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Synthetic approaches and biological functions of 2-deoxyadenosine: A review of nucleoside chemistry and cellular dynamics

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Abstract

2′-Deoxyadenosine (dAdo) resides at the critical junction of de novo and salvage deoxyribonucleotide synthesis while salvage phosphorylation of dAdo replenishes the dATP-pool, the de novo pathway via ribonucleotide reductase (RNR) generates dNTPs from ribonucleotides. Disruption of this balance as typified in adenosine deaminase (ADA) deficiency causes intracellular dAdo and dATP accumulation, potent RNR inhibition, dNTP-pool disruption and replication stress. Concurrently, synthetic modification of the dAdo scaffold has driven the development of analogues that exploit nucleoside transporters, resist deaminative inactivation and selectively accumulate in target cells to modulate DNA repair, immune signalling and mitochondrial DNA maintenance. This review presents a unified account of dAdo's structural and molecular properties, its role in dNTP homeostasis and cellular dynamics, the pathological consequences of salvage/de novo dysregulation, and the latest synthetic and medicinal-chemistry strategies built on the dAdo framework.

Keywords: 2′-Deoxyadenosine salvage, DNTP homeostasis, ribonucleotide reductase inhibition, nucleoside transporter specificity, purine nucleoside analogue design, glycosylation stereochemistry

Introduction

Deoxy nucleosides consist of a deoxyribose sugar connected to either a purine or a pyrimidine base, and they serve as essential building blocks for the creation and repair of DNA. These nucleosides are transformed into deoxyribonucleotide triphosphates (dNTPs), which are the basic units used to construct DNA strands. This transformation occurs through two main metabolic pathways [1, 2].

1. **De Novo Synthesis:** This pathway involves the reduction of ribonucleotides to deoxyribonucleotides via ribonucleotide reductase (RNR), utilizing reducing equivalents from thioredoxin or glutaredoxin systems. It is energy-intensive and tightly regulated to maintain dNTP balance [3].
2. **Salvage Pathway:** In contrast, the salvage pathway recycles free nucleosides or bases released during nucleic acid turnover or absorbed from extracellular sources. This process is catalyzed by specific kinases such as deoxycytidine kinase (dCK), thymidine kinase 1 (TK1), and thymidine kinase 2 (TK2) which phosphorylate nucleosides to their monophosphate forms, subsequently converted to diphosphates and triphosphates. This pathway is energetically efficient and crucial for maintaining dNTP pools, especially under conditions of rapid cell proliferation or DNA repair [4].

1.1 Functional Significance of Salvage Pathways

1. **Metabolic Economy:** Salvage pathways conserve cellular energy by reusing nucleosides and bases, reducing the need for de novo synthesis [5, 6].
2. **Rapid dNTP Provision:** They provide a rapid supply of dNTPs, essential during DNA replication and repair processes [5, 6].
3. **Tissue-Specific Roles:** In mitochondria, where de novo dNTP synthesis is limited, salvage pathways are vital for mitochondrial DNA (mtDNA) replication. However, studies indicate that mitochondrial salvage alone may not suffice for rapid mtDNA replication, necessitating additional sources of dNTPs [5, 7].

4. **Genomic Stability:** Imbalances in dNTP pools, resulting from defective salvage pathways, can lead to replication stress, DNA damage, and genomic instability. For instance, in hematopoietic cells, the activity of salvage kinases like dCK and TK1 is critical for maintaining dCTP pools and preventing replication stress during DNA synthesis [5, 8, 9].

1.2 Clinical Implications and Therapeutic Potential

1. **Mitochondrial DNA Depletion Syndromes:** Deficiencies in mitochondrial salvage enzymes, such as TK2, can lead to mtDNA depletion, resulting in mitochondrial diseases. Enhancing salvage pathways by administering specific deoxynucleosides or inhibiting their catabolism has been proposed as a therapeutic strategy for these conditions [5, 10].
2. **Cancer Therapy:** Given that salvage pathways are often upregulated in proliferating cells, including cancer cells, they present potential therapeutic targets. Inhibiting salvage enzymes could disrupt dNTP homeostasis, impairing DNA replication in rapidly dividing tumor cells [5, 10, 11].
3. **Immunodeficiency Disorders:** Adenosine deaminase (ADA) deficiency leads to the accumulation of dATP, which inhibits RNR, causing a depletion of other dNTPs and resulting in severe combined immunodeficiency (SCID). This highlights the critical balance required in dNTP pools for immune cell function [2, 12].

1.3 Deoxyadenosine (dAdo): Molecular Dynamics and Pathophysiological Implications

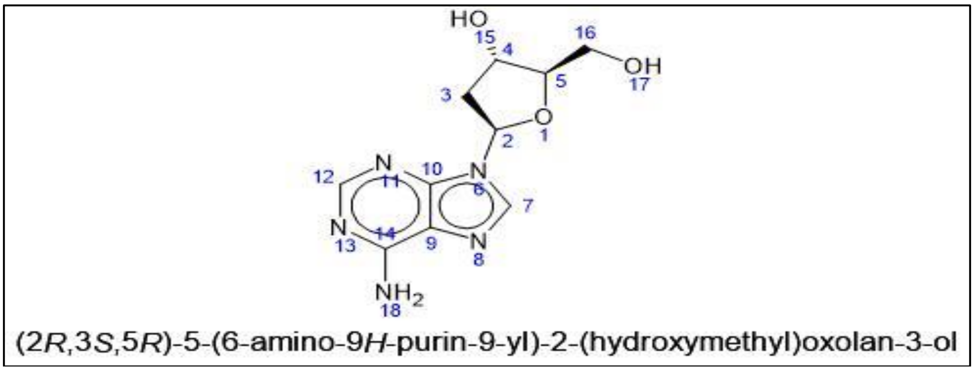

Fig 1: deoxyadenosine

Molecular Architecture and Biosynthesis: Deoxyadenosine (dAdo), or 2′-deoxyadenosine, is a purine nucleoside comprising the adenine base linked via a β-N9 glycosidic bond to a 2′-deoxyribose sugar. This structural configuration specifically, the absence of a hydroxyl group at the 2′ position distinguishes dAdo from adenosine, rendering it suitable for incorporation into DNA rather than RNA. Endogenously, dAdo arises from DNA turnover and nucleotide degradation. It is transported into cells via equilibrative nucleoside transporters (ENTs) and subsequently phosphorylated by adenosine kinase (AdoK) to form dAMP, which can be further converted to dADP and dATP. Alternatively, dAdo can be deaminated by adenosine deaminase (ADA) to form 2′-deoxyinosine, which is further metabolized to uric acid [3, 8, 13].

Metabolic Dysregulation in ADA Deficiency: Adenosine deaminase (ADA) deficiency leads to the accumulation of dAdo and its downstream metabolite, dATP. Elevated dATP levels inhibit ribonucleotide reductase, essential for DNA synthesis, thereby impairing lymphocyte proliferation and function. This inhibition contributes to the immunodeficiency observed in conditions like severe combined immunodeficiency (SCID). Additionally, the accumulation of dAdo and dATP disrupts cellular metabolism by inactivating S-adenosylhomocysteine hydrolase (SAHH), leading to impaired methylation of nucleic acids, proteins, and lipids. This disruption further exacerbates the immunodeficiency and affects other physiological systems, including the renal, neural, skeletal, and pulmonary systems [2, 12, 14-17].

Microbial Metabolism and Toxicity of 5′-Deoxyadenosine: In microbial systems, dAdo is a byproduct of radical S-adenosylmethionine (rSAM) enzyme reactions. For instance, 5′-deoxyadenosine (5′-dAdo) is formed by rSAM enzymes and must be salvaged or recycled to prevent toxicity. Failure to do so can lead to the accumulation of 5′-dAdo, which is toxic to cells [18-20].

Recent studies have identified a salvage pathway for 5′-dAdo in bacteria, involving phosphorylation, isomerization, and aldol cleavage steps. This pathway converts 5′-dAdo to non-toxic metabolites, thereby mitigating its toxic effects.

1.4 Research Gaps and Therapeutic Implications

1. **Uncharacterized Salvage Pathways:** Many dAdo salvage and disposal pathways, particularly in non-mammalian organisms, remain uncharacterized. For example, the salvage of 5′-dAdo is not ubiquitous, raising questions about the fate of this toxic byproduct in species lacking these pathways [21].
2. **Integration of Metabolic Pathways:** The compartmental integration of de novo and salvage pathways in maintaining balanced deoxyribonucleotide triphosphate (dNTP) pools is incompletely understood, especially under stress or in non-dividing cells [22, 23].
3. **Therapeutic Targeting:** Modulation of deoxynucleoside salvage pathways, including dAdo, holds therapeutic potential in conditions such as immune disorders and cancer. However, specificity and systemic effects need to be resolved [24].

1.5 Future Directions

1. **Identification of Novel Enzymes and Pathways:** Discovering new metabolic enzymes and pathways for the disposal or salvage of dAdo and its derivatives, particularly 5′-dAdo, across different organisms will expand our understanding of nucleotide metabolism and its evolutionary diversity [25, 26].
2. **Quantitative Modeling:** Developing better quantitative models to understand how salvage and de novo pathways integrate to maintain balanced dNTP pools in different cellular compartments and under various conditions will improve our understanding of replication stress and genome stability [22, 27].
3. **Exploitation for Therapeutic Benefit:** Investigating how dAdo metabolism or its dysregulation can be leveraged for therapeutic benefit, such as in immune deficiencies or cancer cells that may rely on specific salvage pathways, could reveal novel biomarkers or targets [22].
4. **Species-Specific Variations:** Exploring species-specific variations in dAdo salvage and disposal pathways could have implications in microbiology, biotechnology, and synthetic biology, such as leveraging unusual salvage routes for the production of bioactive compounds [28].

This review seeks to provide a comprehensive understanding of 2′-deoxyadenosine (dAdo), emphasizing its pivotal roles in DNA metabolism, cellular signaling, and its clinical significance. By integrating insights from biochemistry, synthetic chemistry, and medicinal chemistry, the review aims to elucidate the multifaceted functions and therapeutic potential of dAdo.

2. Biochemical and Cellular Functions of 2-Deoxyadenosine

Chemical structure: 2′-Deoxyadenosine (dAdo) is a deoxyribonucleoside composed of the purine base adenine linked via its N9 nitrogen to the C1′ carbon of a 2′-deoxyribose sugar. In the deoxyribose moiety the 2′-hydroxyl (2′-OH) is replaced by hydrogen (2′-H), differentiating it from the ribonucleoside adenosine (which has a 2′-OH). This structural variation underpins many of the biochemical distinctions between deoxynucleosides and ribonucleosides.The β-glycosidic bond (N9-C1′) remains the same as in ribonucleosides; however, the missing 2′-OH decreases the sugar's capacity for intramolecular transesterification (a route of cleavage seen in ribonucleic backbones) and contributes to the enhanced chemical stability of DNA relative to RNA [1, 3, 8, 9, 13].

Relevant physicochemical features for biochemical behavior

1. **Stability vs hydrolysis:** The lack of a 2′-OH reduces the susceptibility of the sugar-base linkage and backbone to intramolecular nucleophilic attack, thereby contributing to the greater chemical stability of deoxyribose-based nucleosides and DNA polymers. (This stability is a general feature of deoxy-nucleosides, including dAdo).
2. **Conformational preferences:** The deoxyribose ring adopts a different sugar-pucker (typically C2′-endo) compared to ribose (which often prefers C3′-endo in RNA). This contributes to the B-form helix geometry of DNA and affects recognition by DNA polymerases, repair enzymes and nucleoside transporters/kinases.
3. **Substrate recognition:** The absence of the 2′-OH changes H-bonding and steric interactions in kinase active sites and in DNA polymerases. For example, deoxyadenosine kinases and deoxynucleoside transporters differ in substrate specificity relative to their ribose counterparts.
4. **Ionisation and UV absorbance:** The adenine base retains the typical absorption maximum ~ 260 nm; physicochemical properties such as solubility, dissociation constants, and interactions with cation/anion environments are generally similar to other purine nucleosides but modified by the sugar. For instance, some chemical-supplier data show ~1.1% solubility in water at 20 °C for the monohydrate form of dAdo.
5. **Metabolic "toxicity" potential:** Elevated intracellular concentrations of dAdo (or its phosphorylated derivatives) can exert toxic effects, indicative of not just substrate availability but metabolite overload. One chemical-supplier catalogue describes that "when present in sufficiently high levels, deoxyadenosine can act as an immunotoxin or metabotoxin." Although phrased as a commercial note, this reflects a real biochemical risk in contexts such as Adenosine deaminase deficiency (ADA deficiency).

In sum, dAdo's structure adenine + 2′-deoxyribose provides the molecular foundation for its dual role as a DNA building block and as a metabolite capable of signaling and toxicity when dys-regulated [1, 3, 8, 9, 13].

2.1 Role in DNA Metabolism and Nucleoside Salvage

Salvage versus de novo synthesis of deoxynucleosides

Cells maintain deoxyribonucleoside triphosphate (dNTP) pools through two main routes:

1. **De novo synthesis:** Ribonucleotide reductase (RNR) reduces ribonucleoside diphosphates (NDPs) to their corresponding deoxyribonucleoside diphosphates (dNDPs). These can be further phosphorylated to dNTPs used for DNA replication and repair [1, 3].
2. **Salvage pathways:** Deoxynucleosides (such as dAdo) generated from DNA catabolism or imported via nucleoside transporters are phosphorylated by deoxynucleoside kinases (e.g., deoxycytidine kinase (dCK) which also acts on dAdo), forming dAMP → dADP → dATP, thereby contributing to the cellular dNTP pool [1, 3, 5, 8].

In this context, *dAdo is a salvage sub-strate-uptake* (via concentrative nucleoside transporters) or intracellular release → phosphorylation → integration into dATP pool. Hence, the balance between de novo and salvage contributions is critical for maintaining dNTP homeostasis, especially during periods of replication or repair [16].

Incorporation into DNA, replication and repair contexts. While free dAdo is not directly incorporated into DNA (it must first be phosphorylated to dATP), the salvage of dAdo is a key entry point into the dATP pool used by DNA polymerases. Elevated salvage flux or blockage of degradation (e.g., via ADA deficiency) leads to dATP accumulation, which can perturb replication/repair dynamics [14].

For example, in human T-lymphoblasts treated with dAdo plus an adenosine deaminase inhibitor, strand breaks accumulated, semiconservative DNA synthesis was inhibited, even though repair synthesis was less affected. Mechanistically, accumulation of dATP (via dAdo) inhibits RNR, depletes other dNTPs and stalls replication forks. This directly implicates salvage of dAdo in replication stress and DNA damage responses [5, 14].

Moreover, in DNA repair contexts, imbalanced dNTP pools lead to mis-incorporation, increased single-strand breaks, stalled replication forks and activation of checkpoint responses. Thus, dAdo through its contribution to dATP pools influences not only replication but also repair fidelity and genome integrity [2].

2.2 Signaling and Cellular Impact

1. **Non-Classical Roles of dAdo and Derivatives:** Ion-Channel Activation**.** Beyond its canonical function as a DNA precursor, 2′-deoxyadenosine (dAdo) serves as a biochemical precursor for signalling metabolites. The derivative 2′-deoxy-ADP-ribose (2′-deoxy-ADPR; 2dADPR) has emerged as a potent endogenous superagonist of the Ca^{2+}-permeable TRPM2 channel (Transient Receptor Potential Melastatin 2) [1, 29]**.**
2. **TRPM2 activation potency**: A 2017 *Nature Chemical Biology* study demonstrated that 2dADPR evokes ~10.4-fold higher TRPM2-mediated whole-cell currents compared to ADP-ribose (ADPR). The enhanced activity results from a slower channel inactivation rate and greater open probability when bound to 2dADPR [29, 30].
3. **Ca^{2+}-sensitivity and kinetics**: A 2024 *Frontiers* study reported a fourfold higher Ca^{2+} sensitivity for 2dADPR ($EC_{50} \approx 190$ nM) relative to ADPR ($EC_{50} \approx 690$ nM), with faster activation kinetics at physiological Ca^{2+} levels [1, 29].
4. **Metabolic-ion channel coupling**: The proposed pathway integrates purine metabolism and Ca^{2+} signalling: dAdo → dATP (via phosphorylation) → 2dADPR (via CD38 and NMNAT-2) → TRPM2 activation → sustained Ca^{2+} influx → apoptotic/stress-response-signaling. This establishes a mechanistic link between nucleotide metabolism and second-messenger signaling [9, 29, 30].
5. **Cellular Effects of Altered dAdo/dATP Levels:** Pathological accumulation of dAdo and dATP, particularly in Adenosine Deaminase (ADA) deficiency, exerts broad cytotoxic effects-most pronounced in lymphoid cells [2, 17].
6. **RNR inhibition and replication block**: Elevated dATP inhibits ribonucleotide reductase (RNR)**,** depleting dNTP pools and suppressing DNA synthesis and repair, leading to apoptosis or necrosis in developing T, B, and NK lymphocytes [12, 16].
7. **Epigenetic and metabolic disruption**: dAdo inhibits S-adenosylhomocysteine (SAH) hydrolase, causing SAH accumulation and inhibition of SAM-dependent methylation of DNA, proteins, and lipids impairing thymocyte differentiation and epigenetic regulation [5].
8. **Mitochondrial and oxidative stress**: Excess dAdo/dATP induces NAD^+ depletion**,** ROS overproduction, and activation of p53 and ATM/ATR checkpoint kinases, culminating in mitochondrial dysfunction and apoptotic signaling [5, 17].
9. **Integrative Signaling Perspective:** Through 2dADPR-mediated TRPM2 activation, dAdo derivatives connect purine metabolism with Ca^{2+}-dependent processes such as apoptosis, inflammasome activation, immune-cell chemotaxis, and oxidative stress response**.** Thus, dAdo metabolism represents a dual regulatory axis governing both nucleic-acid homeostasis and ion-channel-mediated signaling networks [1, 9, 29, 30].

2.3 Pathophysiology of ADA Deficiency

2.3.1 Accumulation of Toxic Metabolites

1. **Deoxyadenosine (dAdo) and dATP:** In the absence of ADA, dAdo accumulates and is phosphorylated to dATP. Elevated dATP levels inhibit ribonucleotide reductase (RNR), leading to a depletion of deoxynucleotide triphosphates (dNTPs), which are essential for DNA synthesis and repair. This inhibition compromises DNA replication and repair mechanisms, resulting in lymphocyte apoptosis and severe combined immunodeficiency (SCID) [2, 12, 16].
2. **S-adenosylhomocysteine (SAH) Accumulation:** dAdo also inhibits SAH hydrolase, leading to an accumulation of SAH. Elevated SAH levels interfere with methylation reactions, affecting gene expression and cellular function [14, 17].

Lymphocyte Dysfunction and Apoptosis

a. **Thymic Apoptosis:** In ADA-deficient mice, there is a significant depletion of T and B lymphocytes, with pronounced apoptosis observed in the thymus. This suggests that developing thymocytes are particularly sensitive to the toxic effects of dAdo and dATP [2, 12, 16].
b. **T Cell Receptor (TCR) Signaling Defects:** Elevated adenosine levels in ADA-deficient mice impair TCR signaling, further compromising T cell development and function [12, 16].
c. **Systemic Manifestations:** Beyond immunodeficiency, ADA deficiency can lead to non-immunological manifestations due to the ubiquitous expression of ADA [2, 12, 17]:

- **Neurological Deficits:** Cognitive impairments and sensorineural hearing loss have been reported in ADA-deficient individuals.
- **Skeletal Abnormalities:** Patients may exhibit growth retardation and skeletal anomalies.
- **Pulmonary Inflammation:** Chronic pulmonary inflammation can occur, contributing to respiratory complications.

2.3.2 Clinical Implications and Therapeutic Approaches

Clinical Interpretation: Infants with ADA deficiency typically present with recurrent infections, failure to thrive, and severe lymphopenia. Without intervention, the condition is fatal in early childhood [5, 10, 12].

Therapeutic Strategies [2, 5, 10, 12]

- **Enzyme Replacement Therapy (ERT):** Involves the administration of recombinant ADA to restore enzyme activity, thereby reducing the accumulation of toxic metabolites.
- **Hematopoietic Stem Cell Transplantation (HSCT):** Provides a long-term solution by replacing the patient's hematopoietic system with ADA-sufficient cells.

- **Gene Therapy:** Emerging as a promising approach, gene therapy aims to correct the underlying genetic defect by introducing a functional ADA gene into the patient's cells.

3. Synthetic strategies for 2-Deoxyadenosine & its derivatives

1. **Classical Glycosylation of Purine Bases:** glycosylation of a purine base (adenine or substituted) with a protected 2deoxyribose derivative (e.g., 1chloro2deoxyribofuranose) via base anion (e.g., K^+ or Na^+ salt) coupling. Stereochemical control remains a major issue obtaining the correct βanomer at N9 and suppressing αanomer and N7 glycoside formation. *For example*, reactions of 2,6dibromopurine base gave 9β, 9α and 7β isomers in meaningful proportions [3, 21, 27, 31, 32].
2. **Example:** The 1989 Kawakami *et al.* paper reports a stereospecific coupling of adenine sodium salt + 1-α-chloro-2-deoxyribose derivative in acetone, affording β-dA in good yield.
3. **Key mechanistic insight:** The deoxyribose donor is less reactive (due to absence of 2′-OH) and more prone to side-reactions (anomerisation, decomposition), and the glycosylation step often requires fine solvent and base tuning to favour β-N9.
4. **Advantages:** Direct access to the nucleoside scaffold, versatile base variation.
5. **Disadvantages:** Moderate yields, significant isomeric mixtures, heavy protecting-group burden, difficult purification.
6. **Modifications on the Purine Ring (2, 6, 8positions)**: Introduce substituents (halogen, methylthio, amine, methoxy) at purine positions C2, C6, C8 either before or after glycosylation [3, 21, 22, 24, 27].
7. **Example:** Use of 2,6-dibromopurine salt + glycosyl donor → mixture of isomers → substitution on bromo positions to yield 2-amino deoxyadenosines.
8. **Example:** 8-Methoxy-2′-deoxyadenosine prepared and incorporated into oligonucleotides for conformational studies.
9. **Mechanistic considerations:** Substituents on the base alter electronic/steric profile → impact glycosylation kinetics, regioselectivity (N9 vs N7), stereochemistry (β/α) and yields. Post-glycosylation substitution requires that the nucleoside scaffold is stable under substitution conditions and protecting groups are compatible.
10. **Advantages:** Diversifies analog library; tunes biological properties (enzyme resistance, cytotoxicity, conformation).
11. **Limitations:** Additional steps altered base reactivity can reduce yield or selectivity may complicate protecting-group scheme and downstream transformations.
12. **Sugarmoiety modifications**: From the nucleoside scaffold, modify sugar moiety: e.g., 2′amino, 2′fluoro, constrained ring, or 5′alterations [3, 22, 24, 27].

Example

N-trifluoroacetyl-2′-amino-2′-deoxyadenosine phosphoramidite for solid-phase synthesis of oligonucleotides.

1. **Phosphate derivatization:** Phosphorylation of nucleoside → 5′monophosphate (dAMP) → diphosphate (dADP) → triphosphate (dATP). Crucial for biochemical incorporation or nucleotide function [3, 24-27].
2. **Advantages:** Enables incorporation into polymers (DNA/RNA), biochemical assays, enzyme substrates; tuning of stability/binding via sugar modifications.
3. **Limitations:** Phosphorylation of deoxynucleosides often low yield, purification of highly charged species is challenging; sugar modifications add synthetic complexity (protecting-group manipulations, reagent cost).

Modern Synthetic Methodologies and Challenges

1. **Protection/deprotection strategy:** Modern methods emphasise minimal steps, selective protection of hydroxy/amino groups, avoiding harsh Lewis acids to reduce anomerisation [21, 22, 31].
2. **Example:** Study reporting anomerisation of 2′-deoxyadenosine derivatives under Lewis acid during O3′-glycosylation.
3. **Biocatalysis / enzymatic synthesis:** Use of nucleoside phosphorylases or transglycosylation: e.g., preparation of purinemodified 2′deoxynucleosides via E. coli nucleoside phosphorylases under optimized glycosyl donor excess and phosphate deficiency (yield 4793%) [22, 25, 26, 31].
4. **Industrial / scaleup constraints:** stereoselectivity (β vs α, N9 vs N7) still problematic; purification of isomeric mixtures; protecting module load high; solvent/metal waste (green chemistry concerns) as noted in review of nucleoside chemistry [22, 27, 28].

Table 1: Summary Table of Key Synthetic Routes

Starting Material	Key Reagents / Conditions	Major Advantages	Major Disadvantages
Purine base (e.g., adenine or halogenated purine) + protected 2′-deoxyribose (e.g., 1-chloro-2-deoxyribofuranose)	Base salt (Na^+/K^+) or silylated base + Lewis-acid glycosylation; e.g. glycosyl chloride in binary solvent mixture for cladribine.	Direct route to nucleoside; well-studied historic method	Mixture of stereoisomers (α/β, N7/N9); complex purification; moderate yields
Purine modified at 2-,6-,8-positions + glycosylation or base modification of nucleoside	Halogenation or substitution on base (e.g., 2,6-dibromopurine → glycosylation → substitution)	Enables-many analogues with biological activity	More steps; base reactivity altered which may reduce yield or complicate glycosylation
2′-Deoxyadenosine (or derivative) + sugar moiety or phosphate derivatisation	Phosphorylation (e.g., $POCl_3$ for dAMP) or sugar modification (2′-F, 2′-NH_2)	Generates nucleotide or modified sugar analogues for advanced applications	Charged species difficult to purify; sugar modifications often need elaborate protection
Modern-methods: protection/deprotection, phosphoramidite, enzymatic methods	Protecting-groups, automated-solid-phase synthesis (e.g., 2′-amino-2′-deoxyadenosine phosphoramidite)	Compatible with oligonucleotide synthesis higher selectivity	Still many steps; cost of reagents; scale-up may be limited

4. Medicinal Chemistry of 2-Deoxyadenosine Derivatives

Rationale for Modifying deoxyadenosine (dAdo): Natural deoxyadenosine (dAdo) is subject to rapid metabolism (especially by adenosine deaminase, ADA) and may not selectively accumulate in target cell types [3, 12, 22, 24-26].

Analogue design goals

- **Mimic** dAdo to exploit nucleoside transporters for cellular uptake.
- **Resist** metabolic inactivation (e.g., ADA deamination) to increase intracellular half-life.
- **Selective accumulation** in target cells (e.g., lymphocytes with high deoxycytidine kinase (DCK) and low 5′-nucleotidase (5′-NTase) activity) in order to maximise therapeutic index.
- In Cladribine the substitution of a chlorine atom at the 2-position of the purine ring renders it resistant to ADA deamination, thereby increasing intracellular persistence.
- Because lymphoid cells (T and B lymphocytes) typically express high DCK and low 5′-NTase, they preferentially activate and accumulate the phosphorylated analogue this differential enzyme expression underpins the selectivity for lymphoid cells.

Structure-Activity Relationships (SAR)

1. **Base modifications (purine ring substituents):** The 2chloro substituent (in Cladribine) is critical for ADA resistance and improved duration of action. Studies in bacterial purinenucleoside phosphorylase (PNP) show that other substituents (e.g., at the 6 or 8positions of the purine) can significantly affect enzymatic substrate/inhibition profiles. *For example,* analogues of 2chloro2′deoxyadenosine with 6benzyloxy substitution showed Ki ~0.5 µM for E. coli PNP [3, 21, 24].
2. **The overall SAR conclusion:** Modifications at the 2, 6 and 8positions can influence (i) substrate affinity for kinases/enzymes, (ii) resistance to degradation, (iii) incorporation into DNA, and (iv) celltype selectivity [21, 24].
3. **Sugar moiety / deoxy vs ribo / 2′,3′ modifications:** Cladribine retains the 2′deoxy ribose sugar, which is important for its incorporation or interaction with DNA synthesis/repair pathways. Preclinical work (e.g., 2′,3′dideoxy analogues) has explored sugar modifications to influence chaintermination, incorporation into DNA, and metabolic stability (see Rosowsky *et al.* 1989, reference). However detailed quantitative SAR for sugar modifications are less frequently reported in the public literature [3, 22, 24, 33].
4. **The key design insight:** Preserving kinase recognition (e.g., by DCK), while modifying sugar moiety to reduce degradation or enhance intracellular retention, but without losing uptake or phosphorylation
5. **Influence on pharmacokinetics, metabolic stability, target selectivity:** Structural modifications that confer ADA resistance (2Cl) improve metabolic stability and thus prolong intracellular exposure [12, 24-26, 31].
6. **Modifying substituents can influence transporter affinity:** For uptake via nucleoside transporters (ENT1, ENT2, CNT3) which in turn affects tissue distribution, uptake kinetics, and cell-type selectivity. For *Cladribine,* uptake is facilitated via ENT1, CNT3 etc. The intracellular accumulation (often 30- to 40-fold higher than extracellular concentrations in lymphocytes) is partly due to structural features enabling retention (phosphorylation, low dephosphorylation) and selective uptake

4.1 Mechanisms of Action

- **Cytotoxic / antimetabolite mechanism (hematologic malignancies):** Cladribine is taken up by nucleoside transporters into target cells, then phosphorylated by DCK (and mitochondrial deoxyguanosine kinase) into monophosphate (CdAMP) → diphosphate → active triphosphate (CdATP) [3, 24-26, 34].
- **In dividing cells:** Accumulation of Cd-ATP results in depletion of the deoxynucleotide triphosphate pool (dATP, dGTP etc), inhibition of DNA synthesis, incorporation into DNA, DNA strand breaks, activation of PARP, NAD/ATP depletion, mitochondrial damage, and apoptotic (caspase-dependent and independent) cell death.
- **In resting lymphocytes:** Even non-dividing cells accumulate Cd-ATP, leading to defective DNA repair of endogenous damage, NAD/ATP depletion, and cell death. Selectivity arises because lymphocytes (high DCK / low 5′-NTase) accumulate more Cd-ATP than non-target cells thus, toxicity is preferential.
- **Immunomodulatory / immunereconstitution mechanism (autoimmune / multiple sclerosis):** Beyond pure cytotoxicity, Cladribine exerts immunomodulatory effects: i.e., in addition to depleting lymphocytes, it modulates subsets of innate immune cells (monocytes, dendritic cells) and affects activation/maturation pathways. As shown in Fissolo *et al.* 2021, Cladribine reduced proliferation and induced apoptosis in lymphocyte subsets (DCKdependent) but also reduced activation of monocytes and dendritic cell lineage (both DCKdependent and DCKindependent) [12, 13, 25, 26, 35].
- **Non-canonical effect:** Cladribine may act as an agonist at adenosine A1/A2A receptors, influencing anti-inflammatory signalling, independent of its phosphorylation. In the context of multiple sclerosis, these dual mechanisms (lymphocyte pool depletion + modulation of innate immune activation) are thought to interrupt the autoimmune cascade, allow immune-reconstitution, and thereby reduce relapse activity and disease progression.

4.2 Pharmacological & Therapeutic Applications

- **Hematologic malignancies:** Cladribine was originally approved for hairy-cell leukemia (HCL) and has been used in other lymphoid malignancies (e.g., chronic lymphocytic leukemia (CLL), low-grade non-Hodgkin lymphomas). Early reviews reported complete response rates for HCL in many studies of 33-92% after single courses.

  The therapeutic strategy exploited Cladribine's ability to kill both dividing and resting lymphocytes in the malignant clone, offering an advantage over many classic cellcyclespecific agents [24, 30, 36, 37].
- **Autoimmune/neuroimmunology (Multiple sclerosis):** Oral Cladribine tablets (cumulative dose 3.5 mg/kg over two years) are indicated for adults with highly active relapsingremitting multiple sclerosis (RRMS). The pivotal phase3 trial (CLARITY) demonstrated

reduction in relapse rate, disability progression, MRI lesion load. The mechanism in MS is thought to be selective B and T lymphocyte pool reduction (especially memory B cells) plus modulation of innate immune cells and resetting of immune homeostasis rather than continuous immunosuppression [12, 13, 35].

- **Emerging / investigational indications:** Analogue design beyond Cladribine (e.g., sugar or base modifications) explores potential antiviral, immunemodulatory or other oncologic uses. For example, 8modified deoxyadenosine analogues for HIV1 reverse transcription inhibition. While clinical translation is yet limited, these represent opportunities. There is interest in neurodegenerative/inflammatory diseases given immunemodulation and possible CNS penetration properties [22, 24-26].

4.3 ADME (Absorption, Distribution, Metabolism, Elimination), Toxicity & Drug-Development Considerations.

ADME / Pharmacokinetics: [24, 36, 37]

- **Absorption:** Oral bioavailability ~39-43% (≈40%) for the 10 mg tablet in MS studies. Median Tmax ~0.5 h (fasted), ~1.5 h (with high-fat meal, delayed) and Cmax reduced ~29% with high-fat meal; AUC essentially unchanged.
- **Distribution:** Low plasma protein binding (~20%). Large volume of distribution (~480-490 L), reflecting widespread tissue uptake and intracellular accumulation. Intracellular concentrations in lymphocytes are ~30-40-fold those in extracellular fluid within 1 hour of exposure. CSF/plasma ratio ~0.25 in small studies.
- **Metabolism:** Cladribine is phosphorylated intracellularly (by DCK) to active nucleotides (Cd-AMP, Cd-ADP, Cd-ATP). Minimal hepatic metabolism; no major (>10%) plasma metabolites identified. The major metabolic/degradation clearance pathways remain incompletely characterised.
- **Elimination:** Terminal half-life ~18-23 h in adults (varies by study) after oral dosing. Renal and non-renal pathways contribute; ~21-35% of an IV dose excreted unchanged in urine; renal clearance exceeds glomerular filtration (suggesting active secretion) in some studies.
- **Toxicity / Safety Considerations:** The primary ontarget toxicity is lymphopenia (reduction in B and T lymphocytes) and associated risk of infections (opportunistic, serious). In MS usage, the nadir of lymphocyte count typically occurs ~2-3 months after start of each annual treatment course; recovery to ≥800 cells/µL median time ~28 weeks. Myelosuppression (neutropenia, thrombocytopenia) in hematologic malignancy use; febrile neutropenia and infections noted in early oncology studies [12, 13, 36, 37].

Long-term safety: Since the drug depletes lymphocytes and modulates immune system, there is theoretical risk of secondary malignancy or prolonged immune-suppression; therefore, monitoring and risk-benefit assessment is important.

For analogue development

Key challenges include achieving celltype selectivity (avoiding excessive depletion of nontarget immune/hematopoietic cells), controlling intracellular accumulation (to avoid offtarget DNA damage), transport/uptake profiling (to avoid offtarget tissues), metabolic stability vs elimination tradeoffs, and immunereconstitution dynamics (timing and recovery of normal immune populations) [21, 22, 25, 26].

Table 2: Key Aspects and Analogue-Design Implications of Cladribine (2-Cl-2′-deoxyadenosine)

Aspect	Cladribine (2-chloro-2′-deoxyadenosine)	Notes for analogue design
Base substitution	2-Cl on purine → ADA resistance	Other substituents (6-, 8-positions) can modulate enzyme binding/activity (e.g., PNP inhibition)
Activation strategy	Pro-drug phosphorylated by DCK → 2-Cd-ATP accumulation in lymphocytes	Analogue design must preserve DCK substrate profile and avoid dephosphorylation by 5′-NTase
Mechanism of cytotoxicity	DNA synthesis/repair inhibition, PARP activation, mitochondrial apoptosis (cytochrome c, AIF)	Sugar modifications can influence DNA incorporation, chain-termination, cytotoxicity
Immunomodulation	Lymphocyte depletion + modulation of monocytes/DCs via DCK-dependent and independent mechanisms (adenosine-receptor agonism)	For analogues, consider innate immune cell effects, inflammatory cytokine modulation
Therapeutic use	Hairy cell leukaemia / other lymphoid malignancies; RRMS immune-reconstitution therapy	Analogues could target other malignancies, antiviral/anti-inflammatory applications
ADME / PK	Oral bioavailability ~40%, intracellular accumulation high in lymphocytes, half-life ~20 h (varies)	Analogues must ensure favourable uptake, distribution, clearance, minimal off-target retention
Safety concerns	Long lymphopenia, infection risk, myelosuppression, possible long-term malignancy risk	Selectivity, immune reconstitution kinetics, transporter/kinase specificity critical

5. Chemical-Biological Interface of dAdo Analogues

Synthesis ↔ Biological Function of dAdo Analogues [3, 21, 25, 26, 31, 38, 39]

a. **Synthetic innovations:** Recent work (e.g., Guinan *et al.*, 2020; Shelton *et al.*, 2016) has advanced the design and synthesis of nucleoside analogues combining base and sugar modifications, prodrug strategies, and chemoenzymatic/biocatalytic methods (Salihovic et. 2025).

b. **Metabolic/functional interplay:** Modifications of 2′-deoxyadenosine (dAdo) influence uptake, salvage, phosphorylation, incorporation, and off-target signalling. For example, accumulation of dAdo → dATP potently inhibits ribonucleotide reductase, blocks deoxynucleotide synthesis and triggers apoptosis in lymphocytes.

c. **Design-impact links:** Synthetic changes (e.g., 2′-fluoro, arabinosyl sugar, base modifications) affect transporter recognition, kinase activation, deaminase/phosphorolysis resistance, incorporation into DNA/RNA, and non-canonical signalling. Thus, "chemistry ↔ metabolism ↔ cellular effect" is a critical axis in analogue design.

Key Challenges & TradeOffs [3, 21, 22, 24-26, 31]

a. **Synthetic complexity vs. manufacturability**: High stereochemical/chemical complexity (sugar, base, prodrug moieties) improves selectivity/potency but constrains yield, cost and scale-up.

b. **Potency vs. selectivity**: Very active analogues risk uptake/activation in healthy tissue → toxicity. Tumor-selectivity (via transporter/kinase expression) is variable.

c. **Efficacy vs. toxicity/resistance**: Strong incorporation/activation may lead to DNA damage, stem-cell toxicity, immune effects. Tumor resistance (e.g., transporter down-regulation, kinase loss, deaminase up-regulation) is common.

Major Knowledge Gaps: Noncanonical signaling roles of dAdo and analogues (immune/metabolic/epigenetic) remain underexplored [2, 12, 24-26, 31].

a. **Long-term safety:** Genomic stability, stem/progenitor cell impact, immune modulation especially for novel sugar/base/prodrug analogues. Synthetic scalability to GMP level and cost-effective manufacturing for complex analogues.

b. **Tumor heterogeneity:** How variable transporter and salvage-enzyme profiles across patients affect uptake/activation of analogues.

c. **Systems-level integration:** How dAdo metabolism intersects with DNA damage response, immunometabolism, one-carbon/methylation networks.

Emerging Trends [12, 21, 22, 24, 38, 40]

a. **Bio catalysis/chemo-enzymatic synthesis**: Use of nucleoside phosphorylases/trans-glycosylases to enhance stereoselectivity and yield.

b. **Prodrug/nanoparticle delivery**: Self-assembling prodrug nanoparticles for targeted analogue delivery (Baroud et . al., 2021).

c. **Multi-target analogue design**: Analogues that not only incorporate into DNA/RNA but also modulate salvage pathways, signaling or epigenetic enzymes.

d. **High-throughput SAR + structural biology**: Combining large-scale synthetic libraries with transporter/kinase/enzyme structural data to optimize design.

e. **Precision medicine approaches**: Stratifying patients by tumor-specific nucleoside transporter/salvage enzyme expression; matching therapies accordingly.

6. Future Directions & Outlook

- **New derivatives**: Novel sugar modifications (e.g., locked sugars, 2′fluoro/azido), purine/base hybrid moieties, tumoractivated prodrugs, analogues for combination therapies (with immune checkpoint inhibitors, repair inhibitors) [3, 14, 15, 21, 24, 40].
- **Technological advances**: Scaleup of enzymatic synthesis in continuousflow/GMP settings, improved purification, robust biocatalysts, highthroughput cellular/ metabolic screening platforms [15, 17, 21, 22, 38].
- **Clinical translation**: Overcoming resistance (monitoring transporter/kinase/deaminase status), mitigating toxicity (biomarkers, longterm followup), improving targeted delivery (ligandtargeted nanoparticles, tumorspecific activation) [4, 12, 24, 40-42].
- **Systemslevel understanding**: Mapping how dAdo and analogues impact cellular networks (DNA damage/repair, methylation, immune signaling, metabolism), considering tumor microenvironment and immune-metabolic interplay [1, 7, 12, 25, 26, 31].

Conclusion

A unified view of dAdo reveals its dual role structurally as a deoxynucleoside, and functionally as a central regulator of dNTP homeostasis via salvage and de novo interplay. Disruption of this interplay underlies pathologies such as ADA deficiency. Meanwhile, synthetic modifications of the dAdo scaffold enable innovative pharmacological strategies exploiting transporter specificity, metabolic resilience and selective intracellular accumulation.

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