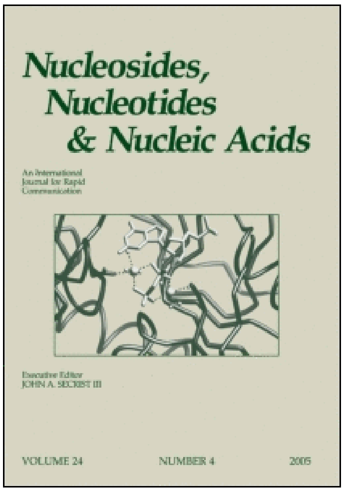

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Synthesis and Structural Analysis of Oxadiazole Carboxamide Deoxyribonucleoside Analogs

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SYNTHESIS AND STRUCTURAL ANALYSIS OF OXADIAZOLE CARBOXAMIDE DEOXYRIBONUCLEOSIDE ANALOGS

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□ *Two novel C-linked oxadiazole carboxamide nucleosides 5-(2′-deoxy-3′,5′-β-D-erythro-pentofuranosyl)-1,2,4-oxadiazole-5-carboxamide (***1***) and 5-(2′-deoxy-3′,5′-β-D-erythro-pentofuranosyl)-1,2,4-oxadiazole-3-carboxamide (***2***) were successfully synthesized and characterized by X-ray crystallography. The crystallographic analysis shows that both unnatural nucleoside analogs* **1** *and* **2** *adapt the C2′-endo ("south") conformation. The orientation of the oxadiazole carboxamide nucleobase moiety was determined as anti (conformer A) and high anti (conformer B) in the case of the nucleoside analog* **1** *whereas the syn conformation is adapted by the unnatural nucleoside* **2***. Furthermore, nucleoside analogs* **1** *and* **2** *were converted with high efficiency to corresponding nucleoside triphosphates through the combination chemo-enzymatic approach. Oxadiazole carboxamide deoxyribonucleoside analogs represent valuable tools to study DNA polymerase recognition, fidelity of nucleotide incorporation, and extension.*

Keywords Azole carboxamide nucleoside; Oxadiazole; Nucleoside triphosphate; C-Deoxyribonucleoside; Crystal structure; DNA polymerase

INTRODUCTION

The azole carboxamide nucleosides have been extensively studied in our laboratory and represent a series of isosteric nucleoside analogs that possess different numbers of heteroatoms (nitrogen, oxygen) variably placed within a 5-membered heterocycle framework resulting in unique distribution of electronic density.[1–3] Overall, azole carboxamide nucleoside analogs also

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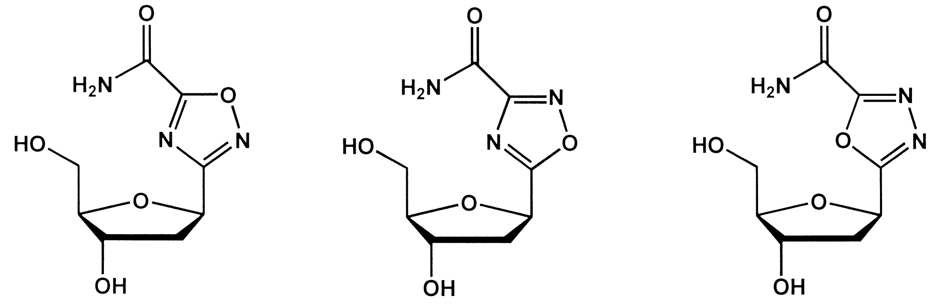
demonstrate size and shape similarities with natural nucleobases which together with unconstrained rotation around the glycosidic and carboxamide bonds provide a potential for degenerate recognition and base pairing through hydrogen bonding and electrostatic interactions.[1,3] Furthermore, it was found that azole carboxamide nucleosides could effectively mimic more than one natural nucleobase in DNA replication.[1,3] Also, due to various heteroatom substitution patterns, the azole carboxamide nucleoside family provides a tool set to study electronic interactions in the active site of DNA polymerases and factors governing fidelity and recognition of replication.

Based on previous studies with the triazole carboxamide series of nucleoside analogs, the presence of a heteroatom at both α and β positions was determined to be essential for the efficient and degenerate recognition and incorporation by *Taq* DNA polymerase.[1,3] In a series of N-nucleosides bearing the azole carboxamide framework, tetrazole carboxamide would seem to be a potential fully degenerate analog as it would have nitrogen atoms at all positions that could be involved in the minor and major grooves interactions. Unfortunately, due to chemical instability of the tetrazole carboxamide nucleobase, synthesis of this analog does not seem to be promising. A C-linked triazole or oxadiazole carboxamide deoxyribonucleoside would place heteroatoms at both α positions in the case of the oxadiazole, which would provide a heteroatom lone electron pair facing the minor groove in both the *syn* and *anti* conformations. In addition, a heteroatom at the β position could either project outward into the major groove or become engaged in interactions with the base pairing partner. The flexibility and length of the C-C1′ bond is close to that of the N-C1′-glycosidic bond and therefore, triazole or oxadiazole C-nucleosides are not likely to lose the structural versatility demonstrated by the triazole carboxamide N-nucleosides.

The series of C-linked oxadiazole carboxamide deoxyribonucleosides **1**-**3** (Figure 1) represents the second generation of the azole carboxamide nucleoside analogs which conserves structural features and expands a spectrum of electronic surface profiles of azole carboxamide nucleosides available for probing interactions of the DNA polymerase with the minor and major grooves of the DNA duplex.

This article describes the synthesis of two oxadiazole carboxamide deoxyribonucleoside analogs (**1** and **2**) and corresponding nucleoside triphosphates. Furthermore, we report the crystal structures and structural analysis of these novel C-deoxyribonucleosides. Unfortunately, the synthesis of the nucleoside analog **3** could not be accomplished due to apparent instability of the 2,5-substituted 1,3,4-oxadiazole moiety. The recognition of oxadiazole carboxamide deoxyribonucleotides **15** and **16** by DNA polymerases was investigated and will be reported elsewhere.



1 **2** **3**

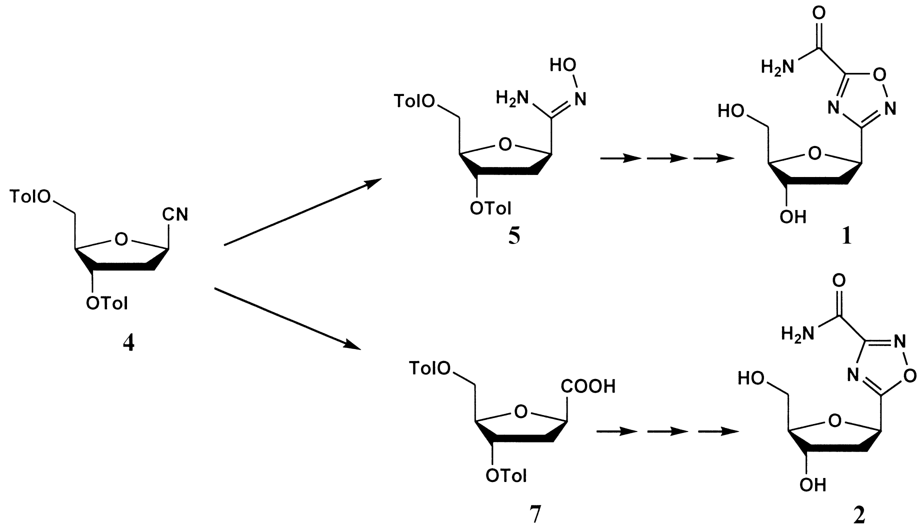
FIGURE 1 Chemical structures of 3-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxamide (**1**), 5-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3-carboxamide (**2**), and 5-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,3,4 oxadiazole-2-carboxamide (**3**).

RESULTS AND DISCUSSION

Synthesis of Oxadiazole Carboxamide Nucleosides 1 and 2

The synthesis of the oxadiazole carboxamide nucleosides **1** and **2** (Figure 1) was approached by building heterocycle scaffolds from a C1′ atom of the protected deoxyribofuranose. One of the common strategies for the synthesis of the 1,2,4-oxadiazole ring is a cyclization reaction of amidoximes with anhydrides or acyl chlorides.[4] A starting material for the stepwise synthesis of both nucleoside analogs was deoxyribofuranosylcyanide (**4**),[5] which was initially converted to an amidoxime derivative (**5**) in the case of nucleoside **1** or a carboxylic acid (**7**) in the case of nucleoside **2** (Scheme 1) followed by cyclization with appropriate acyl chloride or oxime to afford a desired 5-membered oxadiazole ring substituted with the carboxamide group. Free nucleosides **1** and **2** were obtained after deprotection and simultaneous transformation of the ethyl ester group to the carboxamide moiety.

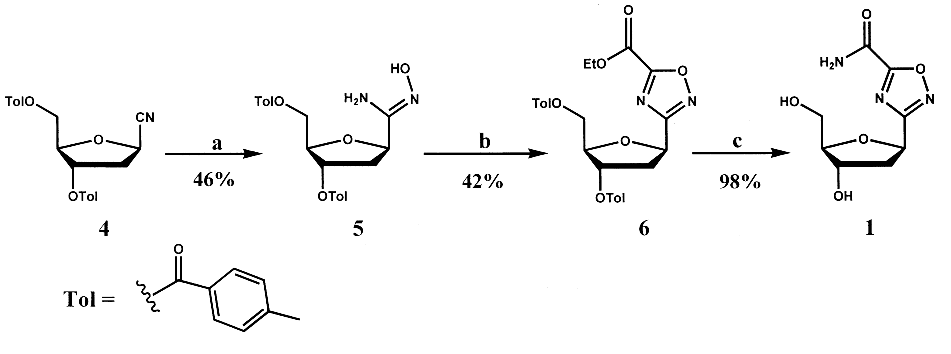
For the synthesis of 3-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxamide **1** (Scheme 2), the allonoamidoxime derivative (**5**) was generated by treatment of deoxyribofuranosylcyanide (**4**) with Na_2CO_3 and hydroxylamine hydrochloride by a method suggested by Revenkar and Robins.[6] Treatment of **5** with ethyl chlorooxoacetate[7] consequently led to cyclization and formation of *bis*-toluoyl protected ethyl-1,2,4-oxadiazole-5-carboxylate deoxyribonucleoside **6** as an anomeric mixture (α/β 20%/80%). The anomers were successfully separated by column liquid chromatography providing the pure β anomer in 42% yield. Deprotection and amidation were accomplished by treatment of **6** with methanolic ammonia in a pressure reactor (Parr) to provide pure 3-(2′-deoxy-3′,5′-



SCHEME 1 Synthesis of oxadiazole carboxamide nucleosides from the common precursor **4**.

β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxamide **1** as a single β anomer.

The generated compound (**1**) was characterized by ^{1}H and ^{13}C NMR spectroscopy. Initial verification of the anomeric conformation was done by the ^{1}H NMR analysis by calculating the sum of $J_{1',2'}$ and $J_{1',2''}$ values for a H′-1 proton.[8–10] Igolen and colleagues also indicated that in the case of C-deoxyribonucleosides, the NMR signals for the anomeric proton in the β configuration were broader with the sum of J values equal to 15.2–15.3 Hz.[9] The ^{1}H NMR analysis for 1,2,4-oxadiazole-5-carboxamide (**1**) clearly

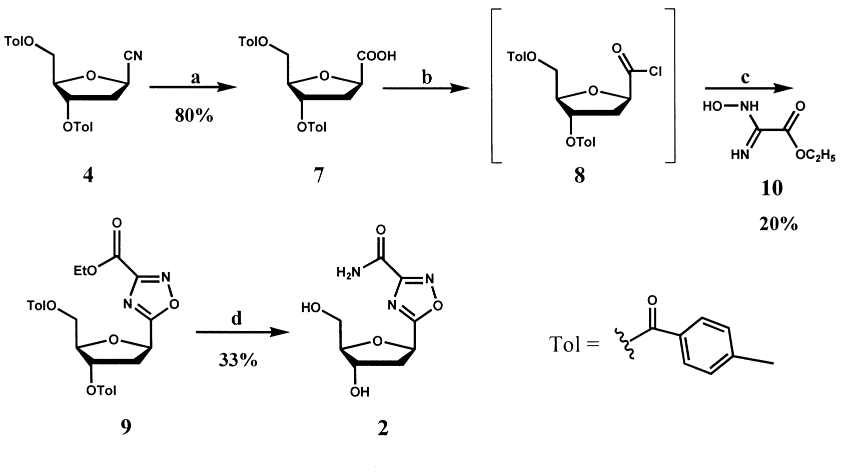


Reagents and Conditions:
a.$NH_2OH{\cdot}HCl$, Na_2CO_3, EtOH, 90-95°C, reflux, 2 hours ; **b**. ClCOCOOEt, Et_3N ;
c. NH_3, MeOH, 100 psi, 50°C, 3 days.

SCHEME 2 Synthesis of 5-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxamide **1**.

demonstrated a "pseudo-triplet" splitting pattern for H-1′ proton with the sum of *J* values equal to 15.7 Hz. Based on these data, the nucleoside **1** was initially assigned the β conformation, which was later confirmed by X-ray crystallographic analysis.

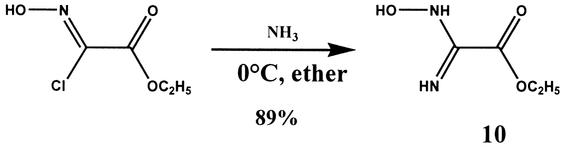
The synthetic approach to 5-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3-carboxamide **2** is illustrated in Scheme 3. In the initial step, the protected deoxyribofuranosylcyanide **4** was hydrolyzed using hydrochloric acid in dioxane according to a method of Bobek and Farkas[11] to provide 1-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-carboxylic acid (**7**). Treatment of **7** with oxalyl chloride[12] in benzene afforded acyl chloride derivative **8**, an intermediate that was neither isolated nor characterized, but was immediately used in the next step. Reaction of ethoxycarbonylformamide oxime (**10**) with the acyl chloride of protected ribofuranose **8** reported by Hennen and Robins for construction of a 1,2,4-oxadiazole-4-carboxylate scaffold[13] was then successfully applied for the synthesis of the protected ethyl-1,2,4-oxadiazole-3-carboxylate deoxyribonucleoside (**9**). Compound **10** was synthesized prior to the reaction by treatment of ethyl chlorooximidoacetate with gaseous ammonia at 0°C according to a procedure reported by Ungnade and Kissinger[14] (Scheme 4) and used after thorough drying in vacuo for 12 h at 25°C. The removal of the toluoyl protecting groups and ammonolysis of the carboxylic ester group was achieved by treatment of the β anomer of the intermediate **9** with methano-



Reagents and Conditions:

a. 4.0M HCl in dioxane, H_2O, 50°C; b. ClC(O)C(O)Cl, DMF, benzene, r.t.;
c. Ethoxycarbonylformamide oxime (**10**), pyridine, 1h at 80°C, 12-16 h at r.t.; d. NH_3, MeOH, 100 psi, room temperature, 24 hours.

SCHEME 3 Synthesis of 5-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3-carboxamide **2**.



10

SCHEME 4 Synthesis of ethoxycarbonylformamide oxime **10**.

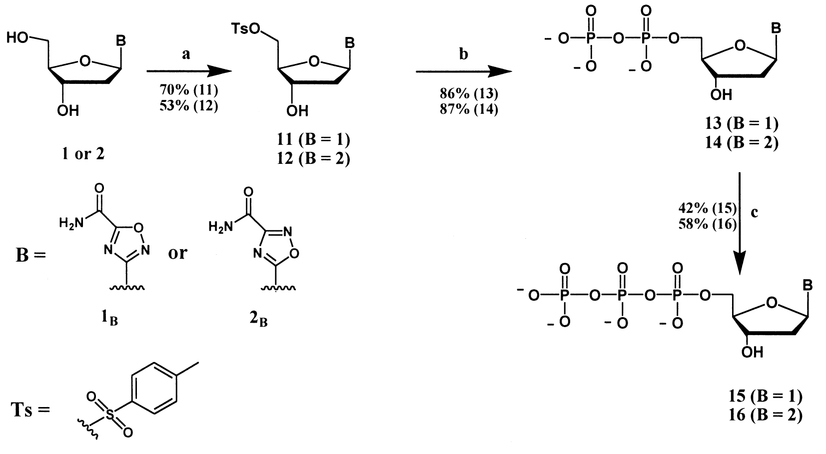
lic ammonia in a pressure reactor (Parr) for 24 h at room temperature. Unexpectedly, the isolated 1,2,4-oxadiazole-3-carboxamide deoxyribonucleoside **2** underwent epimerization during the deprotection step to give a mixture of anomers. The separation of this anomeric mixture was successfully achieved by repetitive recrystallization from absolute ethanol providing a pure β anomer of 1,2,4-oxadiazole-4-carboxamide deoxyribonucleoside (**2**) as a white crystalline compound in the 33% yield.

Deoxyribonucleoside analog **2** was characterized by ^{1}H and ^{13}C NMR. The anomeric proton H-1′ shows a double of doublets (pseudo-triplet) splitting pattern with the sum of J values equal to 15.6 Hz.[9,10] These data allowed preliminary assignment of the β anomeric conformation of the recrystallized nucleoside **2** that was later proved by X-ray crystallography.

Synthesis of Oxadiazole Carboxamide Nucleoside Triphosphates

Triphosphate derivatives of modified nucleosides are extremely valuable because they can serve as biotechnology tools or probes to study recognition specificity of DNA polymerases. However, synthesis and purification of nucleoside triphosphates can be challenging task due to solubility and stability factors.[15] Over the years, a number of methods for chemical phosphorylation of nucleosides has been developed and successfully used. However, the commonly used Yoshikawa procedure[16] and other methods[17,18] that employ the electrophilic phosphorylating reagents were not appropriate in the case of azole carboxamide nucleobases due to side reactions[19] and thus may not be always suitable for nucleosides bearing a modified base moiety due to different chemical reactivity, compatibility with reaction conditions and purification methods.

Recently, a number of azole carboxamide deoxy- and ribonucleosides were efficiently converted to the corresponding nucleoside triphosphates by an elegant method which combines chemical and enzymatic reaction steps to achieve high efficiency of the synthesis and purity of unnatural nucleotides.[19] The chemical step involves generation of a nucleoside diphosphate by a nucleophilic displacement of a 5′ leaving group (tosyl group, Ts) with *tris*(tetra-*n*-butylammonium) hydrogen pyrophosphate ($[n\mathrm{Bu}_4)_4]_3\mathrm{HP}_2\mathrm{O}_7$) (Scheme 5).[20] In a subsequent enzymatic step, a nucleoside diphosphate is phosphorylated by nucleoside diphosphate kinase (NDPK) that utilizes



Reagents and Conditions:
a. TsCl, pyidine, r.t., 24 hours; **b**. $(NBu_4)_3HP_2O_7$, CH_3CN, c. ATP, nucleoside diphosphate kinase (NDPK)

SCHEME 5 Synthesis of a 5′-deoxyribonucleoside diphosphates and triphosphates.

ATP as a donor of the γ phosphate group. Excellent results were achieved with deoxyribonucleosides **1** and **2** which were selectively tosylated at the 5′ hydroxyl group with *p*-toluenesulfonyl chloride in pyridine providing 5′-tosylated deoxyribonucleosides **11** and **12** in 70% and 53% yield, respectively. After the subsequent displacement reaction with *tris*(tetra-*n*-butylammonium) hydrogen pyrophosphate, ion exchange chromatography separation and purification afforded deoxyribonucleoside 5′ diphosphates **13** and **14** (**1**DP, **2**DP) in 86% and 87% yield (Scheme 5).[19]

As was expected, NDPK readily used the modified nucleoside diphosphates **13** and **14** (d**1**DP, d**2**DP) as substrates for phosphorylation completing the conversion to the respective triphosphates in 2–3 h. The isolation and purification of oxadiazole carboxamide nucleoside triphosphates was carried out by anion exchange chromatography to provide high purity nucleotides **15** and **16** in 42% and 58% yield, respectively. Characterization of **1**TP and **2**TP by ^{31}P NMR spectroscopy clearly demonstrated the presence of three sets of signals corresponding to the γ, β, α phosphorus atoms at around −9, −11, and −22 ppm, respectively. These data are in good accordance with literature values for deoxyribonucleoside triphosphate derivatives.[15]

X-Ray Analysis

Crystallization of the nucleosides **1** and **2** was performed by a vapor diffusion technique using methanol and ethyl acetate as solvent 1 and solvent 2, respectively.[21] Crystallographic analysis of nucleoside analogs **1** shows that the crystallographic unit cell contains two molecules (conformers) A and B (Figure 2), whereas one molecule (conformer) of the analog **2** is present in a single crystallographic unit cell (Figure 3). The summarized X-ray data is given in Tables 1 and 2 for the nucleoside analogs **1** and **2**, respectively. Selected geometric parameters (bond distance and certain torsion angles) are presented in Tables 3, 4, and 5. Analysis of the deoxyfuranose conformation was performed by calculating the pseudorotational phase angle (*P*) and the maximum torsion angle ($v_{\max}$) according to Altona and Sundaralingam[22,23] and confirmed using PROSIT (Pseudo-Rotational Online Service and Interactive Tool; cactus.nci.nih.gov/prosit).[24]

Both nucleoside analogs demonstrate a *β*-D-configuration. Conformational analysis of the nucleoside **1** indicates *P* values of 165.05° and 144.97° and $v_{\max}$ values of 37.21° and 40.78° for the A and B conformers, respectively (Table 5), suggesting that the sugar moieties of the both conformers adopt the C2′-*endo* (south) conformation common for deoxyribonucleosides.[23] Specifically, the envelope C2′-*endo* conformation is observed for the A conformer whereas the twisted C2′-*endo*-C1′-*exo* pucker is found for the B conformer. Furthermore, pseudorotational parameters calculated for the analog **2** ($P = 186.9°$ and $v_{\max} = 32.60°$) also indicate that a sugar moiety is twisted in a C2′-*endo*-C3′-*exo* conformation which also falls in the south region of the pseudorotation cycle.[23]

Determination of the glycosidic bond torsion angles for oxadiazole nucleobases was done using the atom sequences that most closely reflect a linked sequence of atoms defined by the glycosidic torsion angle in the nat-

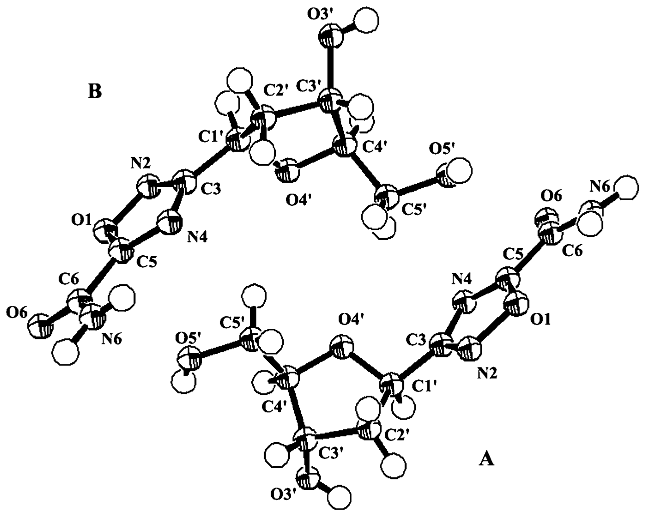


FIGURE 2 An ORTEP diagram of conformers A and B of the nucleoside analog **1**. Ellipsoids are drawn at 50% probability level.

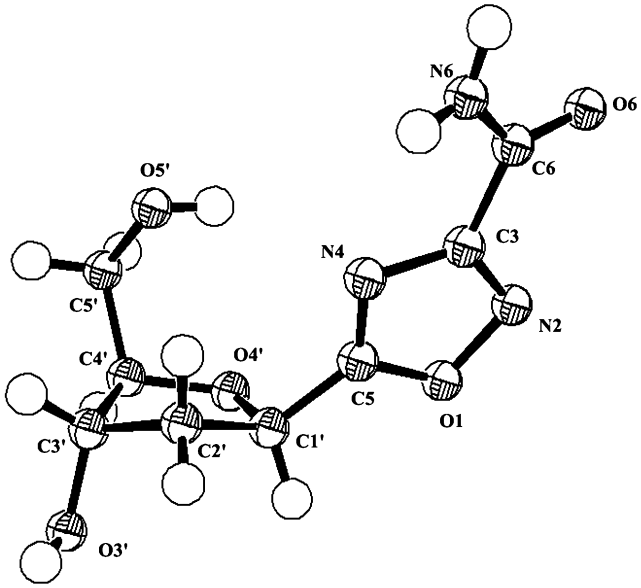


FIGURE 3 An ORTEP diagram of the nucleoside analog **2**. Ellipsoids are drawn at 50% probability level.

ural nucleosides. In the following description of results and discussion, the conformation of the glycosidic bond for 1,2,4-oxadiazole-5-carboxamide deoxyribonucleoside (**1**) will be defined by χ_1 (O4′-C1′-C3-N2) and, similarly, the glycosidic bond angle χ_1 (O4′-C1′-C5-O1) will be used to determine the nucleobase orientation for 1,2,4-oxadiazole-3-carboxamide deoxyribonucleoside (**2**) (Figure 4). According to published data,[23,25] values for χ_1 (O4′-C1′-C3-N2) in the case of both conformers of **1** correspond to the *anti* (**1**A, 122.29°) and *high anti* (**1**B, −85.56°) conformations or anticlinal (+*ac*) and synclinal (−*sc*) by the Klyne-Prelog nomenclature. In the case of the analog **2**, the glycosidic torsion angle χ_1 (O4′-C1′-C5-O1) was found to be −79.53° (Table 5), which corresponds to the *syn* conformation (+*sc*).[23,25] The values for a glycosidic torsion angle χ_2 which was defined by (O4′-C1′-C3-N4) and (O4′-C1′-C5-N4) atom sequences for nucleosides **1** and **2**, respectively, were found to be −59.91°(**1**A)/91.36°(**1**B) and 95.91° (**2**), respectively.

The crystal structure of the nucleoside analog **2** reveals that the 5′ hydroxyl group is oriented towards the nucleobase moiety and the O4′ atom. The orientation and conformation of the C4′-C5′ bond is defined by a torsion angle γ $\{\varphi_{oc}\}$ (O5′-C5′-C4′-C3′) which has a value of 65.51° corresponding to the *gauche* (+*sc*) conformation of the C4′-C5′ bond.[23] As a consequence, the distance between O4′ and O5′ is 2.834 Å that can suggest the presence of H-bonding interactions between 5′-OH and the furanose oxygen. Furthermore, in this conformation, the 5′-OH group is

TABLE 1 Crystal Data and Structure Refinement for the Nucleoside Analog **1**

Empirical formula	$C_8H_{11}N_3O_5$
Formula weight	229.19
Appearance	Whitish flakes
Temperature (K)	150 K
Wavelength	MO K_α (0.71073 Å)
Crystal system	Triclinic
Space group	P1
Unit cell dimension	a = 5.2820 (8) Å, α = 115.217 (7)° b = 9.7287 (13) Å, β = 97.018 (7)° c = 10.8443 (16) Å, γ = 99.543 (6)°
Volume (Å^3)	485.42 (12)
Z	2
Density (calculated) (g/cm^3)	1.568
Absorption coefficient (mm^{-1})	0.123
F(000)	240.0
Crystal size	0.33 × 0.23 × 0.05
Theta range for date collection	4.25°–50.14°
Index (h, k, l) range	−6 to 6, −11 to 11, −12 to 12
Reflections collected	3256
Independent reflections	2599
Absorption correction	Empirical
Max and min transmission	0.418 and 0.994
Refinement method	Full matrix least-squares on F^2
Data/restraints/parameters	2598/0/309
Goodness-of-fit on F^2	1.095
Final R indices [I > 2sigma(I)]	R1 = 0.051, wR2 = 0.124
Absolute structure parameter	0.50
Largest diff. peak and hole (e/Å^{-3})	0.22 and −0.24

in close proximity (3.28 Å) to the oxadiazole ring and may interact weakly with N4. In contrast, the C4′-C5′ bond of the nucleoside analog **1** adapts the -*gauche* (−*sc*) conformation ($\gamma\{\varphi_{oc}\} = -78.23°$ (A) and $-75.00°$ (B)).[23] As a result of this torsion angle, the 5′-CH$_2$OH is facing away from the furanose ring and can not be involved in interactions with the oxadiazole nucleobase. Previously, the +*gauche* (+*sc*) conformation ($\varphi_{oc} = 54.59°$ and $\varphi_{oo} = -66.17°$) was observed for the 5′-CH$_2$OH group in 3-nitropyrrole deoxyribonucleoside (**25**) and the further analysis suggested that the 5′-OH group could be involved in the stabilization of the sugar pucker (C4′-*endo*) and the base conformation (*syn*) of this nucleoside analog.[26]

Survey of Crystal Structure Data for Natural and Modified N- and C-Deoxyribonucleosides

An extensive literature search revealed only limited amounts of crystal structure data obtained for natural and modified N-linked deoxyribonucleosides and very few solved crystal structures for C-linked deoxyribonucleosides. In the case of natural N-deoxyribonucleosides **16** and **17** (Figure 4),

TABLE 2 Crystal Data and Structure Refinement for the Nucleoside Analog **2**

Empirical formula	$C_8H_{11}N_3O_5$
Formula weight	229.19
Appearance	White crystals
Temperature (K)	150 K
Wavelength	MO K_α (0.71073 Å)
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimension	a = 5.14510 (10) Å b = 10.6621 (3) Å c = 17.2450 (7) Å
Volume (Å^3)	946.02 (5)
Z	4
Density (calculated) (g/cm^3)	1.609
Absorption coefficient (mm^{-1})	0.127
F(000)	480.0
Crystal size	0.44 × 0.30 × 0.29
Theta range for date collection	4.49°–55.74°
Index (h, k, l) range	−6 to 6, −13 to 14, −22 to 22
Reflections collected	5421
Independent reflections	2199
Absorption correction	Empirical
Max and min transmission	0.90 and 0.96
Refinement method	Full matrix least-squares on F^2
Data/restraints/parameters	2193/0/162
Goodness-of-fit on F^2	1.036
Final R indices [I > 2sigma(I)]	R1 = 0.029, wR2 = 0.065
Absolute structure parameter	−0.40
Largest diff. peak and hole (e/Å^{-3})	0.15 and −0.17

TABLE 3 Intramolecular Bond Distances for the Nucleoside Analog **1**

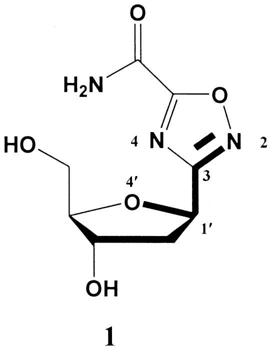
	Distance (Å)	
Bond	Conformer A	Conformer B
O1-C5	1.342 (6)	1.342 (6)
O1-N2	1.423 (5)	1.423 (5)
O6-C6	1.239 (5)	1.215 (6)
O3′-C3′	1.440 (5)	1.444 (5)
O5′-C5′	1.436 (5)	1.426 (5)
O4′-C1′	1.452 (5)	1.434 (5)
O4′-C4′	1.453 (5)	1.446 (5)
N2-C3	1.307 (6)	1.300 (6)
N4-C5	1.293 (6)	1.301 (6)
N4-C3	1.392 (6)	1.382 (6)
N6-C6	1.311 (6)	1.326 (6)
C3-C1′	1.486 (6)	1.496 (6)
C5-C6	1.494 (6)	1.513 (6)
C1′-C2′	1.508 (6)	1.518 (6)
C2′-C3′	1.526 (6)	1.516 (6)
C3′-C4′	1.522 (6)	1.537 (6)
C4′-C5′	1.527 (6)	1.509 (6)

TABLE 4 Intramolecular Bond Distances for the Nucleoside Analog **2**

Bond	Distance (Å)
O1-C5	1.3447 (17)
O1-N2	1.4041 (13)
O6-C6	1.2300 (17)
O3′-C3′	1.4350 (16)
O5′-C5′	1.4242 (18)
O4′-C1′	1.4396 (17)
O4′-C4′	1.4467 (17)
N2-C3	1.3038 (18)
N4-C5	1.2969 (18)
N4-C3	1.3765 (18)
N6-C6	1.3260 (19)
C5-C1′	1.4998 (19)
C3-C6	1.5043 (19)
C1′-C2′	1.521 (2)
C2′-C3′	1.524 (2)
C3′-C4′	1.520 (2)
C4′-C5′	1.5189 (19)

TABLE 5 Values for Selected Torsion Angles and Calculated Pseudorotational Parameters for the Nucleoside Analogs **1** and **2**

Molecule	ν_0 (°)	ν_1 (°)	ν_2 (°)	ν_3 (°)	ν_4 (°)	χ_1 (°)	χ_2 (°)	γ (φ_{oc}) (°)	γ (φ_{oo}) (°)	P (°)	ν_{max} (°)
1A	−30.27	37.65	−30.62	13.4	10.53	122.29	−59.91	−78.23	163.95	165.05	37.21
1B	−23.0	38.8	−39.41	26.83	−2.78	−85.56	91.36	−75.00	167.33	144.97	40.78
2	−8.4	25.71	−32.53	28.28	−12.67	−79.53	95.91	65.51	−54.45	186.9	32.6



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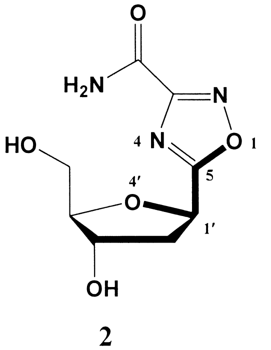

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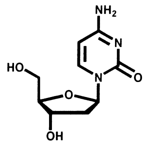
FIGURE 4 Glycosidic bond torsion angles defined for oxadiazole carboxamide nucleosides **1** and **2**.

the glycosidic bond length ranges from 1.472 to 1.474 Å with the nucleobase moiety oriented *anti*. The conformation of the furanose ring observed for the natural deoxyribonucleosides (2′-deoxyriboadenosine and 2′-deoxyribocytidine) is C2′-*endo*, or the south conformation, commonly found in natural deoxyribonucleosides and nucleotides. Interestingly, analysis of the crystal structure of 2′-deoxyribocytidine, which shows two conformers in a single crystal cell, indicates the C3′-*endo* conformation for one of the conformers.[27] This is an unusual result considering that the C3′-*endo* conformation ("north") is characteristic for naturally occurring ribonucleosides.

Modified N-nucleosides, as illustrated by deoxyribonucleosides analogs **18-26**, (Figure 5, Table 6) show the glycosidic bond length between 1.508 Å (**18**) and 1.425 Å (**26**) and the glycosidic bond torsion angle χ places the nucleobase moieties in either *anti* or *high anti* conformation. The *syn* conformation of a nucleobase is observed for the nucleoside analogs **26** and **28**. Compared to nucleosides **16** and **17**, the C-N glycosidic bond was found to be significantly shorter for modified purine and pyrrole nucleosides **19-25** and **26**. Modification of the nucleobase moiety can have great influence on the conformation of the furanose ring. Modified N-deoxyribonucleosides **18-19** display the C3′-*endo* conformation ("north") (Figure 5, Table 6) and the unusual for deoxyribonucleosides C1′-*endo* ring pucker is observed for analogs **20-21** (Figure 5, Table 6). It was suggested that introduction of the electron-withdrawing group (-Br, -I) can result in a stereoelectronic effect (through-bond and through-space interactions) and promote an attractive interaction between the nucleobase moiety (the imidazole ring in the case of compounds **18-20**) and the O4′ of the sugar ring.[28,29] As a consequence, the glycosidic torsion angle shifts to the *high anti* conformational range causing switching of the furanose ring from the C2′-*endo* ("south") to the C3′-*endo* ("north") conformation. The sugar moiety of 3-nitropyrrole deoxyribonucleoside (**26**) is in the C4′-*endo* pucker which is also uncommon for a deoxyribonucleoside and could be a result of the stereoelectronic effect induced by the nitropyrrole.

Rather scarce structural data is available for C-deoxyribonucleosides: only a few crystal structures have been solved. A compound of a special interest would be 2-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-thiazole-4-carboxamide (**28**) because of its close resemblance to the oxadiazole carboxamide analogs.[30,31] Crystallographic analysis of this nucleoside analog indicates that the C1′-C2 bond length has a value of 1.489 Å which is slightly longer than most glycosidic C1′-N bonds found in the natural and modified deoxynucleosides (1.425–1.481 Å) (Table 6). This result is consistent with values obtained for the oxadiazole deoxynucleosides **1** [1.486 Å (A)/1.496 Å (B)] and **2** (1.499 Å) (Table 5). The conformational analysis of the furanose moiety of the thiazole-4-carboxamide provides $P = 173.1°$, which corresponds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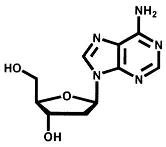
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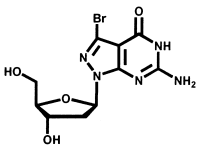
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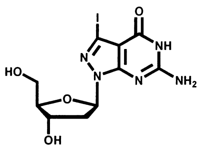
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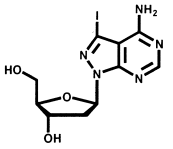
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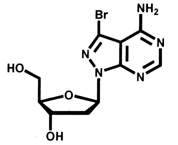
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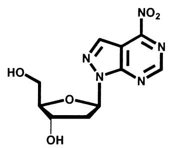
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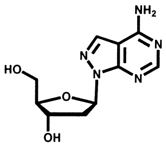
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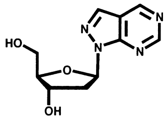
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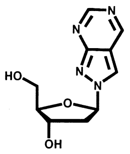
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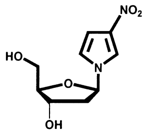
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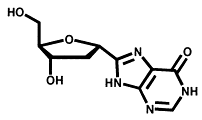
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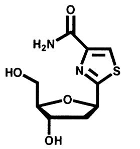
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16	1-*β*-2'-deoxy-D-ribofuranosylcytosine
17	1-*β*-2'-deoxy-D-ribofuranosyladenine
18	3-Bromo-1-[2'-deoxy-*β*-D-*erythro*-pentofuranosyl]-4-propoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-amine
19	1-[2'-deoxy-*β*-D-*erythro*-pentofuranosyl]-3-iodo-4-propoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-amine
20	4-Amino-1-(2'-deoxy-*β*-D-*erythro*-pentofuranosyl)-3-iodo-1*H*-pyrazolo-[3,4-*d*]pyrimidine
21	4-Amino-1-(2'-deoxy-*β*-D-*erythro*-pentofuranosyl)-3-bromo-1*H*-pyrazolo-[3,4-*d*]pyrimidine
22	1-(2'-2-deoxy-*β*-D-*erythro*-pentofuranosyl)-4-nitro-1*H*-indazole
23	4-Amino-1-(2'-deoxy-*β*-D-*erythro*-pentofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine
24	1-(2-deoxy-β–D-*erythro*-furanosyl)-1*H*-pyrazolo[3,4-d]pyrimidine
25	2-(2-deoxy-β–D-*erythro*-furanosyl)-2*H*-pyrazolo[3,4-d]pyrimidine
26	1-(2'-deoxy-*β*-D-ribofuranosyl)-3-nitropyrrole
27	8-(2'-deoxy-*α*-D-*erythro*-pentofuranosyl)-1,7-dihydro-6H-purine-6-one
28	2-(2'-deoxy-*β*-D-*erythro*-pentofuranosyl)thiazole-4-carboxamide

FIGURE 5 Natural and modified deoxyribonucleosides whose crystal structures have been solved.

TABLE 6 Structural and Conformational Parameters for Natural and Modified Deoxyribonucleosides

Nucleoside	Glycosidic bond length (Å)	Glycosidic torsion angle, χ	Nucleobase conformation	Pseudorotational phase angle P (°)	Maximum torsion angle, $v_{\max}$ (°)	Furanose conformation
16 (I)[27]	1.491	201.2° (O1′-C1′-N1-C2)	*Anti*	13.5[a]	36.8[a]	C3′-*endo*-C2′-*exo*, ${}^3T_2$
16 (II)[27]	1.472	222.2° (O1′-C1′-N1-C2)	*Anti*	168.8[a]	38.3[a]	C2′-*endo*, ${}^2T_3$
17[33]	1.474	23.4° (O1′-C1′-N9-C8)	*Anti*	36.9	102.2	C3′-*exo*, E_3
18[39]	1.443	−92.9° (O4′-C1′-N9-C4)	*High anti*	36.78[a]	33.8[a]	C3′-*endo*-C2′-*exo*, ${}^3T_2$
19[39]	1.441	−93.2° (O4′-C1′-N9-C4)	*High anti*	33.16[a]	32.13[a]	C3′-*endo*-C2′-*exo*, ${}^3T_2$
20[29]	1.480	−73.2° (O4′-C1′-N9-C4)	*High anti*	309.4	34.8	C1′-*endo*, ${}^1E$
21[29]	1.473	−74.1° (O4′-C1′-N9-C4)	*High anti*	310.9	35.0	C1′-*endo*, ${}^1E$
22[28]	1.449	−105.3° (O4′-C1′-N9-C7)	*Anti/High anti*	192.6	37.5	C2′-*endo*-C3′-*exo*, ${}^2T_3$
23[32]	1.442	−106.3° (O1′-C1′-N9-C4)	*Anti/High anti*	182.2	41.2	C2′-*endo*-C3′-*exo*, ${}^2T_3$
24[40]	1.448	−100.4° (O1′-C1′-N1-C7)	*High anti*	185.6	40.3	C2′-*endo*-C3′-*exo*, ${}^2T_3$
25[40]	1.481	15.0° (O1′-C1′-N2-N3)	*Anti*	203.3	37.0	C3′-*exo*, ${}_3E/{}^4T_3$
26[26]	1.425	−45.85° (O4′-C1′-N1-C2)	*Syn*	232.9	−24.4	C4′-*endo*, ${}^4E$
27[41], α anomer	1.489	−91.2° (O4′-C1′-N9-C8)	*Anti*	29.04[a]	41.4[a]	C3′-*endo*, ${}^3T_4$
28[30]	1.489	40.8° (O1′-C1′-C2-S)	*Syn*	173.1	37.7	C2′-*endo*-C3′-*exo*, ${}^2T_3$

ND: not determined by authors.

[a] Value was not reported in a primary source but was calculated based on the published data.

to the C2′-*endo* ("south") conformation. The conformation of the thiazole-4-carboxamide moiety relative to the sugar is determined to be *syn* and is possibly favored by intramolecular interactions between the sulfur and O4′ atoms.[30] Thus, the results obtained for oxadiazole deoxyribonucleoside analogs **1** and **2** also correspond well with the structural parameters derived from the crystal structure analysis of natural and modified deoxyribonucleosides (Tables 5 and 6).[26–28,32–36]

EXPERIMENTAL

General Procedures

All chemicals used in nucleoside and nucleoside triphosphate synthesis were purchased from Sigma-Aldrich Chemical Company and were used without any further purification except for oxalyl chloride, which was distilled prior to the synthesis. Solvents involved in reactions under anhydrous conditions were also distilled from an appropriate drying agent (CaH_2) or purchased from Sigma-Aldrich. 2-Deoxy-D-ribose was obtained from Crystal Chem Inc. (Downers Grove, Illinois). Gaseous ammonia (anhydrous grade) was obtained from Indiana Oxygen Company (Indianapolis, IN). Column chromatography and high-performance liquid chromatography (HPLC) were performed with solvents that were either spectroscopic or HPLC grade. Silica gel for column chromatography was obtained either from Silicycle, Quebec, Canada (40–60 μm, 230–400 mesh, 60 Å) or purchased from SAI (Scientific Adsorbents Incorporated, Atlanta, Georgia) (32–63 μm, 60 Å). Thin-layer chromatography was performed using pre-coated polyester or aluminum silica plates with a fluorescent indicator (Sigma-Aldrich, 200–250 μm). Detection was done by UV absorption ($\lambda = 254$ nm) unless otherwise specified. Cellulose TLC was done using plates (0.1 mm thickness) from EM Science. For flash cellulose chromatography Whatman CF-11 fibrous cellulose was used.

Analytical HPLC analysis was performed on Beckman HPLC system (128 Solvent Module, 166 detector) using System Gold Nouveau™ program software. A column employed in HPLC analysis was a SUPELCOSIL LC-18-T column (25 cm × 4 mm, 3 μm). NMR spectra (^1H, ^{13}C, ^{31}P) were recorded either on a Bruker AC250 or on a Bruker ARX300 spectrometer. Acquisition of ^{13}C and ^{31}P spectra was done with broadband gated decoupling. Solvents used in NMR analysis (acetone-d_6, CD_3CN, $CDCl_3$, CD_3OD, CD_2Cl_2, D_2O) were obtained from Cambridge Isotope Laboratories. In the case of ^1H and ^{13}C spectra, the solvent was used as reference, whereas ^{31}P spectra were referenced to external 85% phosphoric acid. For high resolution ^{31}P spectra, a solution of NaEDTA in 10 mM concentration was added to the sample. All mass spectrometry analyses including MALDI were done at the Purdue University Campus-Wide Mass Spectrometry Center. Determination of elemental composition was performed in by the Purdue University Microanalysis Laboratory.

2′-Deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-*erythro*-ribofuranosyl-cyanide (**4**) served as a starting material for synthesis of the nucleoside analogs. This precursor was synthesized according to a previously described method[5] from 1-chloro-2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose.[37] Deoxyribofuranosylcyanide (**4**) was used in the reactions described below as an anomeric mixture (α/β 20%:80%).

Preparation of 3-(2′-Deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxamide (1)

2,5-Anhydro-3-deoxy-4,6-di-*O*-toluoyl-β-D-*erythro*-ribofuranosyl Amidoxime (5). 2′-Deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-ribofuranosyl-cyanide (**4**) (3.022 g, 0.0079 mol) was dissolved in 30 mL of absolute ethanol by heating in a 100 mL round bottom flask in an oil bath. Hydroxylamine hydrochloride (3.87 g, 0.056 mol) was dissolved in 35 mL of ddH_2O followed by addition of anhydrous Na_2CO_3 (2.94 g, 0.0278 mol). This mixture was stirred until all Na_2CO_3 dissolved. The resulting solution was then quickly added to the ethanolic solution of deoxyribofuranosylcyanide and the resulting reaction mixture was stirred under gentle reflux (90–95°C) for 1.5–2.0 h under N_2. Upon completion, ethanol was removed by rotary evaporation and the desired product was extracted with ethyl acetate (3 × 60 mL). The organic layers were combined, washed with 0.4 M HCl (60 mL), cold water (3 × 60 mL), dried over the anhydrous Na_2SO_4, and filtered. The solvent was removed under reduced pressure affording a white foam.

The pure deoxyribose amidoxime **5** was obtained by column chromatography eluting with petroleum ether:acetone (3:1) and affording the desired product as a white foam (1.46 g, 46% yield). TLC (9:1 Benzene/Acetone) $R_f = 0.17$; ^{1}H NMR (250 MHz, Acetone-d_6): δ 8.58 (s, 1H, N-OH), 8.03–7.89 (m, 4H, H-Ar), 7.41–7.25 (m, 4H, H-Ar), 5.61–5.41 (m, 1H, 3′-H), 5.04 (m, 1H, NH_2), 4.67–4.40 (m, 5H, 1′-H, 4′-H, 5′-H), 2.65–2.48 (m, 1H, 2′-H^a), 2.44–2.38 (s, 6H, CH_3), 2.37–2.25 (m, 1H, 2′-H^b). MS (ESI): [M+1] = 413, [M+Na$^+$] = 435.

Ethyl (2′-Deoxy-3′,5′-di-*O*-p-toluoyl-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxylate (6). Amidoxime **5** (0.5 g, 1.2 mmol) was dissolved in 6.7 mL of butyl acetate followed by gradual addition of triethylamine (0.264 g, 2.6 mmol, $\rho = 0.726$ g/mL). Then ethyl chlorooxoacetate (0.364 g, 2.6 mmol, $\rho = 1.222$ g/mL) was added resulting in appearance of a white cloudy precipitate. The reaction mixture was heated under reflux for 2 h and completeness of the reaction was confirmed by TLC ($CHCl_3$) that showed appearance of a single spot corresponding to the desired product

6 ($R_f = 0.28$). The reaction mixture was subsequently cooled and washed twice with water (10–15 mL), dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated in vacuo. The product **6** was eluted with chloroform from the crude reaction mixture as yellow oil (0.394 g, 42% yield). TLC (100% $CHCl_3$), R_f 0.28; ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.032–7.86 (m, 4H, H-Ar), 7.32–7.17 (m, 4H, H-Ar), 5.64 (d, $J = 6$ Hz, 1H, 3′-H), 5.48 (dd, $J = 10, 10$ Hz, 1H, 1′-H), 4.67-4.42 (m, 5H, 1′-H, 4′-H, 5′-H, CH_2CH_3), 2.87–2.69 (m, 1H, 2′-H^a), 2.66–2.53 (m, 1H, 2′-H^b), 2.39 (d, $J = 6$ Hz, 6H, CH_3), 1.43 (t, $J = 7$ Hz, 3H, CH_2CH_3). MS (ESI): [M+1] = 495, [M+Na$^+$] = 517.

3-(2′-Deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxamide (1). Ethyl (2′-deoxy-3′,5′-di-*O*-p-toluoyl-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxylate (**6**) (0.273 g, 0.55 mmol) was dissolved in 20 mL of freshly distilled methanol and cooled to −78°C. Liquid ammonia (~20 mL) was collected to the flask containing the methanolic solution of **6** and the resulting mixture was transferred to a glass lined 500 pressure reactor (Parr), sealed and heated at 50°C (~100 psi) for 48 h, after which appearance of the product was checked by TLC in ethyl acetate:methanol (8:1) ($R_f = 0.47$). The solvent was removed by rotary evaporation and a resulting residue was dissolved in 25 mL of water and 25 mL of dichloromethane. Unreacted starting material and by-products of deprotection were removed by extraction with dichloromethane (5 × 25 mL). The TLC of the aqueous layer indicated a single spot corresponding to a deprotected nucleoside. The aqueous layer was evaporated in vacuo, affording a yellowish-white solid **1** (0.126 g, 98% yield). TLC (8:1 EtOAc/Hex) R_f 0.47; ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.79 (br s, 1H, NH_2, D_2O exchangeable), 8.39 (br s, 1H, NH_2, D_2O exchangeable), 5.24–5.14 (m, 2H, 1′-H, 3′-OH, D_2O exchangeable), 4.70 (t, $J = 5.7$, 1H, 5′-OH, D_2O exchangeable), 4.26 (m, 1H, 3′-H), 3.82 (td, $J = 2.6$ and 5.7 Hz, 1H, 4′-H), 3.43–3.35 (m, 2H, 5′-H), 2.27 (ddd, $J = 5.4, 8.3, 12.9$, 1H, 2′-H^a), 2.15 (ddd, $J = 2.7, 6.6, 12.9$, 1H, 2′-H^b); ^{13}C NMR (300 MHz, CD_3OD): δ 172.54, 170.87, 156.70, 89.77, 73.76, 72.78, 65.74, 40.52. MS (ESI): [M+1] = 230, [M+Na$^+$] = 252. High res. MS (ESI): m/z calcd. for $C_8H_{11}N_3O_5$ 230.0777, actual 230.0785.

Preparation of 5-(2′-Deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3-carboxamide (2)

1-(2′-Deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-carboxylic Acid (7). 2′-Deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-*erythro*-ribofuranosyl-cyanide (**4**) (2 g, 5.3 mmol) was dissolved in 5.3 mL of dioxane followed by addition of 2.12 mL of 4.0 M solution of HCl in 1,4-dioxane and 0.286 g of H_2O (15.9 mmol). This mixture was heated to 50°C for 7–8 h under nitrogen. During the course of the reaction, a white precipitate (NH_4Cl) appeared.

The course of the reaction was followed by TLC using 85:15 benzene:EtOAc. Upon completion, 20 mL of ethyl ether was added to the reaction mixture and the precipitate was removed by filtration. The filtrate was evaporated in vacuo leaving a brown oil. The carboxylic acid **7** was isolated by column chromatography eluting with 15-50% ethyl acetate in toluene gradient affording a yellowish oil (1.67 g, 80% yield). TLC (85:15 benzene:ethyl acetate, R_f 0.2; ^{1}H NMR (250 MHz, CD_3Cl): δ 7.97–7.85 (m, 4H, H-Ar), 7.28–7.18 (m, 4H, H-Ar), 5.49 (d, J = 5.9, 1H, 3′-H), 4.8 (dd, J = 10.3, 10.1 Hz, 1H, 1′-H), 4.72–4.52 (m, 5H, 4′-H, 5′-H), 2.72–2.56 (m, 1H, 2′-H^a), 2.49–2.29 (m, 7H, CH_3, 2′-H^b). MS (ESI): [M+1] = 399, [M+Na$^+$] = 421.

Ethoxycarbonylformamide Oxime (10)[14]. Ethyl chlorooximido acetate (2 g, 13 mmol) was dissolved in 100 mL of anhydrous ethyl ether and cooled to 0°C. Dry ammonia gas was bubbled into the solution for 7–10 min and during this time a white-yellowish precipitate appeared. The reaction mixture was filtered and the filtrate was evaporated in vacuo, leaving a white, slightly yellow hydroscopic fluffy solid **10** (1.53 g, 89% yield). ^{1}H NMR (250 MHz, DMSO-d_6): δ 10.3 (s, 1H, N-OH), 5.71 (s, 2H, NH_2), 4.18 (q, J = 7.2, 2H, OCH_2CH_3), 1.23 (t, J = 7.1, 3H, OCH_2CH_3).

1-(2′-Deoxy-3′,5′-di-*O*-p-toluoyl-*β*-D-*erythro*-pentofuranosyl) Acyl Chloride (8). To a solution of the carboxylic acid **7** (1.58 g, 4 mmol) in anhydrous benzene (15.8 mL) was added a couple of drops of *N,N*-dimethylformamide (DMF) followed by addition of freshly distilled oxalyl chloride (1.0416 mL, 12 mmol). The reaction was run at room temperature under an inert atmosphere (N_2) for 2 h. Then the reaction mixture was evaporated in vacuo to remove the solvent and excess oxalyl chloride. The resulting residue appeared as a yellow-orange oil. The obtained acyl chloride **8** was neither isolated nor characterized but was taken immediately to the next step due to instability of this intermediate.

Ethyl-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-*β*-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3-carboxylate (9). Ethoxycarbonylformamide oxime (**10**) (0.578 g, 4.4 mmol) was added to the yellow oily residue of acyl chloride **8** (theor. 3.9 mmol) followed by addition of 19.9 mL of freshly distilled pyridine. The reaction mixture was heated to 80°C for 1 h under nitrogen while stirring. Initially, the solution turned purple then gradually changed in color to brown-red. Then, the reaction mixture was left at room temperature for 18–16 h while stirring. After 18 h, TLC (hexane:ethyl acetate 3:2) showed a complex mixture of products with one predominant spot (R_f = 0.7). The reaction mixture was concentrated under reduced pressure followed by coevaporation with toluene (2 × 10 mL) to remove residual pyridine leaving a brown-reddish oil. The product was isolated by flash column chromatography and a gradient eluant from 0–20% ethyl acetate in hexanes to afford the compound **9** as a yellow oil (0.4338 g, 20% yield). TLC (3:2 Hex/EtOAc) R_f 0.7; ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.98–7.86 (m, 4H, H-Ar), 7.30–7.12 (m, 4H, H-Ar), 5.56 (d, $J = 5.6$ Hz, 1H, 3′-H), 5.53 (dd, $J = 9.5$, 9.8 Hz, 1H, 1′-H), 4.66–4.42 (m, 5H, 1′-H, 4′-H, 5′-H, CH_2CH_3), 2.97–2.77 (m, 1H, 2′-H^a), 2.77–2.62 (m, 1H, 2′-H^b), 2.4 (d, $J = 6.7$ Hz, 6H, CH_3), 1.42 (t, $J = 7.2$ Hz, 3H, CH_2CH_3). MS (ESI): [M+1] = 495, [M+Na$^+$] = 517. High res. MS (ESI): m/z calcd. for $C_{26}H_{26}N_2O_8$ 495.1767, actual 495.1778.

5-(2′-Deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3-carboxamide (2). Ethyl-(2′-deoxy-3′,5′-di-*O*-p-toluoyl-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**9**) (1.4886 g, 3.0 mmol) was dissolved in 30 mL of freshly distilled methanol and cooled to −78°C. Liquid ammonia (∼40 mL) was collected and added to the methanolic solution of **9**. The resulting mixture was then transferred to a glass lined 500 mL pressure reactor (Parr), sealed and kept at room temperature (∼100 psi). The reaction was allowed to run for 1–2 days after which the reaction was stopped and presence of a desired product was checked by TLC in ethyl acetate:methanol 8:1 ($R_f = 0.5$). The solvent was removed by rotary evaporation and a resulting residue was dissolved in 25 mL of water and 25 mL of dichloromethane. Unreacted starting material and by-products of deprotection were removed by extraction with dichloromethane (5 × 25 mL). The TLC of the aqueous layer indicated a single spot corresponding to deprotected nucleoside. The aqueous layer was evaporated in vacuo affording a white solid **2** (0.3907 g, 56% yield) that appeared to be a mixture of α and β anomers by ^{1}H NMR. Pure β anomer was obtained after repetitive (twice) re-crystallization from absolute ethanol providing the desired product as a white fluffy solid (0.227 g, 33% yield). TLC (8:1 EtOAc/MeOH) R_f 0.5; ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.3 (br s, 1H, NH_2, D_2O exchangeable), 8.1 (br s, 1H, NH_2, D_2O exchangeable), 5.36–5.23 (m, 2H, 1′-H, 3′-OH, D_2O exchangeable), 4.75 (t, $J = 5.8$, 1H, 5′-OH, D_2O exchangeable), 4.23–4.32 (m, 1H, 3′-H), 3.86 (td, $J = 2.2$ and 5.4 Hz, 1H, 4′-H), 3.45–3.35 (m, 2H, 5′-H), 2.44–2.19 (m, 2H, 2′-H^a, 2′-H^b); ^{13}C NMR (300 MHz, CD_3OD): δ 181.79, 164.80, 160.39, 90.07, 73.48, 72.97, 63.48, 40.63. MS (ESI): [M+1] = 230, [M+Na$^+$] = 252. High res. MS (ESI): m/z calcd. for $C_8H_{11}N_3O_5$ 230.0777, actual 230.0776.

Preparation of 2′-Deoxy-β-D-ribofuranosyl-1,2,4-oxadiazole-5-carboxamide-5′-triphosphate (15, 1TP)

2′-Deoxy-β-D-ribofuranosyl-1,2,4-oxadiazole-5-carboxamide-5′-tosylate (11). Oxadiazole carboxamide deoxyribonucleoside **1** (0.100 g, 0.43 mmol) was co-evaporated with dry pyridine (3 × 5 mL) and dried in a vacuum oven overnight to remove residual moisture. The reaction was initiated by addition of 100 mg (0.52 mmol) of *p*-toluenesulfonyl (Ts) chloride followed by addition of 3 mL of dry pyridine. The reaction mixture was stirred under nitrogen at room temperature for 24 h. The completion of the reaction was monitored by TLC (EtOAc:MeOH 8:1), which showed disappearance of starting material and the presence of a product ($R_f = 0.71$). Silica gel (1.5 g) was added to the reaction mixture and pyridine was removed by rotary evaporation. Then, this silica was co-evaporated with methanol (2 × 5 mL) to remove residual pyridine, dried, and applied onto a flash chromatography column. The product was eluted with a gradient eluant from 0–9% methanol in ethyl acetate. Fractions corresponding to a desired product were combined and solvents were removed under reduced pressure affording 5′-tosylate **11** as a white solid (0.117 g, 70% yield). TLC (8:1 EtOAc/MeOH) R_f 0.71; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.67 (br s, 1H, NH_2, D_2O exchangeable), 8.41 (br s, 1H, NH_2, D_2O exchangeable), 7.74 (d, $J = 8.3$, 2H, H-Ar,), 7.42 (d, $J = 8.2$, 2H, H-Ar), 5.42 (d, $J = 4.4$, 1H, 3′-OH, D_2O exchangeable), 5.22 (t, $J = 7.3$, 1H, 1′-H), 4.3–4.18 (m, 1H, 3′-H), 4.17–3.89 (m, 3H, 4′-H, 5′-H), 3.16 (d, $J = 5.4$, 3H, CH_3), 2.34–2.10 (m, 2H, 2′-H^a, 2′-H^b). MS (ESI): [M+1] = 384, [M+Na$^+$] = 406. High res. MS (ESI): m/z calcd. for $C_{15}H_{17}N_3O_7S$ 384.0865, actual 384.0874.

2′-Deoxy-β-D-ribofuranosyl-1,2,4-oxadiazole-5-carboxamide-5′-diphosphate (13, 1DP). The 5′-tosylated derivative **11** (95 mg, 0.248 mmol) was co-evaporated with dry acetonitrile (3 × 3 mL) and dried in the vacuum oven overnight. *Tris*(tetra-*n*-butylammonium) hydrogen pyrophosphate[20] (336 mg, 0.372 mmol) was added under nitrogen followed by addition of 0.3 mL of dry acetonitrile. The reaction mixture was stirred at room temperature under nitrogen for 48 h. The progress and completion of the reaction were monitored by changes in ^{1}H NMR in the aromatic proton region (7–9 ppm) where an upfield shift of peaks corresponding to the tosylate anion (AA′XX′ spin system) was observed. Upon completion, the reaction mixture was diluted with ddH_2O (10 mL) and applied to a DOWEX AG 50W-X8 column (100–200 mesh, 2.5 cm × 10 cm, 83 equiv., NH_4^+ form). The column was eluted with two column volumes of ddH_2O and the eluent containing **1**DP (NH_4^+ salt) was lyophilized affording a yellowish solid.

The crude material was dissolved in minimal amount of 100 mM NH_4HCO_3, loaded on a cellulose column (35 × 2.5 cm), and eluted with CH_3CN/100 mM NH_4HCO_3/NH_4OH (8:3:2). Fractions containing pure **1**DP were determined by cellulose TLC using the same solvent system ($R_f = 0.35$) and combined. The cellulose plates were developed using sulfosalicylic acid-ferric chloride stain (Solution A: 0.2% $FeCl_3$, 40% EtOH; Solution B: 1% sulfosalicylic acid, 60% EtOH).[38] Acetonitrile was removed by evaporation under reduced pressure and the aqueous portion was lyophilized yielding a white fluffy solid **13** (83 mg, 86% yield). TLC 8:3:2 CH_3CN/100 mM NH_4HCO_3/NH_4OH (conc.) R_f 0.35; ^{1}H NMR (300 MHz,

D_2O): δ 5.47 (dd, J = 2.5, 9, 1H, 1′-H), 4.69-4.61 (m, 1H, 3′-H), 4.36–4.23 (m, 1H, 4′-H), 4.05 (t, *J* = 5.8, 2H, 5′-H), 2.64–2.37 (m, 2H, 2′-H^a, 2′-H^b). MS (ESI); M+Na = 411.9921; ^{13}C NMR (300 MHz, D_2O): δ 170.96, 169.64, 156.62, 86.54 (d, *J* = 8.6), 72.67, 72.18, 65.83, 38.52; ^{31}P (300 MHz, D_2O): δ −9.63 (d, *J* = 21.4), −10.91 (d, *J* = 21). MS (ESI): [M+1] = 390, [M+Na$^+$] = 412. High res. MS (ESI): m/z calcd. for $C_8H_{13}N_3O_{11}P_2Na$ 411.9923, found 411.9921.

2′-Deoxy-β-D-ribofuranosyl-1,2,4-oxadiazole-5-carboxamide-5′-triphosphate (15, 1TP). 2′-Deoxy-β-D-ribofuranosyl-1,2,4-oxadiazole-5-carboxamide-5′-diphosphate **13** (145.2 mg, 0.32 mmol) was dissolved in 10 mL of a phosphorylation medium containing triethanolamine (83 mM), $MgCl_2$ (17 mM), KCl (67 mM), ATP (38 mM, 1 equiv.), and phosphoenolpyruvate (PEP) (78 mM) and the pH adjusted to 7.6 using 6 N sodium hydroxide. The phosphorylation reaction was initiated by addition of 50 units of nucleoside diphosphate kinase (NDPK, 2 units/μL) and 62.5 units of pyruvate kinase (2.5 units/μL). This phosphorylation mixture was incubated at 37°C for 2 h. The progress of the reaction was monitored by analytical HPLC (Solvent A: 0.1 M KH_2PO_4, 4 mM TBAP, pH 6.0; Solvent B: 0.1 KH_2PO_4, 4 mM TBAP, 30% MeOH, pH 7.2). After HPLC analysis showed that all **1**DP was converted to triphosphate, 0.5 molar equiv. of ADP (66 mg) was added to the reaction mixture to consume excess PEP. When the reaction was completed, the mixture was filtered to remove proteins and lyophilized, leaving a yellowish solid.

This solid was re-dissolved in 10 mL of 1 M NH_4HCO_3 (pH 8.5) and loaded onto a Bio-Rad boronate affinity gel column (2.5 cm × 5.5 cm, pre-equilibrated with 1 M NH_4HCO_3, pH 8.5 titrated with conc. NH_4OH) and then the column was eluted with the same buffer (1.0 M NH_4HCO_3, pH 8.5) at a flow rate of 1 mL/min. Fractions containing the desired **1**TP, detected by analytical HPLC, were combined and lyophilized. Additional cycles of repetitive lyophilization and pH adjustments (pH 7.2) with carbon dioxide were required to remove the excess ammonia bicarbonate.

Complete purification of **1**TP was achieved using a Q Sepharose FF anion exchange column (2.5 cm × 20 cm; HCO_3^- form). The product (**1**TP) was eluted using a linear gradient (50 mM NH_4HCO_3 → 500 mM NH_4HCO_3, pH 7.8, flow rate 5 mL/min) and monitored by UV (λ = 230 nm). Fractions containing the desired triphosphate were confirmed by HPLC, combined and lyophilized affording **1**TP as white fluffy solid. Finally, this solid was re-dissolved in ddH_2O and passed through a Dowex AG 50W-X8 column to convert **1**TP ammonium salt to a sodium salt, providing **1**TP as a white fluffy solid (72 mg, NH_4^+ salt, 42% yield). ^1H NMR (300 MHz, D_2O): δ 5.47 (dd, *J* = 1.9, 8.8, 1H, 1′-H), 4.7–4.64 (m, 1H, 3′-H), 4.39–4.26 (m, 1H, 4′-H), 4.08 (t, *J* = 6.1, 2H, 5′-H), 2.61–2.36 (m, 2H, 2′-H^a, 2′-H^b);

^{13}C NMR (300 MHz, D_2O): δ 170.977, 169.626, 156.640, 86.568 (d, $J = 8.5$), 72.612, 72.189, 66.066, 38.533; ^{31}P (300 MHz, D_2O): δ −9.32 (d, $J = 19.2$), −11.08 (d, $J = 19.9$), −22.72 (t, $J = 19.3$). MS (ESI): [M+1] = 470. High res. MS (ESI): m/z calcd. for $C_8H_{14}N_3O_{14}P_3$ 469.9767, found 469.9777.

Preparation of 2′-Deoxy-*β*-D-ribofuranosyl-1,2,4-oxadiazole-3-carboxamide-5′-triphosphate (16, 2TP)

2′-Deoxy-*β*-D-ribofuranosyl-1,2,4-oxadiazole-3-carboxamide-5′-tosylate (12). Deoxyribonucleoside **2** (0.300 g, 1.3 mmol) was co-evaporated with dry pyridine (3 × 4 ml) and dried in the vacuum oven overnight. Toluenesulfonyl chloride (0.299 g, 1.6 mmol) and dry pyridine (3 mL) were added to the nucleoside and this reaction mixture was stirred under nitrogen at room temperature for 24 h. The completion of the reaction was monitored by TLC (EtOAc:MeOH 9:1; $R_f = 0.68$). Upon completion, 2.5 g silica was added to the reaction mixture and pyridine was removed by evaporation under reduced pressure and washed with MeOH (2 × 5 mL). This silica was applied onto a flash chromatography column and tosylate **12** was eluted with EtOAc:MeOH (10:1). Fractions corresponding to the desired product were pooled together and the solvent was evaporated under reduced pressure affording the desired product as a white fluffy solid (0.264 g, 53%). TLC (9:1 EtOAc/MeOH) R_f 0.68; ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.3 (br s, 1H, NH_2, D_2O exchangeable), 8.13 (br s, 1H, NH_2, D_2O exchangeable), 7.74 (d, $J = 8.4$, 2H, H-Ar,), 7.43 (d, $J = 8.3$, 2H, H-Ar), 5.47 (d, $J = 4.3$, 1H, 3′-OH, D_2O exchangeable), 5.35 (t, $J = 7.4$, 1H, 1′-H), 4.27–4.17 (m, 1H, 3′-H), 4.16–3.94 (m, 3H, 4′-H, 5′-H), 3.16 (d, $J = 5.3$, 3H, CH_3), 2.38–2.19 (m, 2H, 2′-H^a, 2′-H^b); ^{13}C NMR (300 MHz, DMSO-d_6): 179.10, 163.53, 157.50, 145.04, 131.95, 130.10, 127.59, 84.50, 71.46, 70.95, 70.10, 21.09. MS (ESI): [M+1] = 384, [M+Na$^+$] = 406. High res. MS (ESI): m/z calcd. for $C_{15}H_{17}N_3O_7S$ 384.0865, found 384.0864.

2′-Deoxy-*β*-D-ribofuranosyl-1,2,4-oxadiazole-3-carboxamide-5′-diphosphate (14, 2DP). Tosylate **11** (0.123 g, 0.321 mmol) was co-evaporated with dry acetonitrile (3 × 4 mL) and dried in a vacuum oven overnight (25°C, 76 cmHg). *Tris*(tetra-*n*-butylammonium) hydrogen pyrophosphate[20] (579 mg, 0.64 mmol) was added under nitrogen followed by addition of 0.2 mL of dry acetonitrile. The reaction mixture was stirred at room temperature under nitrogen for 48 h. The progress and completion of the reaction were monitored by changes in ^{1}H NMR in the aromatics region (7–9 ppm) where an upfield shift of peaks corresponding to the tosylate anion (AA′XX′ spin system) was observed. To drive the reaction to completion, an additional 0.5 equiv. of *tris*(tetra-*n*-butylammonium) hydrogen pyrophosphate (144 mg) were added. Upon completion, the reaction mixture was diluted with

ddH_2O (10 mL) and applied to a DOWEX AG 50W-X8 column (100–200 mesh, 2.5 cm × 10 cm, 83 equiv., NH_4^+ form). The column was eluted with two column volumes of ddH_2O and the eluent containing 2DP (NH_4^+ salt) was lyophilized affording a yellowish solid.

The crude material was dissolved in minimal amount of 100 mM NH_4HCO_3, loaded on a cellulose column (35 × 2.5 cm), and eluted with CH_3CN/100 mM NH_4HCO_3/NH_4OH (conc.) (8:3:2). Fractions containing pure **2**DP were determined by cellulose TLC using the same solvent system ($R_f = 0.30$) and combined. The cellulose plates were developed using sulfosalicylic acid-ferric chloride stain (Solution A: 0.2% $FeCl_3$, 40% EtOH; Solution B: 1% sulfosalicylic acid, 60% EtOH).[38] Acetonitrile was removed by evaporation under reduced pressure and the aqueous portion was lyophilized yielding a white fluffy solid **14** (0.109 g, 87%). TLC (8:3:2 CH_3CN/100 mM NH_4HCO_3/NH_4OH) R_f 0.30; ^1H NMR (300 MHz, D_2O): δ 5.56 (dd, $J = 1.5, 8.8$, 1H, 1′-H), 4.56 (p, $J = 1.3$, 1H, 3′-H), 4.36–4.28 (m, 1H, 4′-H), 4.12–3.95 (m, 2H, 5′-H), 2.70–2.47 (m, 2H, 2′-H^a, 2′-H^b); ^{13}C NMR (300 MHz, D_2O): δ 180.55, 163.31, 160.38, 86.90 (d, $J = 9$), 72.51, 72.42, 65.71, 38.60; ^{31}P (300 MHz, D_2O): δ −9.34 (d, $J = 20.5$), −10.98 (d, $J = 20$). MS (ESI): [M+1] = 390, [M−1] = 388. High res. MS (ESI): m/z calcd for $C_8H_{13}N_3O_{11}P_2$ 387.9947, found 387.9945.

2′-Deoxy-β-D-ribofuranosyl-1,2,4-oxadiazole-3-carboxamide-5′-triphosphate (16, 2TP). Nucleoside diphosphate **14** (NH_4^+ salt) (43.2 mg, 97.4 μmol) was dissolved in 10 mL of a phosphorylation medium containing triethanolamine (83 mM), $MgCl_2$ (17 mM), KCl (67 mM), ATP (9.74 mM, 1 equiv.), phosphoenolpyruvate (PEP) (19.5 mM) and the pH adjusted to 7.6 using 6 N sodium hydroxide. The phosphorylation reaction was initiated by addition of 50 units of nucleoside diphosphate kinase (NDPK, 2 units/μL) and 50 units of pyruvate kinase (2.5 units/μL). This phosphorylation mixture was incubated at 37°C for 1 h. The progress of the reaction was monitored by analytical HPLC (Solvent A: 0.1 M KH_2PO_4, 4 mM TBAP, pH 6.0; Solvent B: 0.1 KH_2PO_4, 4 mM TBAP, 30% MeOH, pH 7.2). After HPLC showed that all **2**DP is converted to triphosphate, 0.5 equiv. of ADP (20 mg) was added to the reaction mixture to consume excess PEP. When the reaction was completed, the mixture was filtered to remove proteins and lyophilized, leaving a yellowish solid.

This solid and crude product obtained from another 56.8 mg scale phosphorylation reaction were combined, re-dissolved in 10 mL of 1 M NH_4HCO_3 (pH 8.5), and loaded onto a Bio-Rad boronate affinity gel column (2.5 cm × 5.5 cm, pre-equilibrated with 1 M NH_4HCO_3, pH 8.5 titrated with conc. NH_4OH) followed by elution with the same buffer (1.0 M NH_4HCO_3, pH 8.5) at the flow rate 1 mL/min. Fractions containing the **2**TP (detected by analytical HPLC), were combined and lyophilized. Additional cycles of repetitive lyophilization and pH adjustments (pH 7.2) with carbon dioxide were required to remove the excess of ammonia bicarbonate.

Complete purification of **2**TP was achieved by employing a Q Sepharose FF anion exchange column (2.5 cm × 20 cm; HCO_3^- form). The product (**2**TP) was eluted using a linear gradient (50 mM $NH_4HCO_3 \rightarrow$ 500 mM NH_4HCO_3, pH 7.8, flow rate 5 mL/min) and monitored by UV ($\lambda =$ 230 nm). Fractions containing the desired triphosphate were confirmed by HPLC, combined and lyophilized affording **2**TP as a white fluffy solid (74 mg, NH_4^+ salt, 58% yield). ^1H NMR (300 MHz, D_2O): δ 5.36 (dd, $J = 1.2, 8.4$, 1H, 1′-H), 4.7–4.63 (m, 1H, 3′-H), 4.37–4.28 (m, 1H, 4′-H), 4.15–4.00 (m, 2H, 5′-H), 2.72–2.46 (m, 2H, 2′-H^a, 2′-H^b); ^{13}C NMR (300 MHz, D_2O): δ 180.56, 163.32, 160.397, 86.90 (d, $J = 8.5$), 72.47, 66.05, 38.654; ^{31}P (300 MHz, D_2O): δ −9.30 (d, $J = 19.8$), −11.17 (d, $J = 19.8$), −22.68 (t, $J = 19.8$). MS (ESI): [M+1] = 470, [M+Na^+] = 492, [M−1] = 468. High res. MS (ESI): m/z calcd. for $C_8H_{14}N_3O_{14}P_3$ 468.9689, found 468.9697.

CONCLUSIONS

Oxadiazole carboxamide nucleosides represent a series of nucleobase analogs built on the common 1,4-azole carboxamide framework while displaying an alternative heteroatom substitution pattern and unique electronic surface profiles. C-linked oxadiazole carboxamide nucleosides **1** and **2** were efficiently synthesized from deoxyribofuranosylcyanide as a common precursor. The transformation to corresponding nucleoside triphosphates was successfully accomplished by the combination chemical and enzymatic approach providing unnatural oxadiazole carboxamide deoxyribonucleotides in good yield and excellent purity.

Crystallographic analysis was used to confirm the structures of 5-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-5-carboxamide (**1**) and 5-(2′-deoxy-3′,5′-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3-carboxamide (**2**). The correct chemical composition and anomeric configuration are observed for the synthesized nucleoside analogs. Furthermore, the conformational parameters such as the pseudorotational phase angle *P*, the maximum torsion angle v_m, the furanose ring conformation (pucker), and the glycosidic bond torsion angle χ were determined based on the obtained crystallographic data.

According to the conformational analysis, nucleoside analogs **1** and **2** display the C2′-*endo* (“south”) deoxyribose conformation that is typically observed in the B-form DNA, deoxyribo-nucleotides and -nucleosides. Furthermore, the orientation of the nucleobase moiety was determined as *anti* for **1** (conformer A) and *high anti* for the conformer B of **1** whereas nucleobase of the analog **2** seems to adapt the *syn* conformation.

Importantly, the crystallographic data on unnatural C-deoxyribonucleosides **1** and **2** is also extremely valuable due to the very limited structural information regarding natural and modified C-linked nucleosides. Moreover, this structural study could be important for understanding the mechanisms and factors involved in recognition of azole carboxamide nucleosides.

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