EDAC, DIEA, DCM, DMF, rt, 12 h, 71%; (g) 80% HCO_2H, rt, 12 h, 83%.



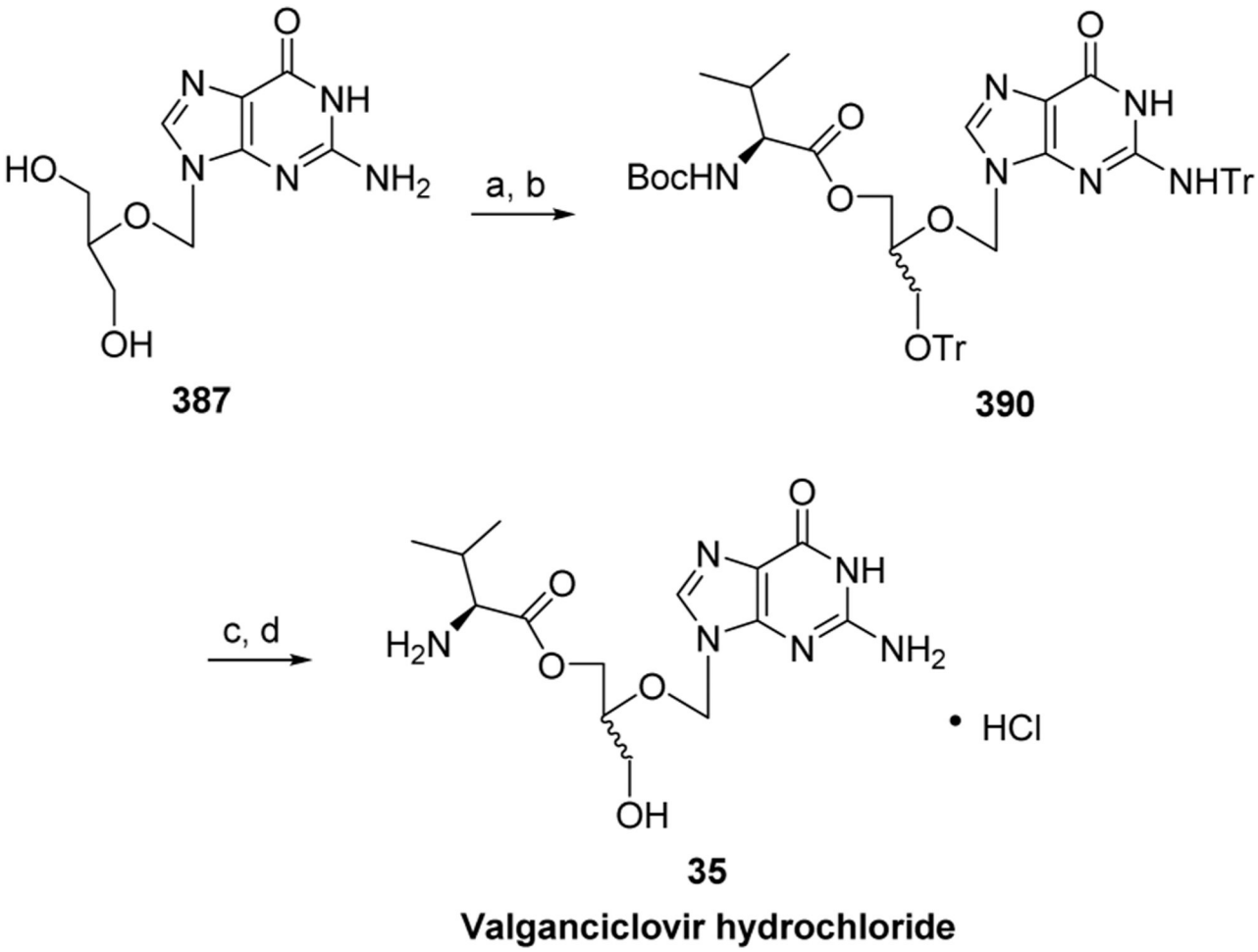
Scheme 68. Synthesis of Ganciclovir 387[a]

[a]Reagents and conditions: (a) BnOH, aq NaOH, rt, 16 h, 63%; (b) HCl (g), $(CH_2O)_n$, DCM, 0 °C, 16 h; (c) KOAc, Me_2CO, rt, 16 h, ~100%; (d) Diacetylguanine, TsOH, sulfolane, 95 °C, 5 days, 31% (N_9-isomer); (e) 20% $Pd(OH)_2/C$, cyclohexene–EtOH, reflux, 32 h; (f) NH_4OH–MeOH (1:1 v/v), rt, 16 h, 86% (2 steps).



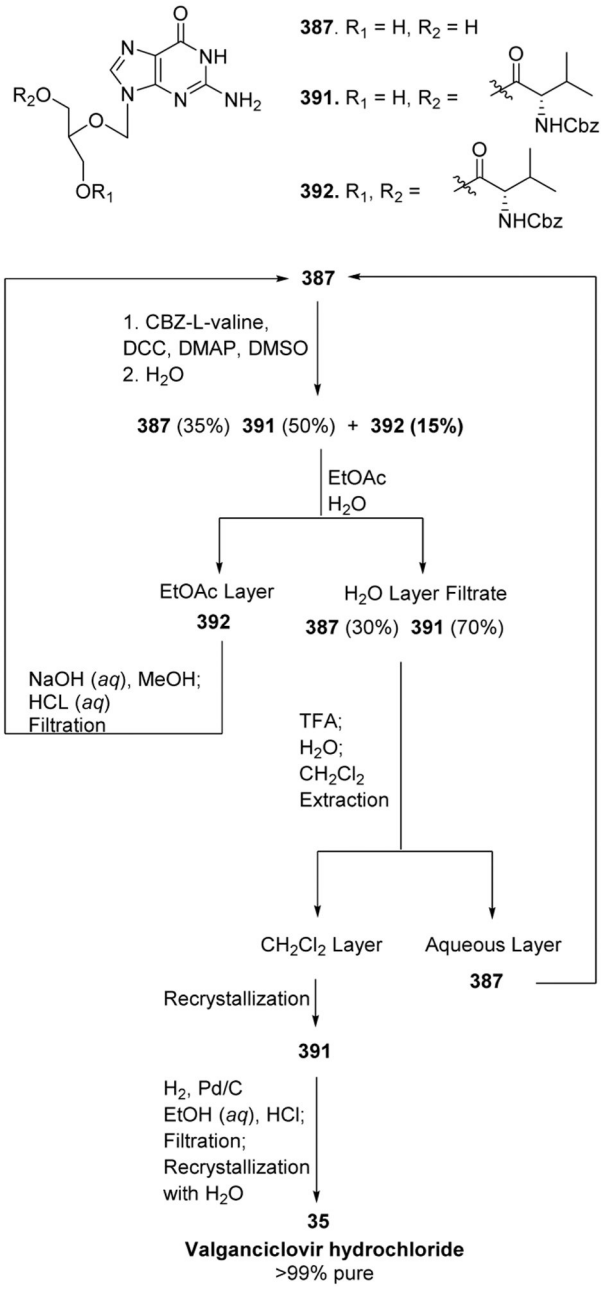
Scheme 69. Synthesis of 389[a]

[a]Reagents and conditions: (a) TsOH, DMF, 95–100 °C, 40 h; (b) Crystallization, 70% (N_9-isomer).



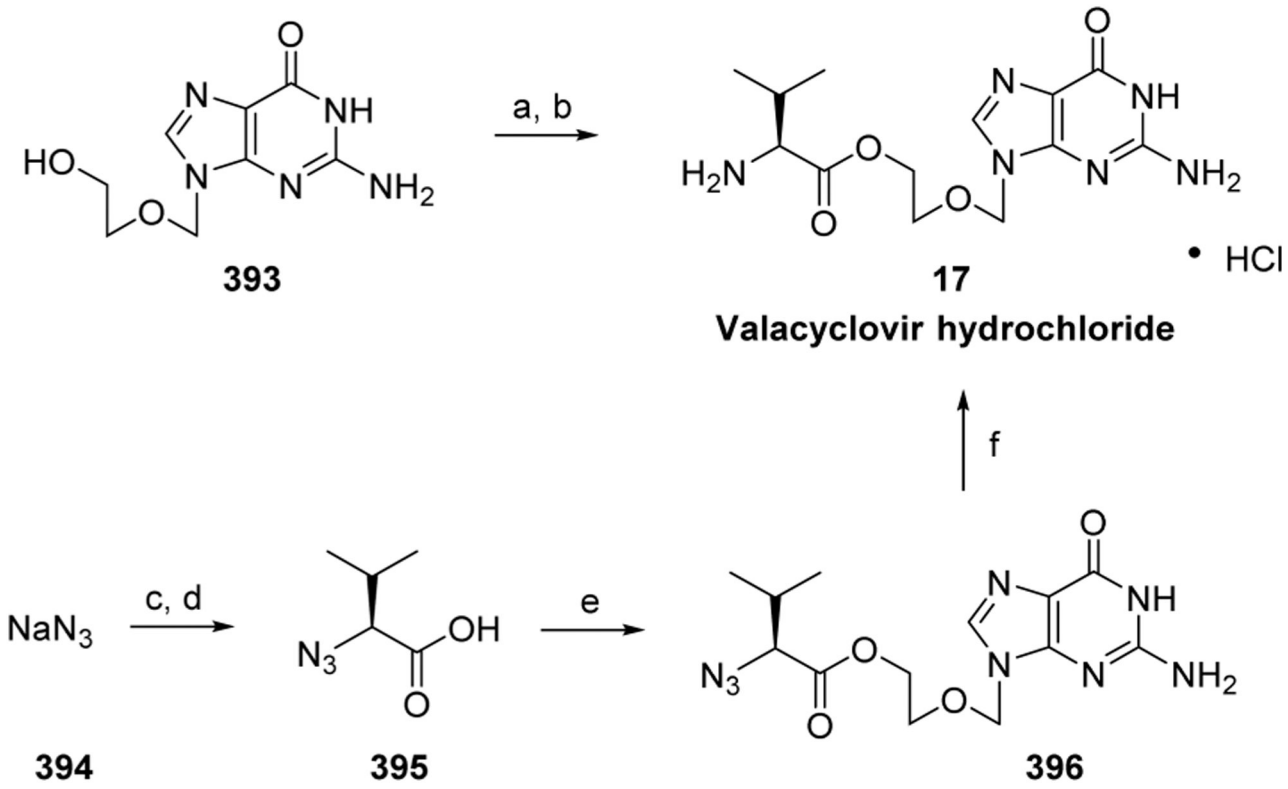
Scheme 70. Synthesis of Valganciclovir Hydrochloride 35[a]

[a]Reagents and conditions: (a) TrCl, DMAP, Et_3N, DMF, 50 °C, overnight; (b) Boc-L-valine, DCC, DMAP, Et_3N, DCM, rt, 16.5 h; (c) TFA, TFE, 20 °C, 5 h; (d) H_2, Pd/C, then HCl/MeOH, 34% (over four steps).



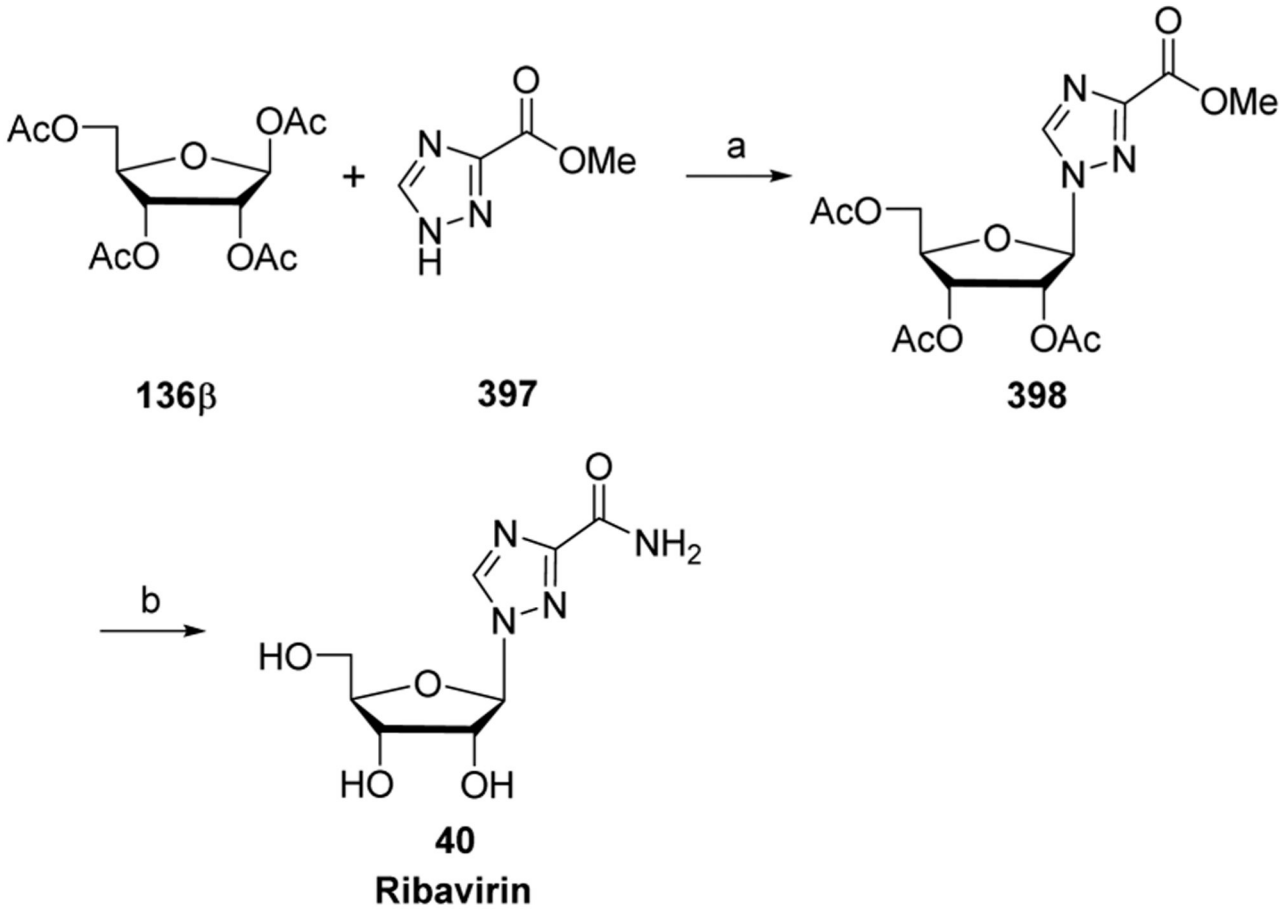
Scheme 71.

Preparation of Valganciclovir Hydrochloride 35



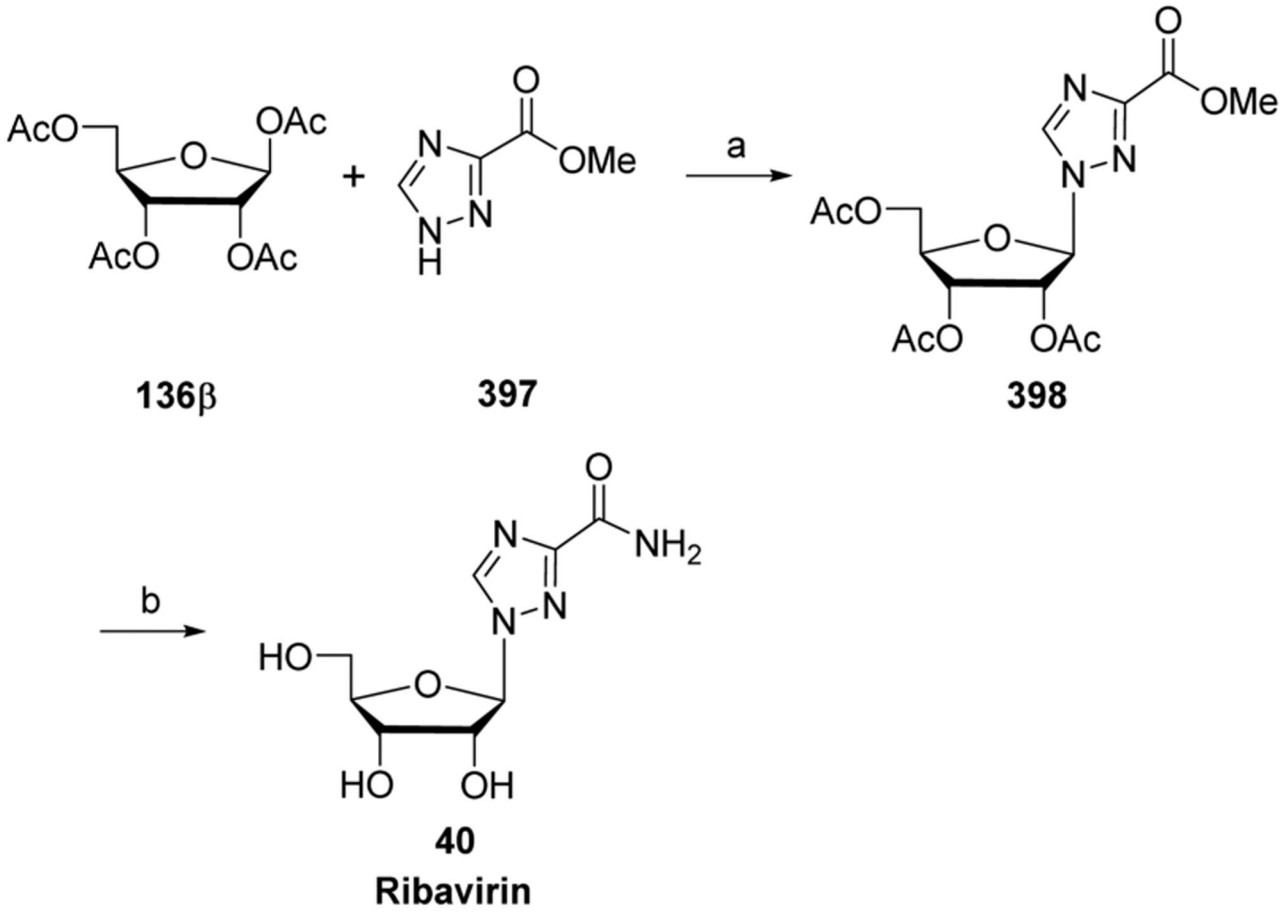
Scheme 72. Synthesis of Valacyclovir Hydrochloride 17[a]

[a]Reagents and conditions: (a) Cbz-L-val-OH, DCC, DMAP, DMF, 60 °C to rt, 12 h; (b) Pd/C, H_2, MeOH, THF, aq HCl, 55% (over two steps); (c) SO_2Cl_2, imidazole, MeCN; (d) L-valine, $CuSO_4$, K_2CO_3, MeCN; (e) Acyclovir, DCC, DMAP, DMF, 10–15 °C, 45 min, 82%; (f) Raney Ni, EtOH, 50–60 °C then aq HCl, 82%.



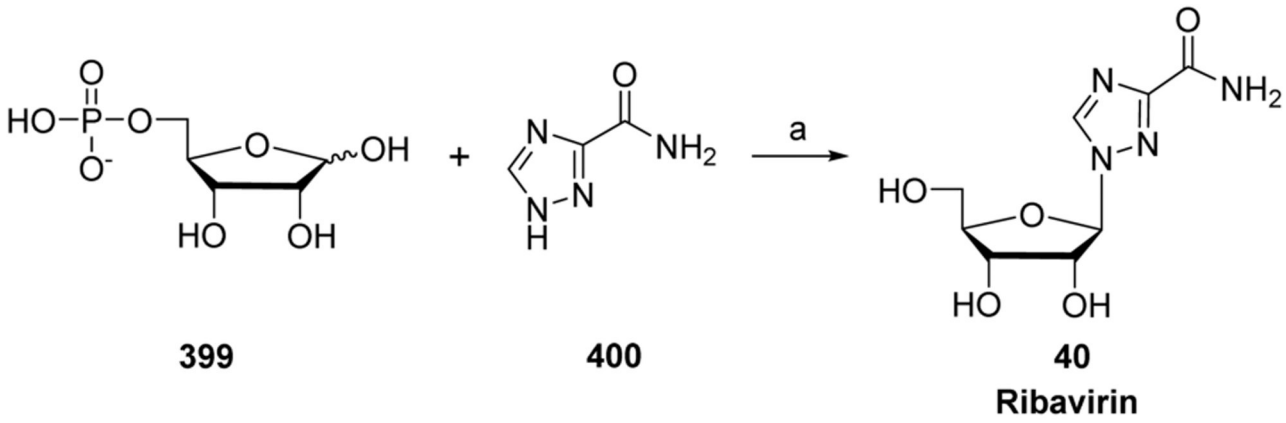
Scheme 73. Synthesis of Ribavirin 40[a]

[a]Reagents and conditions: (a) $SnCl_4$, DCM, 15–20 ° C, then reflux, 2 h, 70%; (b) NaOMe/MeOH, 10 °C, 3 h then NH_3/MeOH, 20 °C, 4 h, 79%.



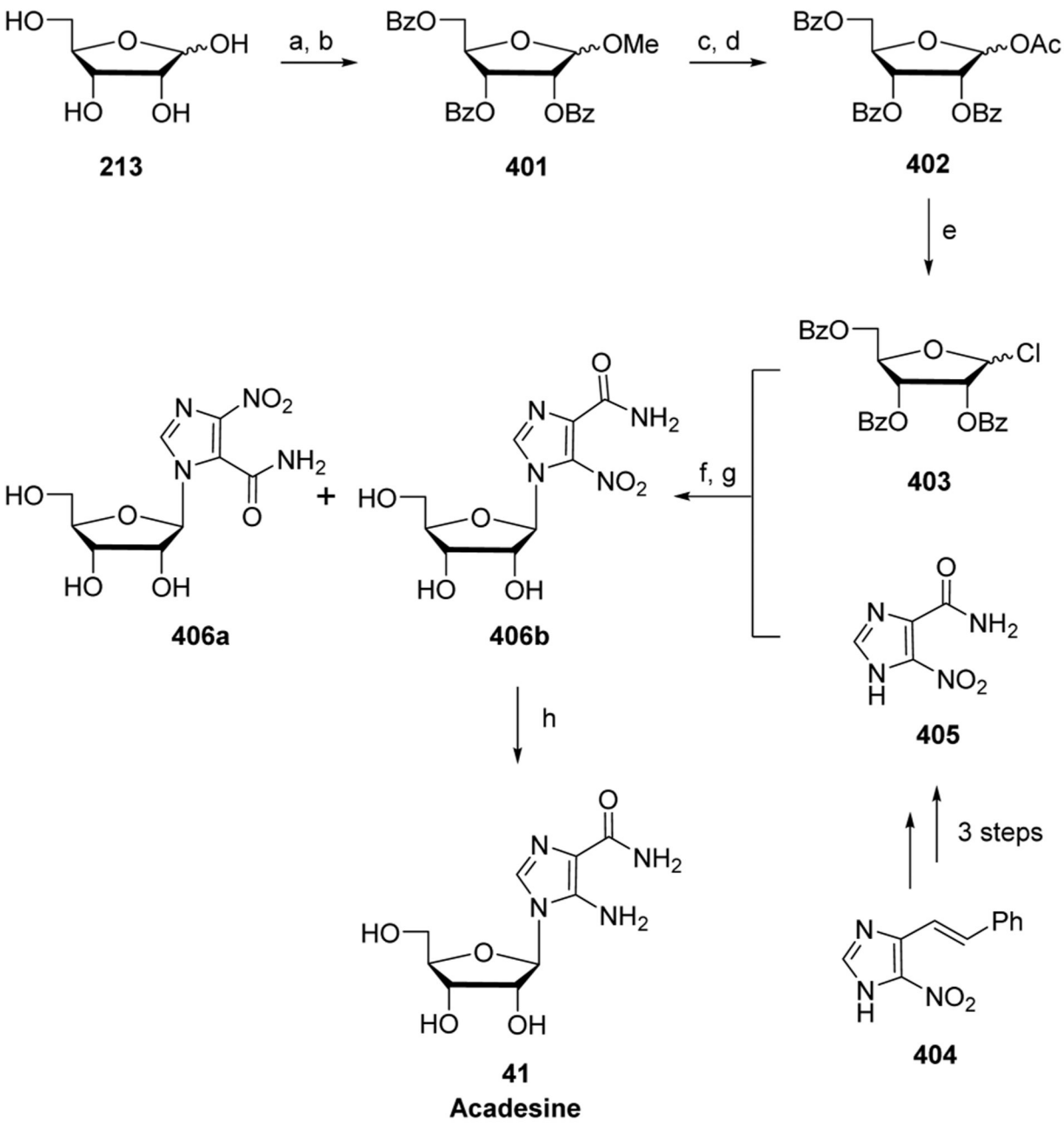
Scheme 74. Large-Scale Synthesis of Ribavirin 40[a]

[a]Reagents and conditions: (a) TfOH, 135 °C, 2 h, vacuum, 92%; (b) NH_3/MeOH, 20 °C, 40 h, 83%.



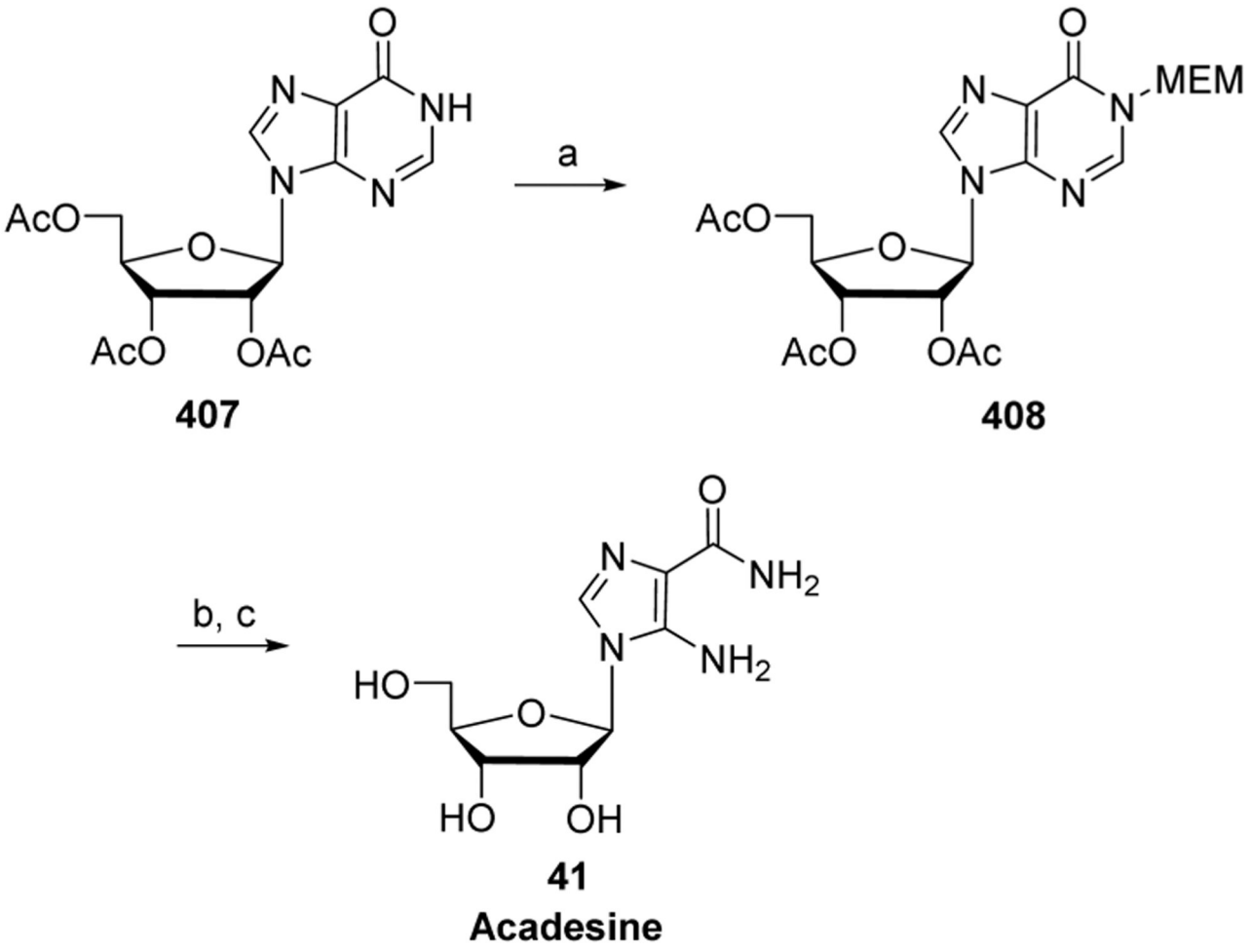
Scheme 75. Synthesis of Ribavirin 40[a]

[a]Reagents and conditions: (a) Phosphopentomutase, purine nucleoside phosphorylase, tris-buffer, 100%.



Scheme 76. Synthesis of Acadesine 41[a]

[a]Reagents and conditions: (a) cat. HCl, MeOH; (b) BzCl, pyr, DCM; (c) HBr/HOAc; (d) Ac_2O, pyr, 48 h, 57% (from D-ribose); (e) HCl/ether, rt, 7 days; (f) AgCl, xylene, reflux, 1 h; (g) NH_3/MeOH, 90 h, 19% (**406a**:**406b** = 1:1); (h) H_2 (1 atm), PtO_2, H_2O, 1 h, 12%.



Scheme 77. Synthesis of Acadesine 41[a]

[a]Reagents and conditions: (a) MEMCl, DIPEA, DCM, 0 °C, 65 min, 86%; (b) NH_4OH, MeOH, rt, 1 h; (c) aq NaOH, reflux, 1 h, 73%.