



HAL
open science

1H-1,2,3-triazolyl-1,6-naphthyridin-7(6H)-ones as Potential Fluorescent Nucleoside Analogues: Synthesis and Optical Properties

Anissa Beghennou, Océane Rondot, Vincent Corcé, Candice Botuha

► **To cite this version:**

Anissa Beghennou, Océane Rondot, Vincent Corcé, Candice Botuha. 1H-1,2,3-triazolyl-1,6-naphthyridin-7(6H)-ones as Potential Fluorescent Nucleoside Analogues: Synthesis and Optical Properties. *Molecules*, 2024, Special Issue Nucleoside and Nucleotide Analogues: Chemical Synthesis and Applications, 29 (3), pp.687. 10.3390/molecules29030687 . hal-04445361

HAL Id: hal-04445361

<https://hal.science/hal-04445361>




Submitted on 7 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Article

1*H*-1,2,3-triazolyl-1,6-naphthyridin-7(6*H*)-ones as Potential Fluorescent Nucleoside Analogues: Synthesis and Optical Properties

Anissa Beghennou [†] , Océane Rondot [†], Vincent Corcé ^{*†}  and Candice Botuha ^{*†} 

Institut Parisien de Chimie Moléculaire, CNRS UMR 9232, Sorbonne Université, F-75252 Paris, France; anissa.beghennou@gmail.com (A.B.); oceane.rondot@sorbonne-universite.fr (O.R.)

* Correspondence: vincent.corce@sorbonne-universite.fr (V.C.); candice.botuha@sorbonne-universite.fr (C.B.)

[†] These authors contributed equally to this work.

Abstract: In this article, we present the synthesis and the optical properties of three original molecules as potential fluorescent ribonucleoside analogues incorporating a 1,6-naphthyridin-7(6*H*)-one scaffold as a fluorescent nucleobase and a 1,2,3-triazole as a linkage. The nucleosides were prepared via a Cu alkyne-azide cycloaddition (CuAAC) reaction between a ribofuranosyl azide and a 4-ethynylpyridine partner. Construction of substituted 1,6-naphthyridin-7(6*H*)-ones was achieved through two additional steps. Optical property studies were investigated on nucleoside analogues. Powerful fluorescence properties have been evidenced with a remarkable change of emissivity depending on the polarity of the solvent, making these molecules suitable as a new class of artificial fluorescent nucleosides for investigating enzyme binding sites as well as probing nucleic acids. In addition, we are convinced that such analogues could be of great interest in the search for new antiviral or antitumoral drugs based on nucleosides.

Keywords: nucleoside; 1,2,3 triazole; CuAAC reaction; fluorescence; solvatochromism



Citation: Beghennou, A.; Rondot, O.; Corcé, V.; Botuha, C. 1*H*-1,2,3-triazolyl-1,6-naphthyridin-7(6*H*)-ones as Potential Fluorescent Nucleoside Analogues: Synthesis and Optical Properties. *Molecules* **2024**, *29*, 687. <https://doi.org/10.3390/molecules29030687>

Academic Editors: Lajos Kovacs, Tuomas Lönnberg, Satu Mikkola and Pasi Virta

Received: 8 December 2023

Revised: 24 January 2024

Accepted: 30 January 2024

Published: 1 February 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Nucleic acid structure and dynamics are of fundamental importance in understanding biological processes in cells [1–3]. In this context, nucleoside derivatives and analogues have been designed to interfere with cell metabolism and are being developed as antiviral, anticancer, and antibacterial agents [4–6] or as tools for several purposes, such as investigating enzyme binding sites, protein interactions, DNA features, and perturbations [7,8].

DNA-based fluorescent structures capable of labeling nucleic acids are powerful tools to investigate DNA interactions and have been used in a variety of applications in chemical biology [9–12].

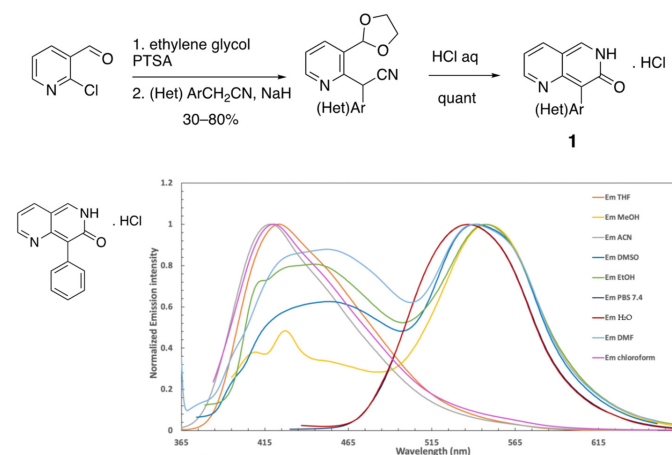
In this context, numerous environmentally sensitive unnatural fluorescent nucleosides have been developed to fluorescently label nucleic acids via binding, intercalation, or covalent bonds [13,14]. The design of fluorescent nucleosides is a great challenge and has stimulated much research in various fields of photophysics, synthetic chemistry, and computational studies. The main fluorescence properties should include a change in fluorescence intensity or a shift in the emission maximum when interacting with the DNA environment while maintaining the other key optical properties, such as large Stokes Shift, high brightness, and high quantum yield.

In this field, unnatural fluorescent nucleosides possessing heterocyclic nucleobases are widely represented. EthenoA [15], Coumarin nucleobase analogues [16], Nile red, imidazophenanthrene, and other types of heterocyclic nucleobases have been developed [17–20]. In particular, 1,2,3-triazolyl nucleoside analogues, which consist in connecting the appropriate aromatic ring or purine residue to a 1,2,3-triazole moiety, have found a growing

interest due to their powerful antiviral activities [21–23]. 4-substituted-1,2,3 triazolo nucleotide analogues are also well known for antitumoral activities as inhibitors of human ecto-5'-nucleosidase CD73, a cell-surface protein associated with adenosine metabolism that promotes tumor progression [24]. Interestingly, a nucleoside analogue incorporating a diaminopyrimidine linked to a 1,2,3-triazole [25], also called click fleximer [26,27], was found to be luminescent and a promising tool to investigate enzyme binding sites and to characterize protein and nucleic acid interactions (Figure 1). Indeed, unlike the classic bicyclic fused system of the parent purine nucleobase, the two heterocyclic components of the click fleximers are attached by a C–C bond that permits conformational mobility. As a consequence, the relative flexibility of nucleobase enables it to adapt rapidly to the spatial requirement of an enzyme binding site. The design of flexible bioprobes will clearly provide a better understanding of the conformational effects of enzymes, and elucidate the structure of ligand binding sites in biologically important enzyme systems [28]. Other synthetic fleximer derivatives whose heterocyclic bases contain one or more than one planar ring capable of interacting with their environment, via π stacking and/or H-bonding, have been discovered. For example, a fluorescent triazolyl containing a pyrene ring has shown interesting fluorescence properties and capacity to interact with BSA via hydrophobic and electrostatic bindings [29] (Figure 1). Interestingly, an environment-sensitive fluorescent nucleoside analogue bearing a thienyl ring linked to a 3-hydroxychromone (3HCnt) as ESIPT dye has been successfully incorporated into a DNA sequence with minimal perturbation. In addition, the authors demonstrated that 3HCnt can monitor local conformation changes of oligonucleotides upon interaction with HIV-1 nucleocapsid protein [30].

Naphthyridines [31] are important scaffolds used for biological applications [32,33]. These structures are also known as fluorescent probes [34] or as luminescent materials [35]. 1,8-Naphthyridine C-nucleoside and their base-pairing properties have been reported [36]. Among these structures, naphthyridinones are also well known for their biological properties [37]. Only the 1,8-naphthyridinones have been particularly studied as nucleobase analogues. 1,8-naphthyridin-2(1*H*)-ones were described as efficient bicyclic and tricyclic fluorescent-based analogues of thymines [38] and were further incorporated into DNA [39,40]. The naphthyridinones thus appear as promising scaffolds to be developed as new fluorescent nucleosides.

We have recently reported the short synthesis of 1,6-naphthyridin-7(6*H*)-ones **1**, a fluorescent heteroaromatic scaffold with adequate photophysical properties to be incorporated as the nucleobase into a nucleoside (Scheme 1). Indeed, 1,6-naphthyridin-7(6*H*)-one scaffold shows a visible absorption, a solvatochromism, an acidochromism, large Stokes shifts, and high quantum yields depending on the solvent and media. These naphthyridinones are also able to generate dual fluorescence in polar solvent from an intramolecular proton transfer at the excited state mechanism (ESIPT) coming from their lactim-lactam forms [41] (Scheme 1).



Scheme 1. Our reported 8-substituted 1,6-naphthyridin-7(6*H*)-ones **1** for Ar = Ph and their emission spectra in various solvents, adapted with permission from Ref. [41]. 2023, Royal Chemical Society (RCS).

Therefore, the recent development in the field of triazole-linked fluorescent nucleoside conjugates and the photophysical properties of 1,6-naphthyridin-7(6*H*)-ones encouraged us to design and synthesize fused 1,6-naphthyridin-7(6*H*)-ones with triazole and to study their photophysical properties. We thus expect to develop a new class of fluorescent nucleoside analogues with potentially powerful biological properties as well as to use them as molecular probes to investigate enzyme binding sites or the structural characteristics of nucleic acids.

Herein, we described the synthesis of three original 1,2,3-triazole nucleoside conjugates (TzNat) containing fluorescent 1,6-naphthyridin-7(6*H*)-ones as the nucleobase using CuAAC click chemistry (Figure 1). Photophysical properties (absorption, emission quantum yield) of TzNat **A**, **B**, and **C** were studied in various solvents (Figure 1).

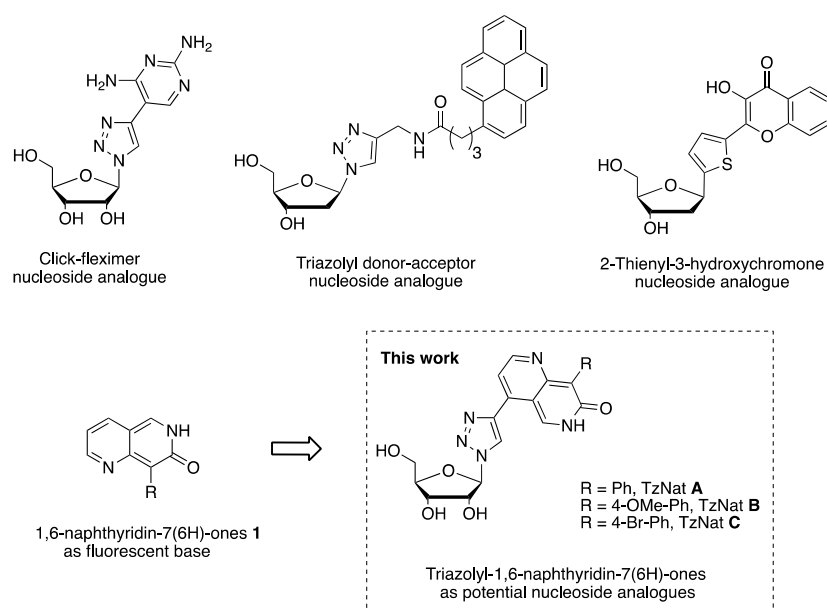


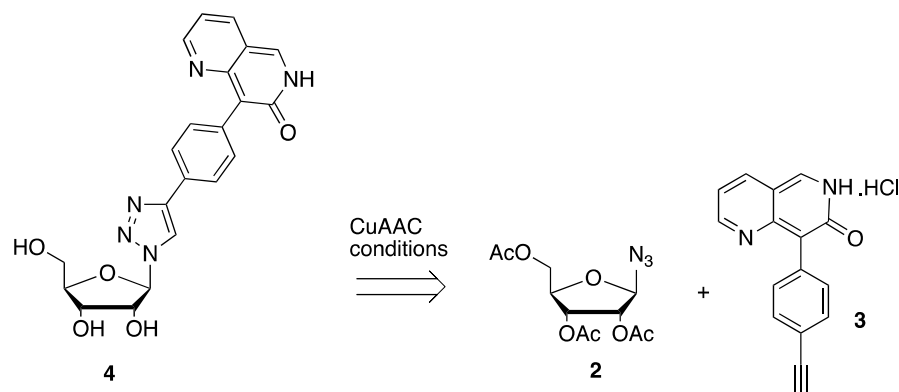
Figure 1. Selection of unnatural fluorescent nucleoside analogues reported in the literature [25,29,30,41] and the design of 1,2,3-triazolyl-1,6-naphthyridin-7(6*H*)-ones nucleoside analogues TzNat **A** to **C** presented in this work.

2. Results

2.1. Fluorescent Nucleosides Synthesis

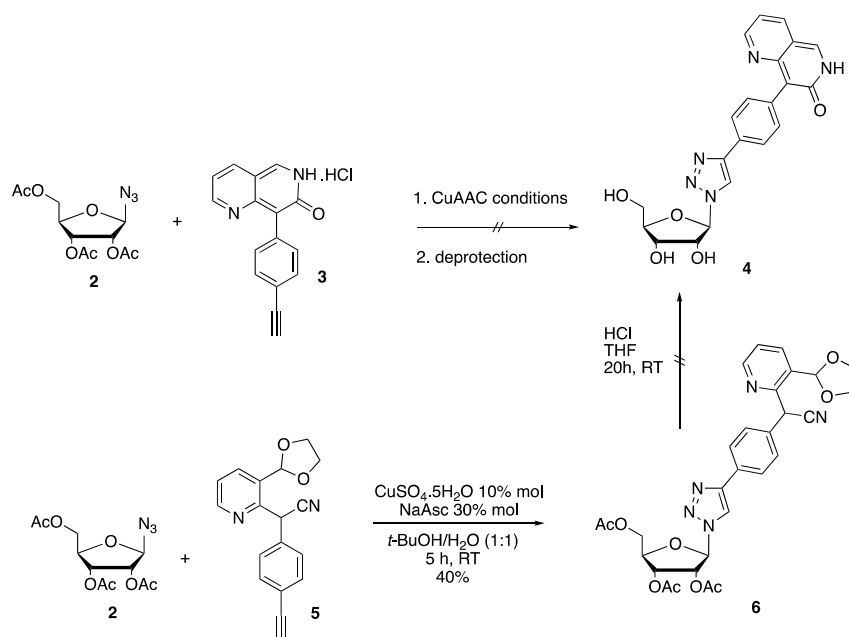
Heteroaromatic nucleoside analogues possessing 1,2,3 triazoles as a linker between the nucleobase and the ribose are generally designed and synthesized using CuAAC click chemistry [42–44] to be used as Furo[2,3-*b*]pyrazine nucleoside analogues [45], nucleoside–iridium conjugates [46], Hydroxyanthracene triazolyl glycoconjugates [47], or as ligands for Cu(I) catalysis in the synthesis of quinazolinones [48]. Therefore, to design the naphthyridinone–ribose conjugate TzNat, we chose to use a CuAAC click reaction.

First, in our earlier design we attempted to link the fluorescent naphthyridinone core at the 8-position to the ribose by a 1,2,3 triazole using a CuAAC reaction to get 1,2,3-triazolyl naphthyridinone–ribose conjugate **4**. To do so, we developed a strategy using azido ribofuranose **2** as an azide partner and 8-(4-ethynylphenyl)-6,7-dihydro-1,6-naphthyridin-7-one **3** as an alkyne partner (Scheme 2).



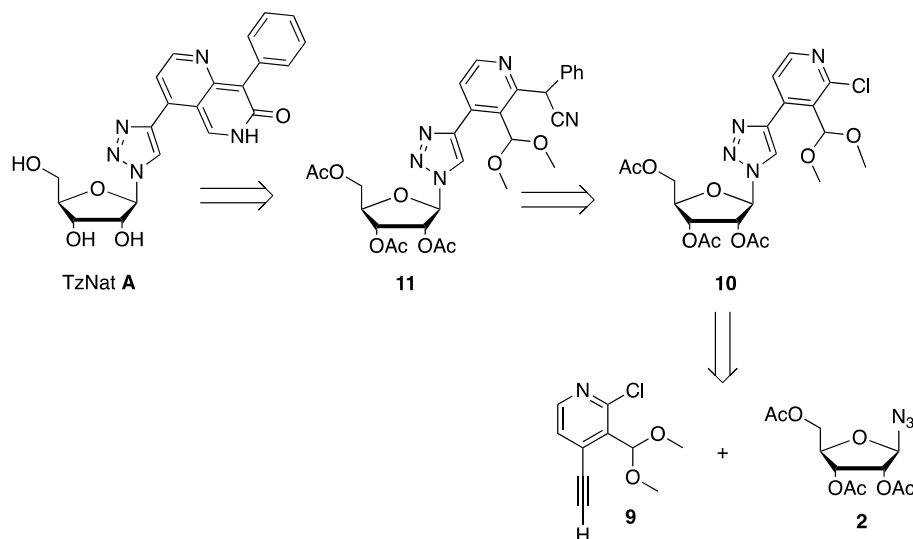
Scheme 2. Retrosynthetic approach for the synthesis of 1,2,3-triazolyl naphthyridinone–ribose conjugate **4**.

Synthesis of 8-(4-ethynylphenyl)-1,6-naphthyridin-7(6H)-one **3** was achieved following our previously reported strategy from commercially available 2-chloro-3-formylpyridine. The azide partner **2** was prepared from 1,2,3,4-tetra-*O* acetyl β -D-ribofuranose following a reported procedure [49]. However, attempts to realize the CuAAC click reaction between the two partners failed. The classical procedure using a combination of CuSO_4 and sodium ascorbate in tertbutanol/water at various temperatures failed. The use of CuI or more activated $\text{Cu}(\text{iMes})\text{Cl}$ in organic solvent with a base remained unsuccessful (Scheme 3 and Table S1 in Supplementary Materials). We hypothesized that the naphthyridinone bicycle **3** was not compatible with the click conditions because of the high reactivity of the pyridone moiety acting as a ligand with the copper atom. To overcome this issue, the click reaction was performed on the opened form **5** [41], the synthetic precursor of the 8-(4-ethynylphenyl)-1,6-naphthyridin-7(6H)-one **3** [41], and the azide **2** using standard conditions. Under these conditions, the click compound **6** was obtained in 40% yield. The last step consisted of an acidic treatment to promote the concomitant deprotection of the ribose, the acetal removal, and the nitrile function hydrolysis–cyclization. Unfortunately, this step did not give satisfactory results and led to an inseparable mixture containing a majority of the starting product, a very small amount of the expected product **4**, and a portion containing the mono-deacetylated sugar unit (Scheme 3).



Scheme 3. Attempts to use click reaction to prepare triazole conjugate **4** from acetylated ribosyl azide **2**.

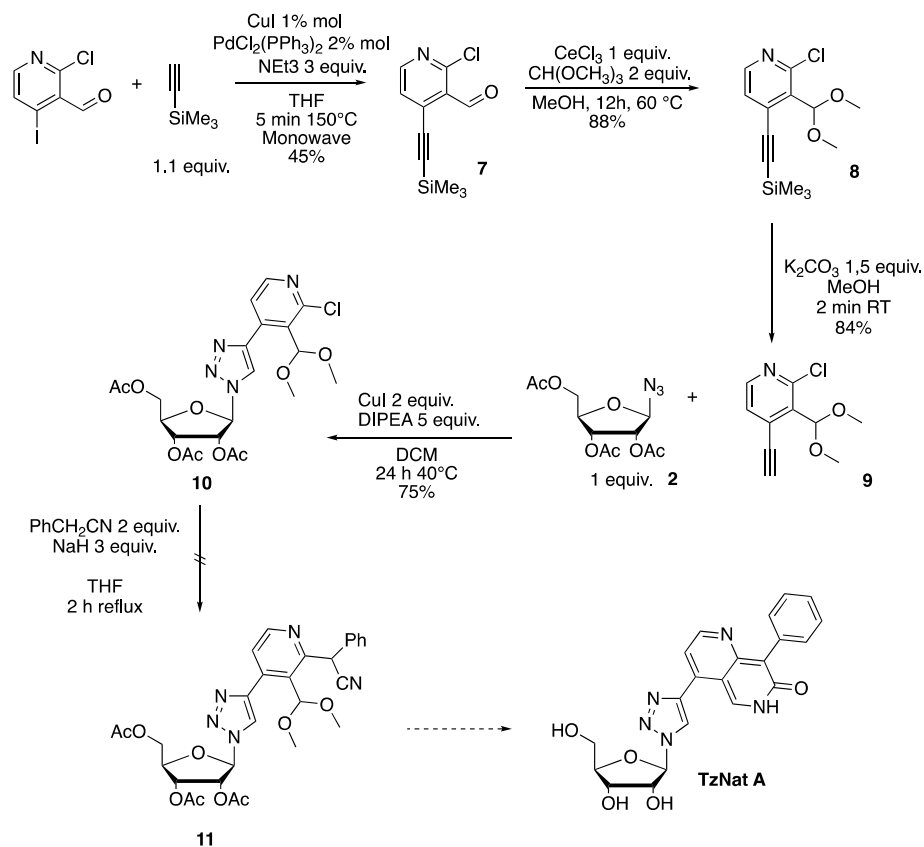
Encouraged by this last result, we slightly modified the strategy to target triazole conjugate **A** or TzNat **A**, which has the advantage of being easily modified at the 8-position of the naphthyridinone ring, and to allow further photophysical modification. Therefore, we have envisioned performing the click reaction with the 2-chloro-3-(dimethoxymethyl)-4-ethynylpyridine **9** as the alkyne partner and the azide ribose tetraacetate **2** in the first intention (Scheme 4).



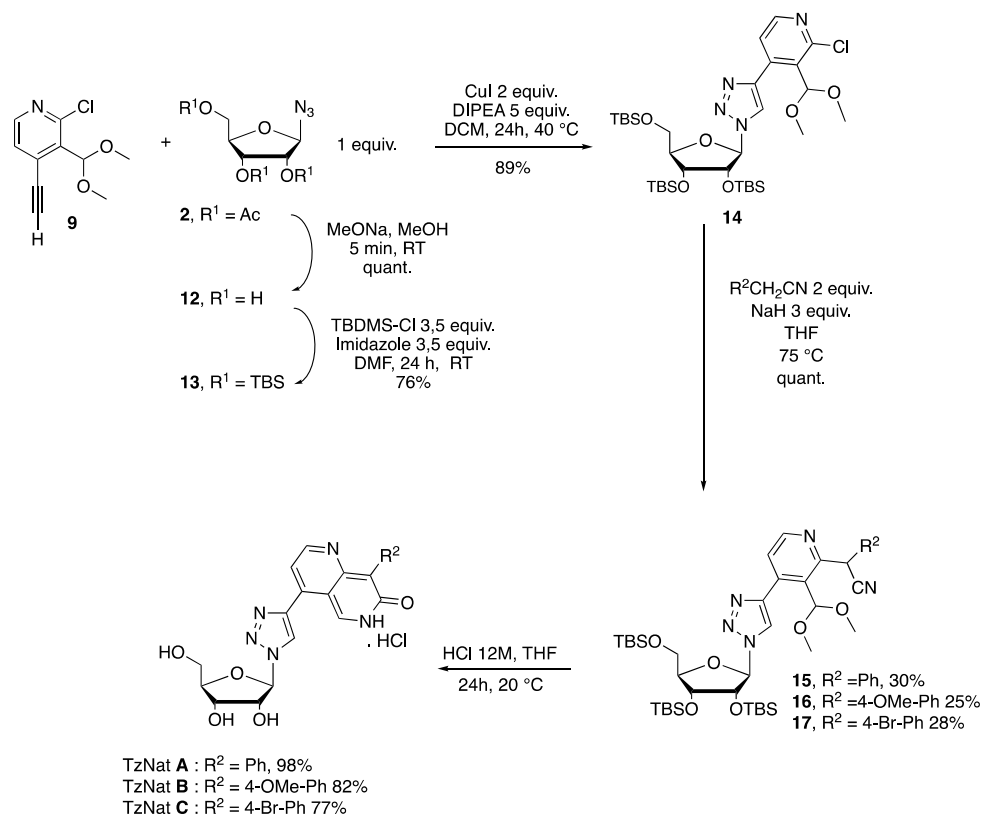
Scheme 4. Retrosynthetic analysis of the synthesis of triazole conjugate TzNat **A**.

The synthesis of alkyne partner **9** was achieved in two steps. The mono-alkynylated product **7** was successfully prepared by a regioselective Sonogashira cross-coupling reaction performed in 5 min at 150 °C using a monowave apparatus. The aldehyde function of **7** was then protected as dimethoxyacetal under the mild conditions developed by Luche using methanol, cerium chloride, and trimethylorthoformate as the water scavenger [50]. Deprotection of the alkyne function appeared very sensitive due to the high reactivity of the triple bond. Two minutes were necessary to cleave the C–Si bond with K₂CO₃ in methanol at room temperature and obtain compound **9**. The ribofuranosyl azide partner **2** was reacted with **9** by a CuAAC click reaction with CuI, diisopropylethylene diamine in dichloromethane for 24 h at reflux, affording the desired triazole **10** in good yield. The triazole **10** was subsequently engaged in a nucleophilic aromatic substitution with phenylacetonitrile and sodium hydride. However, the reaction was unsuccessful. The presence of acetate protecting groups of the ribose part was suspected to be part of the observed lack of reactivity, due to its electron-withdrawing character and its propensity to be saponified under basic conditions (Scheme 5).

Therefore, we changed the ribose acetate protecting groups for the more stable tert-butyltrimethylsilylether protecting groups. Starting from 2,3,5-triacetate-β-D-ribofuranosyl azide **2**, the protected tertbutyltrimethylsilylether ribose azide **13** was obtained in two steps using sodium methoxide, followed by the addition of tertbutyltrimethylsilylchloride and imidazole, with excellent yield (Scheme 6). Next, the CuAAC click reaction between the azide **13** and the alkyne **9** proceeded smoothly, affording the desired triazole **14** in good yield. The reaction of **14** with phenylacetonitrile in the presence of sodium hydride gave the substituted product **15** in 30% yield. Finally, the nucleoside naphthyridinone TzNat **A** was obtained as its chlorohydrate salt in quantitative yield after acidic treatment of compound **15**. This strategy was employed to successfully prepare TzNat **B** possessing a 4-methoxyphenyl substituent and TzNat **C** bearing a 4-bromophenyl, starting from the same precursor **14** and using 4-methoxyphenylacetonitrile and 4-bromophenylacetonitrile respectively for the nucleophilic aromatic substitution step (Scheme 6).



Scheme 5. First attempt for the synthesis of TzNat A.



Scheme 6. Successful strategy to prepare TzNat A, B, and C.

The structures of nucleosides TzNat A, B, and C were confirmed by NMR spectroscopy using 1D (^1H , JMOD and ^{13}C -DEPT) and 2D (COSY, HSQC, HMBC) experiments (See Supplementary Materials).

2.2. Optical Properties of TzNat Molecules

Optical properties of TzNat molecules as their chlorohydrate salts were studied in different solvents (Table 1). As their related compounds 1,6-Naphthyridin-7(6H)-ones contain a 2-hydroxypyridine system, the ribonucleoside TzNat may exist under lactim-enol and lactam-keto tautomeric forms in the ground state [41].

Table 1. UV–Vis Absorption and Fluorescence Emission Properties of Compounds TzNat A–C in various solvents.

| Compd | UV–Vis ^[b] | | | Fluorescence ^[c] | |
|---------|--------------------------|---------------------------------------|-----------------------|---|----------------------------------|
| | Solvent ^[a] | λ_{abs} (ϵ) | λ_{em} | Stokes ^[d] Shifts in cm^{-1} | Φ_{F} ^[e] |
| TzNat A | CHCl_3 | 358 (8.8), 479 (5.8) | 471 | 6701 | <0.01 |
| | CH_2Cl_2 | 356 (10.3), 476 (4.7) | 474 | 6992 | <0.01 |
| | THF | 369 (6.4), 479 (0.6) | 461 | 5809 | 0.32 |
| | acetone | 363 (5.3), 474 (0.5) | 460 | 5943 | 0.20 |
| | CH_3CN | 360 (5.1), 465 (1.9) | 458 | 6179 | 0.11 |
| | DMF | 369 (5.2), 480 (1.1) | 413, 478, 560 | 6179 | 0.01 |
| | DMSO | 374 (3.6), 483 (1.1) | 421, 475, 560 | 5685 | <0.01 |
| | EtOH | 374 (3.8), 451(3.8) | 477 | 5773 | <0.01 |
| | H_2O | 427 (4.9) | 563 | 5657 | <0.01 |
| | PBS | 427 (6.6) | 558 | 5498 | <0.01 |
| TzNat B | CHCl_3 | 317 (17.2), 461 (6.7) | 492 | 11,220 | <0.01 |
| | CH_2Cl_2 | 325 (16.3), 498 (4.9) | 499 | 10,729 | <0.01 |
| | THF | 378 (7.6), 490 (0.4) | 489 | 6500 | 0.34 |
| | acetone | 369 (8.1), 488 (1.6) | 492 | 6775 | 0.22 |
| | CH_3CN | 369 (7.6), 468 (3.1) | 494 | 6867 | 0.18 |
| | DMF | 379 (9.5), 486 (2.2) | 499 | 6345 | 0.14 |
| | DMSO | 380 (7.1), 490 (1.9) | 502 | 6395 | 0.12 |
| | EtOH | 386 (3.2), 457 (4.0) | -- | -- | -- |
| | H_2O | 433 (8.1) | -- | -- | -- |
| | PBS | 431 (7.3) | -- | -- | -- |
| TzNat C | CHCl_3 | 356 (12.5), 461 (6.4) | 470 | 6813 | <0.01 |
| | CH_2Cl_2 | 356 (9.8), 454 (6.1) | 467 | 6676 | <0.01 |
| | THF | 369 (8.6), 479 (1.1) | 460 | 5361 | 0.31 |
| | acetone | 363 (8.4), 470 (1.7) | 459 | 5761 | 0.25 |
| | CH_3CN | 363 (5.4), 470 (2.1) | 458 | 5714 | 0.11 |
| | DMF | 372 (9.2), 470 (1.7) | 476, 556 | 5873 | <0.01 |
| | DMSO | 371 (9.2), 472 (2.7) | 477, 558 | 5989 | <0.01 |
| | EtOH | 378 (4.6), 452 (5.5) | 479 | 5578 | <0.01 |
| | H_2O | 427 (7.5) | 561 | 5593 | <0.01 |
| | PBS | 429 (7.1) | 561 | 5484 | <0.01 |

^[a]. All TzNat have been pre-solubilized in DMSO at 10^{-3} M and used it as stock solution to prepare 10^{-5} M diluted solutions in solvents. ^[b]. Absorption maxima measured in solution (10^{-5} M) at room temperature in nm (extinction coefficient at absorption maxima in $10^3 \text{ M}^{-1} \text{ cm}^{-1}$). ^[c]. Fluorescence wavelengths measured at room temperature at a concentration of 10^{-5} M after excitation at the maximum absorption wavelength. ^[d]. Stokes shift in cm^{-1} defined as the wavelength difference between the absorption and emission peak maxima. ^[e]. Absolute fluorescence quantum yields were measured.

As shown in the UV–Vis spectra in Figure 2, TzNat A to C show similar absorption profiles. The wavelength of the maximum absorption depends strongly on the polarity of the solvent. In non-polar solvent and polar aprotic solvent, the maximum absorption is centered between 320 and 386 nm, assigned to a π – π^* transition with molar extinction coefficients ranging from 17,200 to $3200 \text{ M}^{-1} \text{ cm}^{-1}$. Interestingly, a bathochromic shift of this band is observed for all the TzNat from nonpolar solvent (CHCl_3 , CH_2Cl_2) to polar

aprotic solvent (THF, DMSO, DMF, DMSO, acetone). This phenomenon is more pronounced for TzNat **B** possessing a para-methoxyphenyl group, for which a red-shift of 70 nm was found for the position of the maximum absorption in EtOH or DMSO relating to nonpolar solvent CHCl_3 or CH_2Cl_2 . This observed red-shift of the maximum absorption band from non-polar to polar solvent can be rationalized by the existence of an intramolecular charge transfer generated between the electron-donating group para-methoxy phenyl and the 1,2,3-triazole ring acting as an acceptor group [51].

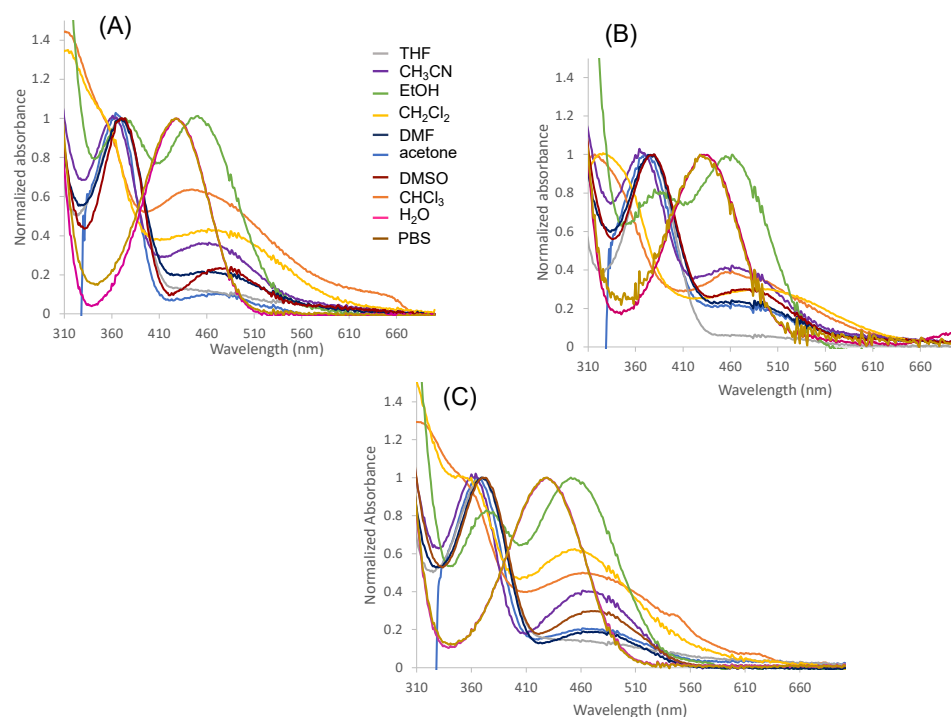


Figure 2. Normalized UV-Vis absorption spectra of TzNat **A–C** (at 10^{-5} M) in different solvents: (A) TzNat **A**, (B) TzNat **B**, (C) TzNat **C**.

A less intense band ($\epsilon = 6700$ to $400 \text{ M}^{-1} \cdot \text{cm}^{-1}$) centered at 459–474 nm is also observed in all solvents and is enhanced in non-polar solvent (CHCl_3 , CH_2Cl_2) as well as in protic solvent EtOH. However, in aqueous solvent (H_2O and PBS), maximum absorption wavelengths of TzNat **A** to **C** ranging from 425 to 435 nm are recorded. This band is blue-shifted by 20 nm compared with that recorded in ethanol.

Overall, the UV properties of these molecules are in accordance with those recorded for 1,6-Naphthyridin-7(6*H*)-ones [41]. The maximum absorption ranging from 320 to 386 nm can be assigned to $\pi \rightarrow \pi^*$ transition of the lactim form, whereas the absorption shift ranging from 425 to 474 nm could be related to the lactam form stabilized by intermolecular hydrogen bond with solvent.

The fluorescence properties of TzNat molecules were studied in solvents with different polarity (Table 1). Stokes shifts and fluorescence quantum yields (Φ) were determined in each solvent. The corresponding spectra are represented in Figure 3. Spectra recorded upon excitation at 320–386 nm in polar aprotic solvent acetone, THF, DMF, and DMSO show an emission band for the three molecules ranging from 458–502 nm, with modest Stokes shifts of 5300 to 6500 cm^{-1} and quantum yield up to $\Phi = 0.34$ for TzNat **B** in THF. In particular, TzNat **B** shows a red-shift of emission wavelength (+10 nm) and a higher quantum yield compared to TzNat **A** and **C** when placed in a medium of increased polarity. The red-shift can be explained by dipole–dipole interactions between the excited fluorophore and the surrounding molecules decreasing the excited-state energy, which is more noticeable for molecule **B** possessing a donor–acceptor system. In addition, the dramatic increase in emission quantum yield ($\times 15$) in DMF and DMSO observed for TzNat **B** is in accordance

with the presence of an intramolecular charge transfer (ICT) stabilized in a high polar solvent as mentioned above.

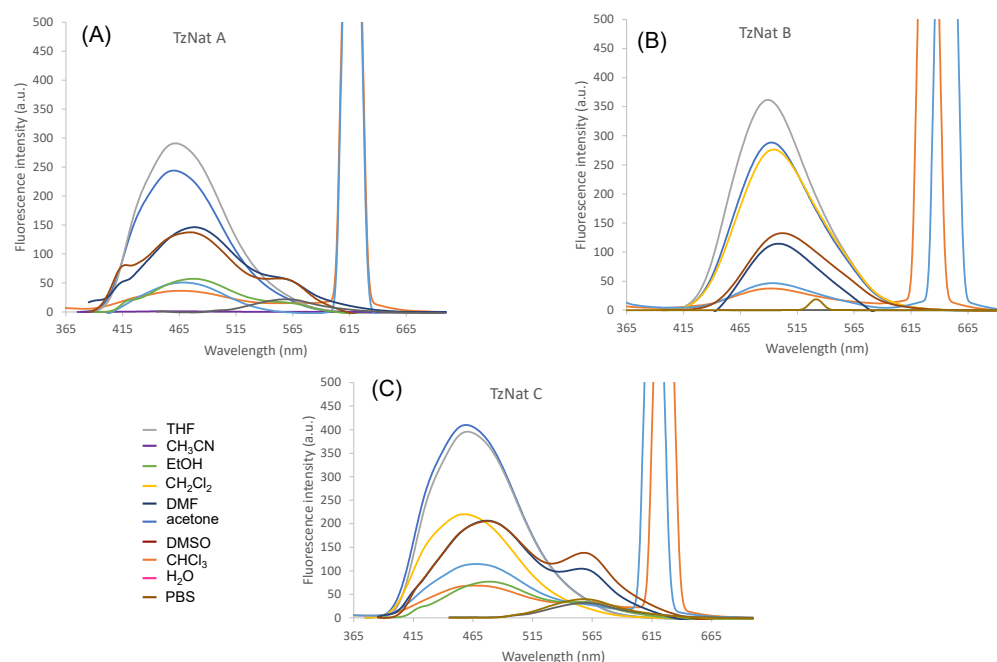


Figure 3. Fluorescence spectra of TzNat A–C (at $C = 10^{-5}$ μM) in different solvents: (A) TzNat A, (B) TzNat B, (C) TzNat C. The sharp peaks around 615 nm are the 2nd harmonic of the excitation wavelength.

Interestingly, for TzNat A and C possessing a phenyl and a para-bromophenyl group respectively, an additional emission band is observed in DMSO and DMF at 560 nm.

Surprisingly, upon excitation of the lactim form at 320–386 nm in non-polar solvent CHCl_3 and CH_2Cl_2 and in polar protic solvent EtOH, TzNat A, B, and C show very weak fluorescence emission centered at 470–490 nm, accompanied by a drop in quantum efficiency ($\Phi < 0.01$). Fluorescence quenching was also observed in aqueous solvent H_2O and PBS buffer after excitation at 420–430 nm corresponding to the lactam form ($\Phi < 0.01$).

By analogy with parent 1,6-Naphthyridin-7(6H)-ones, the solvatochromic behavior of TzNat molecules, showing distinct bands in absorption and emission depending on the polarity of the solvent, could be rationalized by the presence of lactam and lactim forms in equilibrium. A lactim form would correspond to the high energy band at 458–502 nm which is favored in polar aprotic solvent, with a red-shift for TzNat B of 30 nm related to TzNat A and C.

The lactam form of TzNat generated by a proton transfer of the lactim form isomer at the excited state is thought to be responsible for the low-energy band at 560 nm in polar solvent for molecules substituted by a phenyl (TzNat A) and a para-bromo phenyl group (TzNat C). However, the lactam form is not observed for TzNat B due to the ICT.

The quench of fluorescence in protic solvent could be explained by the nonradiative relaxation of the excited fluorophores enhanced by the polarity of the environment and hydrogen bonding with protic solvent molecules. Therefore, a twisted intramolecular charge transfer (TICT) resulting from the shift of the π -electron density on the donor-acceptor system at the excited state could also be involved to explain the quenching of the fluorescence in a protic solvent [52]. The twisted conformation is usually enhanced in a highly polar solvent possessing H-bonds and leads to full charge separation and a bathochromic shift. Indeed, in H_2O , the weak emission band ($\Phi < 0.01$) of the lactam form is observed red-shifted to 560 nm, and a second band of weak intensity centered at 560 nm is also observed in DMSO and DMF, which could be attributed to the TICT emission from the lactam form (Figure 4).

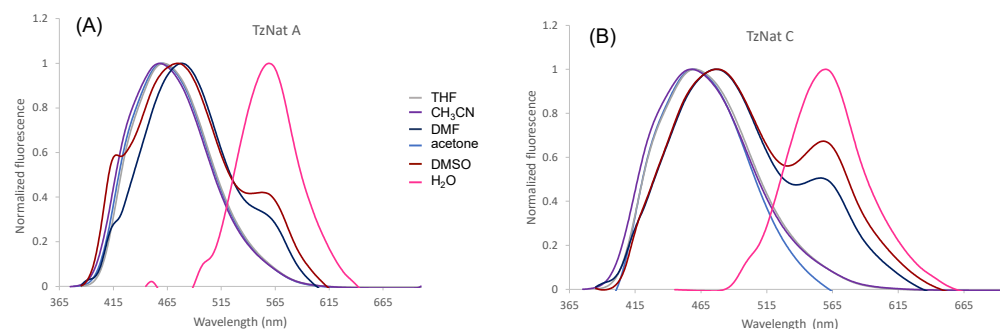


Figure 4. Normalized Fluorescence spectra of TzNat A and C (at 10^{-5} M) in polar solvents: (A) TzNat A, (B) TzNat C.

Nucleosides TzNat A, B, and C are environmentally sensitive fluorescent molecules, which are emissive in polar aprotic solvents with an enhanced quantum yield in the presence of charge transfer (TzNat B) and much less emissive in H_2O . This dramatic change of emissivity can be used to probe interactions with surrounding molecules or as a reported group to study interactions with enzymes in incorporated oligomer [25].

Conformational studies of the triazole nucleosides TzNat A–C with computational methods will yield additional information about favored conformers, and help to rationalize the optical behavior and give evidence of the TICT mechanism. In-depth photo-physical studies of these compounds is currently under investigation.

3. Materials and Methods

3.1. General Information

All reagents and solvents were purchased from commercial suppliers, Sigma-Aldrich, TCI (Europe), Alfa Aesar, or Fluorochem. The monowave reactor is a conventionally heated synthesis reactor from Anton Paar. Infra-red spectra were recorded on ATR VariGATR crystal Ge. All reactions were monitored by TLC on silica gel 60 F254 plates and revealed using UV lamp (l 254 nm). Flash chromatography was carried out on a Merck silica gel 60 F 254. Reactions involving air- or moisture-sensitive reagents were carried out under an inert atmosphere (argon) with oven-dried glassware. THF was distilled over sodium/benzophenone. All final compounds were analyzed by high-resolution ESI mass spectrometry (HRMS) in MeOH using a LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with an electrospray ion source. NMR spectra were recorded on a Bruker Avance spectrometer at 300 or 400 MHz for 1H and 100 MHz for ^{13}C . 2D NMR experiments such as 1H - 1H COSY and 1H - ^{13}C HSQC experiments were performed to enable signal attributions. UV-Vis absorption spectra were measured using a Cary 50 (Varian) spectrophotometer at 20 °C. Emission spectra were performed on a JASCO J-815 CD spectrofluorometer at 20 °C. Quantum yields were determined with a Fluoromax+ (Jobin Yvon) equipped with a quanta phi integration sphere. Data were treated with the dedicated software provided by the supplier. Measurements were performed at room temperature. Synthesis and data concerning compounds 3 and compounds 5 are already described [29].

3.2. Attempts to Prepare Triazole Conjugate 4 from Acetylated Ribosyl Azide 2 and Compounds 5

3.2.1. Synthesis of 2,3,5-Tri-O-acetyl- β -D-ribofuranosyl Azide 2

Trimethylsilyl azide (2.9 mL, 21.98 mmol) and tin chloride 1 M in heptane (4.7 mL, 4.7 mmol) were added to a stirred solution of 1,2,3,5-Tetra-O-acetyl- β -D-ribofuranose (5 g, 15.7 mmol) in dry DCM (40 mL). The reaction mixture was stirred at rt under argon for 20 h. The solution was washed with Na_2CO_3 + NaCl (10 mL + 20 mL), and the aqueous layer was extracted with DCM (2×30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The resulting residue was purified by silica gel chromatography (Cyclohexane/EtOAc 7/3) to afford the desired compound as

a colorless oil. $M_{\text{pure}} = 4.8$ g, Yield: quantitative. $^1\text{H NMR}$ (300 MHz, Chloroform- d) δ 5.45–5.22 (m, 2H, H_5), 5.12 (s, 1H, H_1), 4.54–4.23 (m, 2H, H_3/H_4), 4.23–4.00 (m, 1H, H_2), 2.21–1.93 (m, 9H, OAc). HRMS (ESI+): Calcd for $\text{C}_{11}\text{H}_{15}\text{N}_3\text{O}_7\text{Na}$ 324.0802; found 324.0805 $[\text{M} + \text{Na}]^+$. Spectral data of **2** were in agreement with the literature [49].

3.2.2. Synthesis of (2*R*,3*R*,4*R*,5*R*)-2-(4-(4-((3-(1,3-dioxolan-2-yl)pyridin-2-yl)(cyano)methyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-5-(acetoxymethyl)tetrahydrofuran-3,4-diyl Diacetate **6**

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (4.2 mg, 10% mmol) and sodium ascorbate (10 mg, 30% mmol) were added to a stirred solution of compound **5** (50 mg, 0.17 mmol) and azide **2** (51 mg, 0.17 mmol) in $t\text{BuOH}/\text{H}_2\text{O}$ (150 μL v/v 1:1). The reaction mixture was stirred at rt for 5 h. Then, an aqueous solution of EDTA 0.1 M (2 mL) was added to the mixture, and the solution was extracted with EtOAc (3×5 mL). The combined organic layers were washed with an aqueous solution of EDTA 0.1 M (3×5 mL), dried over MgSO_4 , filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (Cyclohexane/EtOAc 6/4, $R_f = 0.21$) to afford the title compound as a white oil. $M = 40$ mg. Yield: 40%. $^1\text{H NMR}$ (400 MHz, Chloroform- d) δ 8.66 (d, $J = 4.7$ Hz, 1H, H_{11}), 8.03 (s, 1H, H_{18}), 7.91 (d, $J = 7.6$ Hz 1H, H_3), 7.81 (d, $J = 8.0$ Hz, 2H, $H_{14/15}$), 7.55 (d, $J = 8.0$ Hz, 2H, $H_{12/13}$), 7.30 (dd, $J = 8, 4.8$ Hz, 1H, H_2), 6.18 (d, $J = 3.7$ Hz, 1H, H_{17}), 6.00–5.84 (m, 3H, $H_{6/9/4'}$), 5.63 (t, $J = 5.4$ Hz, 1H, $H_{3'}$), 4.50 (m, 1H, $H_{2'}$), 4.25 (dd, $J = 12.4, 4.4$ Hz, 1H, $H_{5'a}$), 4.17–4.04 (m, 5H, $H_{7/8/5'b}$), 2.19–1.99 (m, 9H, OAc). $^{13}\text{C NMR}$ (100 MHz, Chloroform- d) δ 170.7, 169.8, 169.7, 153.6, 150.7, 147.8, 136.1, 135.3, 131.5, 130.5, 129.3, 126.8, 123.5, 119.4, 101.2, 90.5, 81.4, 74.7, 71.17, 65.8, 65.7, 63.2, 60.8, 47.7, 21.1, 20.9, 20.8, 14.6. IR (cm^{-1}): 2971, 2360, 1747, 1445, 1373, 1229, 1112, 1073, 805. HRMS (ESI+) Calcd for $\text{C}_{29}\text{H}_{29}\text{N}_5\text{O}_9\text{H}$ 592.2038; found 592.2035 $[\text{M} + \text{H}]^+$.

3.3. Synthesis of the Alkyne Partner **9** for the Click Reaction

3.3.1. Synthesis of 2-Chloro-4-((trimethylsilyl)ethynyl)nicotinaldehyde **7**

In a monowave vial (10 mL), 4-iodo-2-chloro-3-pyridine carboxaldehyde (1 g, 3.74 mmol) was dissolved in THF (6 mL). Ethynyltrimethylsilane (570 μL , 4.11 mmol), CuI (7 mg, 0.037 mmol), $\text{PdCl}_2(\text{PPh}_3)_3$ (52 mg, 0.075 mmol), and NEt_3 (1.58 mL, 11.22 mmol) were added into the vial. The reaction was heated using the mode “as fast as possible” at 150 $^\circ\text{C}$ for 10 min. The mixture was filtered on celite[®] and washed with DCM. The residue was purified by silica gel chromatography (cyclohexane/ Et_2O 5%, $R_f = 0.57$) to afford the title compound as a yellow-brown solid. $M = 400$ mg. Yield: 45%. $^1\text{H NMR}$ (300 MHz, Chloroform- d) δ 10.56 (s, 1H), 8.48 (d, $J = 5.0$ Hz, 1H), 7.42 (d, $J = 5.1$ Hz, 1H), 0.32 (s, 9H). $^{13}\text{C NMR}$ (100 MHz, Chloroform- d) δ 188.2, 152.2, 152.0, 135.3, 128.9, 127.1, 109.8, 98.3, -0.6 . HRMS (ESI+) Calcd for $\text{C}_{11}\text{H}_{12}\text{ClNOSiH}$ 238.0449; found: 238.0450 $[\text{M} + \text{H}]^+$.

3.3.2. Synthesis of 2-Chloro-3-(dimethoxymethyl)-4-((trimethylsilyl)ethynyl)pyridine **8**

Compound **7** (455 mg, 1.94 mmol) dissolved in MeOH (5 mL) was added to a stirred solution of CeCl_3 (479 mg, 1.94 mmol) and trimethyl orthoformate $\text{CH}(\text{OCH}_3)_3$ (1.37 mL, 6.26 mmol) in MeOH (2 mL). The reaction mixture was heated at 60 $^\circ\text{C}$ until complete conversion of the starting material. The solution was neutralized with a saturated aqueous solution of NaHCO_3 (10 mL). The aqueous layer was extracted with DCM (2×10 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated under pressure to afford the title compound without further purification as an oil. $M = 493$ mg. Yield: 89%. $R_f = 0.42$ (cyclohexane/ Et_2O 8/2). $^1\text{H NMR}$ (300 MHz, Chloroform- d) δ 8.30 (d, $J = 5.0$ Hz, 1H), 7.29 (d, $J = 5.0$ Hz, 1H), 5.81 (s, 1H), 3.49 (s, 6H), 0.31 (s, 9H). $^{13}\text{C NMR}$ (100 MHz, Chloroform- d) δ 149.2, 134.0, 133.0, 126.7, 106.8, 104.1, 100.2, 56.1, -0.1 . HRMS (ESI+) Calcd for $\text{C}_{13}\text{H}_{18}\text{ClNO}_2\text{SiH}$ 284.0868; found: 284.0869 $[\text{M} + \text{H}]^+$.

3.3.3. Synthesis of 2-Chloro-3-(dimethoxymethyl)-4-ethynylpyridine **9**

K_2CO_3 (623 mg, 4.5 mmol) was added to a stirred solution of compound **8** (850 mg, 3 mmol) in MeOH (20 mL). The reaction mixture was stirred at rt for 2 min. The solution

was immediately quenched with water (20 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure to afford a crude product which was engaged in the next step without further purification. M = 556 mg. Yield: 84%. Rf = 0.25 (cyclohexane/Et₂O 8/2). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.32 (d, *J* = 5.0 Hz, 1H), 7.36 (d, *J* = 5.0 Hz, 1H), 5.76 (s, 1H), 3.60 (s, 1H), 3.49 (s, 6H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 151.0, 148.8, 132.9, 132.2, 127.3, 103.6, 87.3, 78.9, 55.8. IR (cm⁻¹): 3217, 2995, 2940, 2838, 2107, 1573, 1531, 1371, 1210, 1184, 1060, 961, 827. HRMS (ESI+) Calcd for C₁₀H₁₀ClNO₂H 212.0473, found: 212.0473 [M + H]⁺.

3.4. Synthesis of Target 1,2,3 Triazole Nucleoside TzNat A, B, and C

3.4.1. Synthesis of (2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(4-(2-chloro-3-(dimethoxymethyl)pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-3,4-diyl Diacetate **10**

CuI (79 mg, 0.42 mmol), DIPEA (182 μL, 1.05 mmol) and compound **9** (65 mg, 0.21 mmol) were added to a stirred solution of compound **2** (45.5 mg, 0.21 mmol) in DCM (3 mL). The reaction mixture was stirred at 40 °C for 7 h. Then an aqueous solution of EDTA 0.1 M (10 mL) was added to the mixture which was stirred for 1 h until the organic layer was colorless. The organic layer was dried over MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by silica gel chromatography (Cyclohexane/EtOAc 1/1) to afford the title compound as a colorless oil, m_{pure} = 70 mg. Yield: 65%. ¹H NMR (300 MHz, Chloroform-*d*) δ 8.50 (s, 1H), 8.38 (d, *J* = 5.1 Hz, 1H), 8.04 (d, *J* = 5.1 Hz, 1H), 6.18 (d, *J* = 3.6 Hz, 1H), 5.90–5.84 (m, 2H), 5.64 (t, *J* = 5.4 Hz, 1H), 4.51–4.39 (m, 2H), 4.28–4.13 (m, 1H), 3.39 (s, 6H), 2.14–2.00 (m, 9H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 170.4, 169.4, 169.2, 151.7, 149.5, 143.2, 140.3, 126.6, 123.9, 104.8, 90.0, 80.9, 77.2, 74.4, 70.8, 62.9, 56.1, 56.0, 20.6, 20.4, 20.4. HRMS (ESI+) Calcd for C₂₁H₂₅ClN₄O₉Na 535.1202; found: 535.1199 [M + Na]⁺.

3.4.2. Synthesis of β-D-ribofuranosyl Azide **12**

A freshly prepared solution of MeONa (1 M in MeOH) was added to a stirred solution of 2,3,5-tri-*O*-acetate-β-D-ribofuranosyl azide (4.8 g, 15.9 mmol) in MeOH (90 mL). The reaction mixture was stirred for 5 min at rt and quenched with DOWEX resin. The suspension was vigorously stirred for 10 min, and the resin was filtered and washed with DCM. The desired compound was obtained as a pure colorless oil without purification. M_{pure} = 2.7 g. Yield: quantitative. ¹H NMR (300 MHz, Methanol-*d*₄) δ 5.21 (d, *J* = 1.9 Hz, 1H), 4.10–4.05 (m, 1H), 4.02–3.97 (m, 1H), 3.84–3.82 (m, 1H), 3.76 (m, 1H), 3.63 (dd, *J* = 12.0, 5.5 Hz, 1H). HRMS (ESI+): Calcd for C₅H₈N₃O₄ 174.0520; found 174.0522 [M – H]⁻. Spectral data of **12** were in agreement with the literature [53].

3.4.3. Synthesis of 2,3,5-Tri-*O*-(tertbutyldimethylsilyle)-β-D-ribofuranosyl Azide **13**

Imidazole (3.9 g, 57 mmol) and tertbutyldimethylchlorosilane (8.2 g, 54 mmol) were added to a stirred solution of D-ribofuranosyl azide (2.7 g, 15.4 mmol) in DMF (30 mL). The reaction mixture was stirred for 24 h at rt. The mixture was quenched with a saturated aqueous solution of NaHCO₃, and the aqueous layer was extracted with DCM (3 × 40 mL). The combined organic layers were washed with water (3 × 50 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (Cyclohexane/Et₂O 5%, Rf = 0.20) to afford the title compound as a white solid. M = 6.1 g. Yield: 76%. ¹H NMR (300 MHz, Chloroform-*d*) δ 5.10 (d, *J* = 2.7, 1H, H_{1'}), 4.20 (dd, *J* = 5.9, 4.0 Hz, 1H, H_{3'}), 4.01 (dt, *J* = 6.2, 3.2, 1H, H_{4'}), 3.82 (m, 1H, H_{2'}), 3.82 (dd, *J* = 11.5, 3.4 Hz, 1H, H_{5'}), 3.67 (dd, *J* = 11.5, 3.4 Hz, 1H, H_{5'}), 0.92 (s, 9H, H_{7'}), 0.91 (s, 9H, H_{7'}), 0.90 (s, 9H, H_{7'}), 0.11–0.08 (s, 18H, H_{6'}). ¹³C NMR (100 MHz, Chloroform-*d*) δ 94.7 (CH_{1'}), 84.3 (CH_{4'}), 76.5 (CH_{2'}), 71.1 (CH_{3'}), 62.1 (CH_{5'}), 25.9 (CH_{7'}), 25.8 (CH_{7'}), 25.8 (CH_{7'}), -4.4, -4.6, -5.5 (CH_{6'}). IR (cm⁻¹): 2951, 2931, 2859, 2117, 1472, 1142, 1128, 1073, 999, 777. HRMS (ESI+): Calcd for C₂₃H₅₁N₃O₄Si₃Na 540.3080. Found 540.3080 [M + Na]⁺.

3.4.4. Synthesis of 4-(1-((2*R*,3*R*,4*R*,5*R*)-3,4-bis((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)-2-chloro-3-(dimethoxymethyl)pyridine **14**

Under inert condition, in a sealed tube, CuI (1.06 g, 5.56 mmol), DIPEA (2.40 mL, 14 mmol), and compound **13** (1.58 g, 3.06 mmol) were added portionwise to a stirred solution of compound **9** (588 mg, 2.78 mmol) in DCM (23 mL). The reaction mixture was stirred at 40 °C for 24 h. Then, the solution was cooled to room temperature and an aqueous solution of EDTA 0.1 M (50 mL) was added to the mixture, which was stirred for 1 h until the organic layer was colorless. The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with a solution of EDTA 0.1 M until the aqueous layer was colorless, with water (20 mL) and brine (20 mL). Then, it was dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude was purified by silica gel chromatography (cyclohexane/Et₂O 20%, R_f = 0.30) to afford the title compound as a yellow oil. M = 1.8 g. Yield: 89%. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.40 (s, 1H, H₁₀), 8.38 (d, *J* = 5.1 Hz, 1H, H₅), 8.01 (d, *J* = 5.1 Hz, 1H, H₄), 5.91 (d, *J* = 4.8 Hz, 1H, H_{1'}), 5.84 (s, 1H, H₆), 4.80 (t, *J* = 4.8 Hz, H_{2'}), 4.35 (t, *J* = 3.9 Hz, 1H, H_{3'}), 4.15 (q, *J* = 4.6, 4.3 Hz, 1H, H_{4'}), 3.80 (dd, *J* = 11.1, 5.4 Hz, 1H, H_{5'}), 3.75 (dd, *J* = 11.1, 5.4 Hz, 1H, H_{5'}), 3.37 (s, 3H, H₇), 3.41 (s, 3H, H₈), 0.97 (s, 9H, H_{7'}), 0.87 (s, 9H, H_{7'}), 0.84 (s, 9H, H_{7'}), 0.12 (s, 6H, H_{6'}), 0.04 (s, 3H, H_{6'}), 0.03 (s, 3H, H_{6'}), 0.01 (s, 3H, H_{6'}), −0.14 (s, 3H, H_{6'}). ¹³C NMR (100 MHz, Chloroform-*d*) δ 151.8 (C₃), 149.5 (CH₅), 142.8 (C₁), 140.9 (C₉), 128.0 (C₂), 127.4 (CH₁₀), 124.1 (CH₄), 104.9 (CH₆), 92.3 (CH_{1'}), 86.3 (CH_{4'}), 76.3 (CH_{2'}), 72.5 (CH_{3'}), 63.1 (CH_{5'}), 56.2 (CH_{7/8}), 56.1 (CH_{7/8}), 27.0 (CH_{7'}), 18.5 (C_{8'}), 18.1 (C_{8'}), 18.1 (C_{8'}), −4.3 (CH_{6'}), −4.6 (CH_{6'}), −5.0 (CH_{6'}), −5.3 (CH_{6'}), −5.4 (CH_{6'}), 5.86 (CH_{6'}). IR (cm^{−1}): 2953, 2930, 2896, 2857, 1253, 1110, 1076, 836, 777. HRMS (ESI+) Calcd for C₃₃H₆₁ClN₄O₆Si₃Na 751.3480. Found 751.3480 [M + Na]⁺.

3.4.5. Synthesis of 2-(4-(1-((2*R*,3*R*,4*R*,5*R*)-3,4-bis((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)-3-(dimethoxymethyl)pyridin-2-yl)-2-phenylacetonitrile **15**

Under inert conditions, in a sealed tube, sodium hydride (3 equiv., 60% in oil) was added to a stirred solution of compound **14** (255.3 mg, 0.35 mmol) in dry THF (0.18 M). Phenylacetonitrile (80 μL, 0.7 mmol) was added in one portion, and the reaction mixture was refluxed for 20 h. The solution was cooled to room temperature and quenched by addition of water. The compound was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (Cyclohexane/Et₂O 8/2, R_f = 0.21) to afford the title compound as a yellow oil and as a mixture of two diastereoisomers: 55/45. M = 85 mg. Yield: 30%. ¹H NMR (400 MHz, CDCl₃) δ 8.62 (d, *J* = 2.5 Hz, 1H, H₅, dia 1), 8.60 (d, *J* = 2.5 Hz, 1H, H₅, dia 2), 8.09 (s, 1H, H₁₀, dia 1 or dia 2), 8.08 (s, 1H, H₁₀, dia 1 or dia 2), 7.60–7.58 (m, 2H, H_{12,16}, dia 1 or dia 2), 7.58–7.56 (m, 2H, H_{12,16}, dia 1 or dia 2), 7.37–7.34 (m, 2H, H_{13,15}, dia 1), 7.34–7.31 (m, 2H, H_{13,15}, dia 2), 7.30–7.28 (m, 2H, H₁₄, dia 1), 7.28–7.26 (m, 2H, H₁₄, dia 2), 7.22 (d, *J* = 5.0 Hz, 1H, H₄, dia 1 or dia 2), 7.20 (d, *J* = 5.0 Hz, 1H, H₄, dia 1 or dia 2), 6.49 (s, 1H, H₁₁, dia 1 or dia 2), 6.48 (s, 1H, H₁₁, dia 1 or dia 2), 6.08 (d, *J* = 2.4 Hz, 1H, H_{1'}, dia 1), 6.06 (d, *J* = 2.3 Hz, 1H, H_{1'}, dia 2), 5.83 (s, 1H, H₆, dia 1 or dia 2), 5.83 (s, 1H, H₆, dia 1 or dia 2), 4.64–4.59 (m, 1H, H_{2'}, dia 2), 4.60–4.58 (m, 1H, H_{2'}, dia 1), 4.30–4.27 (m, 1H, H_{3'}, dia 2), 4.27–4.24 (m, 1H, H_{3'}, dia 1), 4.20–4.18 (m, 1H, H_{4'}, dia 1), 4.18–4.16 (m, 1H, H_{4'}, dia 2), 3.93–3.91 (m, 1H, H_{5'}, dia 2), 3.90–3.88 (m, 1H, H_{5'}, dia 1), 3.79 (d, *J* = 2.9 Hz, 1H, H_{5'}, dia 1), 3.77 (d, *J* = 2.9 Hz, 1H, H_{5'}, dia 2), 3.49 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.45 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.42 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.40 (s, 3H, H₇ or H₈, dia 1 or dia 2), 0.95 (s, 1H, H_{7'}, dia 1 or dia 2), 0.94 (s, 9H, H_{7'}, dia 1 or dia 2), 0.89 (s, 9H, H_{7'}, dia 1 or dia 2), 0.88 (s, 9H, H_{7'}, dia 1 or dia 2), 0.86 (s, 9H, H_{7'}, dia 1 or dia 2), 0.85 (s, 9H, H_{7'}, dia 1 or dia 2), 0.13 (s, 3H, H_{6'}, dia 1 or dia 2), 0.12 (s, 3H, H_{6'}, dia 1 or dia 2), 0.12 (s, 3H, H_{6'}, dia 1 or dia 2), 0.11 (s, 3H, H_{6'}, dia 1 or dia 2), 0.08 (s, 3H, H_{6'}, dia 1 or dia 2), 0.08 (s, 3H, H_{6'}, dia 1 or dia 2), 0.08 (s, 3H, H_{6'}, dia 1 or dia 2), 0.07 (s, 3H, H_{6'}, dia 1 or dia 2), 0.01 (s, 3H, H_{6'}, dia 1), 0.00

(s, 3H, H_{6'}, dia 2), −0.14 (s, 3H H_{6'}, dia 1), −0.16 (s, 3H, H_{6'}, dia 2). ¹³C NMR (101 MHz, CDCl₃) δ 156.3 (C₁, dia 1 or dia 2), 156.3 (C₁, dia 1 or dia 2), 149.8 (C₅, dia 1 or dia 2), 149.8 (C₅, dia 1 or dia 2), 144.6 (C₉, dia 1 or dia 2), 144.6 (C₉, dia 1 or dia 2), 138.8 (C₃, dia 1 or dia 2), 138.7 (C₃, dia 1 or dia 2), 136.0 (C₁₇, dia 1 or dia 2), 136.0 (C₁₇, dia 1 or dia 2), 130.1 (C₂, dia 1 or dia 2), 130.1 (C₂, dia 1 or dia 2), 128.8 (C_{12,16} or C_{13,15}, dia 1 or dia 2), 128.7 (C_{12,16} or C_{13,15}, dia 1 or dia 2), 128.7 (C_{12,16} or C_{13,15}, dia 1 or dia 2), 127.8 (C_{12,16} or C_{13,15}, dia 1 or dia 2), 127.8 (C₁₄, dia 1 or dia 2), 127.8 (C₁₄, dia 1 or dia 2), 123.0 (C₄, dia 1 or dia 2), 122.9 (C₄, dia 1 or dia 2), 122.6 (C₁₀, dia 1 or dia 2), 122.6 (C₁₀, dia 1 or dia 2), 120.3 (CN, dia 1 or dia 2), 120.2 (CN, dia 1 or dia 2), 103.2 (C₆, dia 1 or dia 2), 103.2 (C₆, dia 1 or dia 2), 92.9 (C_{1'}, dia 1 or dia 2), 92.8 (C_{1'}, dia 1 or dia 2), 86.8 (C_{4'}, dia 1 or dia 2), 86.6 (C_{4'}, dia 1 or dia 2), 77.2 (C_{2'}, dia 1 or dia 2), 77.2 (C_{2'}, dia 1 or dia 2), 72.6 (C_{3'}, dia 1 or dia 2), 72.4 (C_{3'}, dia 1 or dia 2), 62.9 (C_{5'}, dia 1 or dia 2), 62.8 (C_{5'}, dia 1 or dia 2), 56.3 (C₇ or C₈, dia 1 or dia 2), 56.2 (C₇ or C₈, dia 1 or dia 2), 55.9 (C₇ or C₈, dia 1 or dia 2), 55.9 (C₇ or C₈, dia 1 or dia 2), 41.6 (C₁₁, dia 1 or dia 2), 41.5 (C₁₁, dia 1 or dia 2), 26.1 (3C_{7'}, dia 1 or dia 2), 26.1 (3C_{7'}, dia 1 or dia 2), 25.9 (3C_{7'}, dia 1 or dia 2), 25.9 (3C_{7'}, dia 1 or dia 2), 25.9 (3C_{7'}, dia 1 or dia 2), 25.9 (3C_{7'}, dia 1 or dia 2), 18.1 (3C_{8'}, dia 1 or dia 2), 18.1 (3C_{8'}, dia 1 or dia 2), −4.3 (2C_{6'}, dia 1 or dia 2), −4.3 (2C_{6'}, dia 1 or dia 2), −4.5 (2C_{6'}, dia 1 or dia 2), −4.5 (2C_{6'}, dia 1 or dia 2), −5.0 (2C_{6'}, dia 1 or dia 2), −5.0 (2C_{6'}, dia 1 or dia 2). IR (cm^{−1}): 2953, 2930, 2857, 2359, 2341, 2254, 1595, 1494, 1259, 1253, 1109, 1074, 836, 779. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₄₁H₆₇N₅O₆Si₃H 810.4472. Found 810.4476.

3.4.6. Synthesis of 2-(4-(1-((2*R*,3*R*,4*R*,5*R*)-3,4-bis((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)-3-(dimethoxymethyl)pyridin-2-yl)-2-(4-methoxyphenyl)acetonitrile 16

Under inert condition, in a sealed tube, sodium hydride (3 equiv., 60% in oil) was added to a stirred solution of compound 14 (295 mg, 0.4 mmol) in dry THF (0.2 M). 4-methoxyphenylacetonitrile (108.5 μL, 0.80 mmol) was added in one portion, and the reaction mixture was refluxed for 48 h. The solution was cooled to room temperature and quenched by addition of water. The compound was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (Cyclohexane/Et₂O 7/3, R_f = 0.28) to afford the title compound as a yellow oil and as a mixture of two diastereoisomers: M = 85.2 mg. Yield: 25%. ¹H NMR (300 MHz, CDCl₃) δ 8.62 (d, *J* = 1.8 Hz, 1H, H₅, dia 1), 8.61 (d, *J* = 1.8 Hz, 1H, H₅, dia 2), 8.09 (s, 1H, H₁₀, dia 1 or dia 2), 8.08 (s, 1H, H₁₀, dia 1 or dia 2), 7.55–7.51 (m, 2H, H_{13,17}, dia 1 or dia 2), 7.51–7.48 (m, 2H, H_{13,17}, dia 1 or dia 2), 7.21 (d, *J* = 5.2 Hz, 1H, H₄, dia 2), 7.19 (d, *J* = 5.6 Hz, 1H, H₄, dia 1), 6.89–6.86 (m, 2H, H_{13,16}, dia 1 or dia 2), 6.86–6.83 (m, 2H, H_{13,16}, dia 1 or dia 2), 6.42 (s, 1H, H₁₁, dia 1 or dia 2), 6.41 (s, 1H, H₁₁, dia 1 or dia 2), 6.08 (d, *J* = 1.9 Hz, 1H, H_{1'}, dia 1), 6.06 (d, *J* = 1.7 Hz, 1H, H_{1'}, dia 2), 5.82 (s, 1H, H₆, dia 1 or dia 2), 5.82 (s, 1H, H₆, dia 1 or dia 2), 4.64–4.60 (m, 1H, H_{2'}, dia 2), 4.60–4.56 (m, 1H, H_{2'}, dia 1), 4.30–4.26 (m, 1H, H_{3'}, dia 2), 4.26–4.23 (m, 1H, H_{3'}, dia 1), 4.20–4.18 (m, 1H, H_{4'}, dia 1), 4.18–4.15 (m, 1H, H_{4'}, dia 2), 3.94–3.90 (m, 1H, H_{5'}, dia 2), 3.90–3.87 (m, 1H, H_{5'}, dia 1), 3.83–3.74 (m, 1H, H_{5'}, dia 1), 3.78 (s, 3H, H₁₈, dia 1 or dia 2), 3.78 (s, 3H, H₁₈, dia 1 or dia 2), 3.77–3.74 (m, 1H, H_{5'}, dia 2), 3.49 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.45 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.41 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.39 (s, 3H, H₇ or H₈, dia 1 or dia 2), 0.94 (s, 9H, H_{7'}, dia 1 or dia 2), 0.94 (s, 9H, H_{7'}, dia 1 or dia 2), 0.89 (s, 9H, H_{7'}, dia 1 or dia 2), 0.87 (s, 9H, H_{7'}, dia 1 or dia 2), 0.86 (s, 9H, H_{7'}, dia 1 or dia 2), 0.85 (s, 9H, H_{7'}, dia 1 or dia 2), 0.12 (s, 3H, H_{6'}, dia 1 or dia 2), 0.12 (s, 3H, H_{6'}, dia 1 or dia 2), 0.11 (s, 3H, H_{6'}, dia 1 or dia 2), 0.11 (s, 3H, H_{6'}, dia 1 or dia 2), 0.08 (s, 3H, H_{6'}, dia 1 or dia 2), 0.08 (s, 3H, H_{6'}, dia 1 or dia 2), 0.07 (s, 3H, H_{6'}, dia 1 or dia 2), 0.00 (s, 3H, H_{6'}, dia 1 or dia 2), 0.00 (s, 3H, H_{6'}, dia 1 or dia 2), −0.15 (s, 3H, H_{6'}, dia 1 or dia 2), −0.17 (s, 3H, H_{6'}, dia 1 or dia 2). ¹³C NMR (75 MHz, CDCl₃) δ 159.2 (C₁₅, dia 1 or dia 2), 159.1 (C₁₅, dia 1 or dia 2), 156.5 (C₁, dia 1 or dia 2), 156.5 (C₁, dia 1 or dia 2), 149.8 (C₅, dia 1 or dia 2), 149.8 (C₅, dia 1 or dia 2), 144.7 (C₉, dia 1 or dia 2), 144.6 (C₉, dia 1 or dia 2), 138.7 (C₃, dia 1 or dia 2), 138.7 (C₃, dia 1 or dia 2), 130.0 (C₂, dia 1 or dia 2), 130.0 (C₂, dia 1 or dia 2), 129.9 (C_{13,14}, dia 1 or dia 2), 129.9 (C_{13,14}, dia 1 or dia 2), 128.1

(C₁₂, dia 1 or dia 2), 128.1 (C₁₂, dia 1 or dia 2), 122.9 (C₄, dia 1 or dia 2), 122.9 (C₄, dia 1 or dia 2), 122.6 (C₁₀, dia 1 or dia 2), 122.5 (C₁₀, dia 1 or dia 2), 120.5 (CN, dia 1 or dia 2), 120.5 (CN, dia 1 or dia 2), 114.0 (C_{14,16}, dia 1 or dia 2), 114.0 (C_{14,16}, dia 1 or dia 2), 103.2 (C₆, dia 1 or dia 2), 103.2 (C₆, dia 1 or dia 2), 92.9 (C_{1'}, dia 1 or dia 2), 92.8 (C_{1'}, dia 1 or dia 2), 86.8 (C_{4'}, dia 1 or dia 2), 86.6 (C_{4'}, dia 1 or dia 2), 77.4 (C_{2'}, dia 1 or dia 2), 77.3 (C_{2'}, dia 1 or dia 2), 72.6 (C_{3'}, dia 1 or dia 2), 72.4 (C_{3'}, dia 1 or dia 2), 62.9 (C_{5'}, dia 1 or dia 2), 62.8 (C_{5'}, dia 1 or dia 2), 56.2 (C₇ or C₈, dia 1 or dia 2), 56.2 (C₇ or C₈, dia 1 or dia 2), 55.9 (C₇ or C₈, dia 1 or dia 2), 55.9 (C₇ or C₈, dia 1 or dia 2), 55.4 (C₁₈, dia 1 or dia 2), 55.4 (C₁₈, dia 1 or dia 2), 40.7 (C₁₁, dia 1 or dia 2), 40.7 (C₁₁, dia 1 or dia 2), 26.1 (3C, C_{7'}, dia 1 or dia 2), 26.1 (3C, C_{7'}, dia 1 or dia 2), 25.9 (3C, C_{7'}, dia 1 or dia 2), 25.9 (3C, C_{7'}, dia 1 or dia 2), 25.8 (3C, C_{7'}, dia 1 or dia 2), 18.5 (C_{6'}, dia 1 or dia 2), 18.5 (C_{6'}, dia 1 or dia 2), 18.2 (C_{6'}, dia 1 or dia 2), 18.2 (C_{6'}, dia 1 or dia 2), 18.1 (C_{6'}, dia 1 or dia 2), 18.1 (C_{6'}, dia 1 or dia 2), −4.3 (C_{6'}, dia 1 or dia 2), −4.3 (C_{6'}, dia 1 or dia 2), −4.5 (C_{6'}, dia 1 or dia 2), −4.5 (C_{6'}, dia 1 or dia 2), −4.5 (C_{6'}, dia 1 or dia 2), −4.5 (C_{6'}, dia 1 or dia 2), −5.0 (C_{6'}, dia 1 or dia 2), −5.0 (C_{6'}, dia 1 or dia 2), −5.1 (C_{6'}, dia 1 or dia 2), −5.1 (C_{6'}, dia 1 or dia 2) −5.3 (C_{6'}, dia 1 or dia 2), −5.3 (C_{6'}, dia 1 or dia 2). IR (cm^{−1}): 2952, 2930, 2857, 2359, 2342, 2254, 1596, 1254, 1108, 1073, 836, 778. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₄₂H₆₉N₅O₇Si₃H 840.4578. Found 840.4581.

3.4.7. Synthesis of 2-(4-(1-((2*R*,3*R*,4*R*,5*R*)-3,4-bis((*tert*-butyldimethylsilyloxy)-5-(((*tert*-butyldimethylsilyloxy)methyl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)-3-(dimethoxymethyl)pyridin-2-yl)-2-(4-bromophenyl)acetonitrile 17

Under inert condition, in a sealed tube, sodium hydride (3 equiv., 60% in oil) was added to a stirred solution of compound 14 (297 mg, 0.41 mmol) in dry THF (0.2 M). 4-bromophenylacetonitrile (155.8 mg, 0.82 mmol) was added in one portion, and the reaction mixture was refluxed for 72 h. The solution was cooled to room temperature and quenched by addition of water. The compound was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (Cyclohexane/Et₂O 7/3, R_f = 0.24) to afford the title compound as an orange oil and as a mixture of two diastereoisomers. M = 102 mg. Yield: 28%. ¹H NMR (300 MHz, CDCl₃) δ 8.60 (d, *J* = 1.4 Hz, 1H, H₅, dia 1), 8.59 (d, *J* = 1.4 Hz, 1H, H₅, dia 2), 8.10 (s, 1H, H₁₀, dia 1 or dia 2), 8.10 (s, 1H, H₁₀, dia 1 or dia 2), 7.48–7.47 (m, 4H, H_{13,14,16,17}, dia 1 or dia 2), 7.47–7.46 (m, 4H, H_{13,14,16,17}, dia 1 or dia 2), 7.21 (d, *J* = 5.0 Hz, 1H, H₄, dia 2), 7.19 (d, *J* = 5.0 Hz, 1H, H₄, dia 1), 6.45 (s, 1H, H₁₁, dia 1 or dia 2), 6.44 (s, 1H, H₁₁, dia 1 or dia 2), 6.09 (d, *J* = 1.6 Hz, 1H, H_{1'}, dia 1 or dia 2), 6.07 (d, *J* = 1.4 Hz, 1H, H_{1'}, dia 1 or dia 2), 5.85 (s, 1H, H₆, dia 1 or dia 2), 5.85 (s, 1H, H₆, dia 1 or dia 2), 4.61 (d, *J* = 4.6 Hz, 1H, H_{2'}, dia 1), 4.58 (d, *J* = 4.8 Hz, 1H, H_{2'}, dia 2), 4.29–4.26 (m, 1H, H_{3'}, dia 1), 4.26–4.23 (m, 1H, H_{3'}, dia 2), 4.20–4.18 (m, 1H, H_{4'}, dia 2), 4.18–4.16 (m, 1H, H_{4'}, dia 1), 3.93 (dd, *J* = 3.7, 1.5 Hz, 1H, H_{5'}, dia 1), 3.89 (dd, *J* = 3.7, 1.5 Hz, 1H, H_{5'}, dia 2), 3.80 (d, *J* = 2.8 Hz, 1H, H_{5'}, dia 2), 3.76 (d, *J* = 2.8 Hz, 1H, H_{5'}, dia 1), 3.51 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.47 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.42 (s, 3H, H₇ or H₈, dia 1 or dia 2), 3.39 (s, 3H, H₇ or H₈, dia 1 or dia 2), 0.95 (s, 9H, H_{7'}, dia 1 or dia 2), 0.94 (s, 9H, H_{7'}, dia 1 or dia 2), 0.89 (s, 9H, H_{7'}, dia 1 or dia 2), 0.88 (s, 9H, H_{7'}, dia 1 or dia 2), 0.86 (s, 9H, H_{7'}, dia 1 or dia 2), 0.85 (s, 9H, H_{7'}, dia 1 or dia 2), 0.13 (s, 3H, H_{6'}, dia 1 or dia 2), 0.12 (s, 3H, H_{6'}, dia 1 or dia 2), 0.11 (s, 3H, H_{6'}, dia 1 or dia 2), 0.11 (s, 3H, H_{6'}, dia 1 or dia 2), 0.10 (s, 3H, H_{6'}, dia 1 or dia 2), 0.09 (s, 3H, H_{6'}, dia 1 or dia 2), 0.08 (s, 3H, H_{6'}, dia 1 or dia 2), 0.08 (s, 3H, H_{6'}, dia 1 or dia 2), 0.00 (s, 3H, H_{6'}, dia 1 or dia 2), 0.00 (s, 3H, H_{6'}, dia 1 or dia 2), −0.15 (s, 3H, H_{6'}, dia 1 or dia 2), −0.17 (s, 3H, H_{6'}, dia 1 or dia 2). ¹³C NMR (75 MHz, CDCl₃) δ 155.8 (C₁, dia 1 or dia 2), 155.8 (C₁, dia 1 or dia 2), 149.9 (C₅, dia 1 or dia 2), 149.8 (C₅, dia 1 or dia 2), 144.6 (C₉, dia 1 or dia 2), 144.5 (C₉, dia 1 or dia 2), 138.8 (C₃, dia 1 or dia 2), 138.7 (C₃, dia 1 or dia 2), 135.1 (C₁₂, dia 1 or dia 2), 135.1 (C₁₂, dia 1 or dia 2), 131.8 (C_{14,16} or C_{13,17}, dia 1 or dia 2), 131.8 (C_{14,16} or C_{13,17}, dia 1 or dia 2), 130.5 (C_{14,16} or C_{13,17}, dia 1 or dia 2), 130.5 (C_{14,16} or C_{13,17}, dia 1 or dia 2), 130.3 (C₂, dia 1 or dia 2), 130.2 (C₂, dia 1 or dia 2), 123.0 (C₄, dia 1 or dia 2), 123.0 (C₄, dia 1 or dia 2), 122.5 (C₁₀, dia 1 or dia 2), 122.5 (C₁₀, dia 1 or dia 2), 122.0 (C₁₅, dia 1 or dia 2), 122.0 (C₁₅, dia 1 or dia 2), 119.8 (CN, dia 1 or dia 2), 119.8 (CN, dia 1 or dia 2), 103.2 (C₆, dia 1 or dia 2),

103.2 (C₆, dia 1 or dia 2), 92.9 (C_{1'}, dia 1 or dia 2), 92.9 (C_{1'}, dia 1 or dia 2), 86.9 (C_{4'}, dia 1 or dia 2), 86.6 (C_{4'}, dia 1 or dia 2), 77.5 (C_{2'}, dia 1 or dia 2), 77.3 (C_{2'}, dia 1 or dia 2), 72.6 (C_{3'}, dia 1 or dia 2), 72.4 (C_{3'}, dia 1 or dia 2), 62.9 (C_{5'}, dia 1 or dia 2), 62.8 (C_{5'}, dia 1 or dia 2), 56.4 (C₇ or C₈, dia 1 or dia 2), 56.4 (C₇ or C₈, dia 1 or dia 2), 56.0 (C₇ or C₈, dia 1 or dia 2), 56.0 (C₇ or C₈, dia 1 or dia 2), 41.0 (C₁₁, dia 1 or dia 2), 41.0 (C₁₁, dia 1 or dia 2), 26.1 (3C, C_{7'}, dia 1 or dia 2), 26.1 (3C, C_{7'}, dia 1 or dia 2), 25.9 (2C, C_{7'}, dia 1 or dia 2), 25.8 (2C, C_{7'}, dia 1 or dia 2), 18.5 (C_{8'}, dia 1 or dia 2), 18.5 (C_{8'}, dia 1 or dia 2), 18.2 (C_{8'}, dia 1 or dia 2), 18.2 (C_{8'}, dia 1 or dia 2), 18.1 (C_{8'}, dia 1 or dia 2), 18.1 (C_{8'}, dia 1 or dia 2), −4.3 (2C, C_{6'}, dia 1 or dia 2), −4.3 (2C, C_{6'}, dia 1 or dia 2), −4.4 (C_{6'}, dia 1 or dia 2), −4.4 (C_{6'}, dia 1 or dia 2), −4.5 (C_{6'}, dia 1 or dia 2), −4.5 (C_{6'}, dia 1 or dia 2), −4.5 (C_{6'}, dia 1 or dia 2), −4.5 (C_{6'}, dia 1 or dia 2), −5.0 (C_{6'}, dia 1 or dia 2), −5.0 (C_{6'}, dia 1 or dia 2), −5.0 (C_{6'}, dia 1 or dia 2), −5.0 (C_{6'}, dia 1 or dia 2). IR (cm^{−1}): 2930, 2857, 2360, 2341, 2255, 1596, 1256, 1109, 1043, 836, 735. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₄₁H₆₆BrN₅O₆Si₃H 888.3577. Found 888.3576.

3.4.8. Synthesis of 4-(1-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1H-1,2,3-triazol-4-yl)-8-phenyl-1,6-naphthyridin-7(6H)-one Hydrochloride Salt TzNat A

A 37% *w/w* aqueous solution of hydrochloric acid (3 equiv.) was added to a stirred solution of compound **15** (1.25 g, 1.54 mmol) in THF (0.1 M). The solution was stirred at room temperature for 24 h. The mixture was triturated in Et₂O and filtered to yield the TzNat A as a hydrochloride salt. Red powder, M = 695 mg. Yield: 98%. ¹H NMR (400 MHz, CH₃OD) δ 10.34 (s, 1H, H₆), 9.36 (s, 1H, H₁₆), 8.77 (d, *J* = 5.8 Hz, 1H, H₁), 7.88 (d, *J* = 5.7, 1H, H₂), 7.70–7.45 (m, 5H, H_{10/11/12/13/14}), 6.23 (d, *J* = 3.7 Hz, 1H, H_{1'}), 4.65 (dd, *J* = 4.3, 4.3 Hz, 1H, H_{2'}), 4.40 (t, *J* = 5.1 Hz, 1H, H_{3'}), 4.23 (dt, *J* = 4.1, 4.0 Hz 1H, H_{4'}), 3.91 (dd, *J* = 12.3, 3.1 Hz, 1H, H_{5'}), 3.78 (dd, *J* = 12.3, 3.1 Hz, 1H, H_{5'}). ¹³C NMR (101 MHz, DMSO) δ 160.9 (C₇), 155.0 (C₁), 147.9 (C₆), 143.6 (C₃, C₁₅), 134.2 (C_{8,9}), 131.9 (C_{10,14}), 128.0 (C_{11,13}) 127.4 (C₁₂), 125.6 (C₁₆), 117.4 (C₂), 114.2 (C_{4,5}), 93.1 (C_{1'}), 86.6 (C_{4'}), 75.6 (C_{2'}), 70.7 (C_{3'}), 61.6 (C_{5'}). IR (cm^{−1}): 3349 (O-H), 2963, 2924, 2360, 2341, 1631, 1589, 1263, 1101, 814. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₂₁H₁₉N₅O₅H 422.1459. Found 422.1458.

3.4.9. Synthesis of 4-(1-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1H-1,2,3-triazol-4-yl)-8-(4-methoxyphenyl)-1,6-naphthyridin-7(6H)-one Hydrochloride salt TzNat B

A 37% *w/w* aqueous solution of hydrochloric acid (3 equiv.) was added to a stirred solution of compound **16** (68 mg, 0.08 mmol) in THF (0.1 M). The solution was stirred at room temperature for 24 h. The mixture was triturated in Et₂O and filtered to yield the TzNat B as a hydrochloride salt. Red powder, M = 30 mg. Yield: 82%. ¹H NMR (400 MHz, CH₃OD) δ 10.32 (s, 1H, H₆), 9.36 (s, 1H, H₁₆), 8.77 (d, *J* = 6.0 Hz, 1H, H₁), 7.88 (d, *J* = 5.9 Hz, 1H, H₂), 7.47–7.40 (m, 2H, H_{10,14} or H_{11,12}), 7.21–7.14 (m, 2H, H_{10,14} or H_{11,12}), 6.23 (d, *J* = 3.7 Hz, 1H, H_{1'}), 4.65 (dd, *J* = 4.9, 3.7 Hz, 1H, H_{2'}), 4.40 (t, *J* = 5.1 Hz, 1H, H_{3'}), 4.23 (dt, *J* = 5.2, 3.5 Hz, 1H, H_{4'}), 3.91 (s, 3H, H₁₇), 3.90 (dd, *J* = 12.3, 3.9 Hz, 1H, H_{5'}), 3.78 (dd, *J* = 12.3, 3.9 Hz, 1H, H_{5'}). ¹³C NMR (101 MHz, DMSO) δ 160.6 (C₇), 158.5 (C_{12,8}), 154.0 (C₁), 147.9 (C₃), 144.1 (C₆), 143.0 (C₁₅), 132.6 (C_{10,14} or C_{11,13}), 126.0 (C₁₆), 125.1 (C₉), 116.6 (C_{2'}), 113.7 (C_{4,5}), 113.3 (C_{10,14} or C_{11,13}), 92.6 (C_{1'}), 86.2 (C_{4'}), 75.2 (C_{2'}), 70.2 (C_{3'}), 61.1 (C_{5'}), 55.1 (C₁₇). IR (cm^{−1}): 3348, 2880, 2857, 2360, 2341, 1630, 1587, 1248, 1179, 1101, 1049, 824. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₂₂H₂₁N₅O₆H 452.1565. Found 452.1563.

3.4.10. 8-(4-Bromophenyl)-4-(1-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1H-1,2,3-triazol-4-yl)-1,6-naphthyridin-7(6H)-one Hydrochloride Salt TzNat C

A 37% *w/w* aqueous solution of hydrochloric acid (3 equiv.) was added to a stirred solution of compound **17** (86 mg, 0.09 mmol) in THF (0.1 M). The solution was stirred at room temperature for 24 h. The mixture was triturated in Et₂O and filtered to yield the Tz Nat C as a hydrochloride salt. Orange-red powder, M = 37 mg. Yield: 77%. ¹H NMR (400 MHz, CH₃OD) δ 10.37 (s, 1H, H₆), 9.36 (s, 1H H₁₆), 8.78 (d, *J* = 6.0 Hz, 1H, H₁), 7.89 (d,

$J = 6.0$ Hz, 1H, H₂), 7.82–7.76 (m, 2H, H_{10,14} or H_{11,12}), 7.47–7.41 (m, 2H, H_{10,14} or H_{11,12}), 6.22 (d, $J = 3.7$ Hz, 1H, H_{1'}), 4.65 (dd, $J = 4.9, 3.7$ Hz, 1H, H_{2'}), 4.40 (t, $J = 5.1$ Hz, 1H, H_{3'}), 4.22 (dt, $J = 5.2, 3.4$ Hz, 1H, H_{4'}), 3.90 (dd, $J = 12.3, 3.1$ Hz, 1H, H_{5'}), 3.78 (dd, $J = 12.3, 3.9$ Hz, 1H, H_{5'}). ¹³C NMR (101 MHz, DMSO) δ 160.2 (C₇), 154.9 (C₁), 152.7 (C₄), 149.6 (C₅), 147.3 (C₆), 143.2 (C₁₅), 141.2 (C₃), 133.7 (C_{10,14} or C_{11,13}), 133.2 (C₉), 130.4 (C_{10,14} or C_{11,13}), 125.4 (C₁₆), 120.2 (C₁₂), 116.9 (C₂), 113.5 (C₈), 92.5 (C_{1'}), 86.6 (C_{4'}), 75.1 (C_{2'}), 70.2 (C_{3'}), 61.1 (C_{5'}). IR (cm⁻¹): 3304, 3085, 2980, 2888, 2360, 2341, 1641, 1588, 1261, 1101, 1073, 678. HRMS (ESI) m/z : [M + H]⁺ Calcd for C₂₁H₁₈BrN₅O₅H 500.0564. Found 500.0561.

4. Conclusions

We have synthesized, using a Cu alkyne-azide cycloaddition (CuAAC) reaction, three original molecules as potential fluorescent ribonucleoside analogues, incorporating 1,6-naphthylidene-7(6H)-one as the fluorescent nucleobase and a 1,2,3-triazole as a linkage to a ribofuranosyl. Optical properties of these molecules have been studied in solvents of different polarity. Importantly, these molecules are fluorescent, showing a dual emission in highly polar aprotic solvent. In addition, a remarkable change of emissivity depending on the polarity of the solvent has been observed. We posit that these optical properties are useful in developing these molecules as flexible fluorescent probes for studying binding sites of enzymes and protein–nucleic acid interaction. In addition, such analogues could be of great interest in the search for new antiviral or antitumoral drugs based on nucleosides.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules29030687/s1>.

Author Contributions: Conceptualization, C.B. and V.C.; methodology, C.B. and V.C.; Formal analysis, C.B., A.B., O.R. and V.C.; investigation, C.B., V.C., A.B. and O.R.; resources, C.B. and V.C.; writing—original draft preparation, C.B.; writing—review and editing, C.B. and V.C.; project administration, C.B. and V.C.; funding acquisition, C.B. All authors have read and agreed to the published version of the manuscript.

Funding: Centre National de la Recherche Scientifique (CNRS) and Sorbonne Université.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article and its Supplementary Information Files.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Tinoco, I. Nucleic Acid Structures, Energetics, and Dynamics. *J. Phys. Chem.* **1996**, *100*, 13311–13322. [[CrossRef](#)]
2. Bottaro, S.; Di Palma, F.; Bussi, G. The role of nucleobase interactions in RNA structure and dynamics. *Nucleic Acids Res.* **2014**, *42*, 13306–13314. [[CrossRef](#)]
3. Minchin, S.; Lodge, J. Understanding biochemistry: Structure and function of nucleic acids. *Essays Biochem.* **2019**, *63*, 433–456. [[CrossRef](#)]
4. Chu, C.K.; Baker, D.C. *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Plenum Press: New York, NY, USA, 1993.
5. Thomson, J.M.; Lamont, I.L. Nucleoside Analogues as Antibacterial Agents. *Front. Microbiol.* **2019**, *20*, 952. [[CrossRef](#)]
6. Jordheim, L.; Durantel, D.; Zoulim, F.; Dumontet, C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug. Discov.* **2013**, *12*, 447–464. [[CrossRef](#)] [[PubMed](#)]
7. Kimoto, M.; Cox, R.S., III; Hirao, I. Unnatural base pair systems for sensing and diagnostic applications. *Expert. Rev. Mol. Diagn.* **2011**, *3*, 321–331. [[CrossRef](#)] [[PubMed](#)]
8. Krueger, A.T.; Kool, E.T. Redesigning the architecture of the base pair: Toward biochemical and biological function of new genetic sets. *Chem. Biol.* **2009**, *16*, 242–248. [[CrossRef](#)] [[PubMed](#)]
9. Xu, W.; Chan, K.M.; Kool, E.T. Fluorescent nucleobases as tools for studying DNA and RNA. *Nat. Chem.* **2017**, *11*, 1043–1055. [[CrossRef](#)] [[PubMed](#)]
10. Michel, B.Y.; Dziuba, D.; Benhida, R.; Demchenko, A.P.; Burger, A. Probing of Nucleic Acid Structures, Dynamics, and Interactions with Environment-Sensitive Fluorescent Labels. *Front. Chem.* **2020**, *8*, 112. [[CrossRef](#)] [[PubMed](#)]

11. Burns, D.D.; Teppang, K.L.; Lee, R.W.; Lokensgard, M.E.; Purse, B.W. Fluorescence turn-on sensing of DNA duplex formation by a tricyclic cytidine analogue. *J. Am. Chem. Soc.* **2017**, *139*, 1372–1375. [[CrossRef](#)] [[PubMed](#)]
12. Schmidt, O.P.; Mata, G.; Luedtke, N.W. Fluorescent base analogue reveals T-Hg^{II}-T base pairs have high kinetic stabilities that perturb DNA metabolism. *J. Am. Chem. Soc.* **2016**, *138*, 14733–14739. [[CrossRef](#)] [[PubMed](#)]
13. Klöcker, N.; Weissenboeck, F.P.; Rentmeister, A. Covalent labeling of nucleic acids. *Chem. Soc. Rev.* **2020**, *49*, 8749–8773. [[CrossRef](#)] [[PubMed](#)]
14. Dziuba, D. Environmentally sensitive fluorescent nucleoside analogues as probes for nucleic acid–protein interactions: Molecular design and biosensing applications. *Methods Appl. Fluoresc.* **2022**, *4*, 10. [[CrossRef](#)] [[PubMed](#)]
15. Secrist, J.A., III; Barrio, J.R.; Leonard, N.J.A. Fluorescent modification of adenosine triphosphate with activity in enzyme systems: 1,N 6-ethenoadenosine triphosphate. *Science* **1972**, *175*, 646–647. [[CrossRef](#)]
16. Coleman, R.S.; Madaras, M.L. Synthesis of a novel coumarin C-riboside as a photophysical probe of oligonucleotide dynamics. *J. Org. Chem.* **1998**, *63*, 5700–5703. [[CrossRef](#)]
17. Morales-Rojas, H.; Kool, E.T. A porphyrin C-nucleoside incorporated into DNA. *Org. Lett.* **2002**, *4*, 4377–4380. [[CrossRef](#)]
18. Okamoto, A.; Tainaka, K.; Fujiwara, Y. Nile Red nucleoside: Design of a solvatofluorochromic nucleoside as an indicator of micropolarity around DNA. *J. Org. Chem.* **2006**, *71*, 3592–3598. [[CrossRef](#)]
19. Gao, J.; Strassler, C.; Tahmassebi, D.; Kool, E.T. Libraries of composite polyfluors built from fluorescent deoxyribosides. *J. Am. Chem. Soc.* **2002**, *124*, 11590–11591. [[CrossRef](#)]
20. Hirao, I.; Kimoto, M.; Yamashige, R. Natural versus artificial creation of base pairs in DNA: Origin of nucleobases from the perspectives of unnatural base pair studies. *Acc. Chem. Res.* **2012**, *45*, 2055–2065. [[CrossRef](#)]
21. Andreeva, O.V.; Garifullin, B.F.; Zarubaev, V.V.; Slita, A.V.; Yesaulkova, I.L.; Saifina, L.F.; Shulaeva, M.M.; Belenok, M.G.; Semenov, V.E.; Kataev, V.E. Synthesis of 1,2,3-triazolyl nucleoside analogues and their antiviral activity. *Mol. Divers.* **2021**, *25*, 473–490. [[CrossRef](#)]
22. Alvarez, R.; Velázquez, S.; San-Félix, A.; Aquaro, S.; De Clercq, E.; Perno, C.F.; Karlsson, A.; Balzarini, J.; Camarasa, M.J. 1,2,3-Triazole-[2',5'-bis-O-(tert-butyl dimethylsilyl)-beta-D-ribofuranosyl]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (TSAO) analogues: Synthesis and anti-HIV-1 activity. *J. Med. Chem.* **1994**, *37*, 4185–4194. [[CrossRef](#)]
23. Chitpepu, P.; Sirivolu, V.R.; Seela, F. Nucleosides and oligonucleotides containing 1,2,3-triazole residues with nucleobase tethers: Synthesis via the azide-alkyne 'click' reaction. *Bioorg. Med. Chem.* **2008**, *16*, 8427–8439. [[CrossRef](#)] [[PubMed](#)]
24. Ghoteimi, R.; Braka, A.; Rodriguez, C.; Cros-Perrial, E.; Tai Nguyen, V.; Uttaro, J.P.; Mathé, C.; Chaloin, L.; Ménétrier-Caux, C.; Jordheim, L.P.; et al. 4-Substituted-1,2,3-triazolo nucleotide analogues as CD73 inhibitors, their synthesis, in vitro screening, kinetic and in silico studies. *Bioorg. Chem.* **2021**, *107*, 104577. [[CrossRef](#)] [[PubMed](#)]
25. St. Amant, A.H.; Bean, L.A.; Guthrie, J.P.; Hudson, R.H. Click fleximers: A modular approach to purine base-expanded ribonucleoside analogues. *Org. Biomol. Chem.* **2012**, *10*, 6521–6525. [[CrossRef](#)] [[PubMed](#)]
26. Seley, K.L.; Zhang, L.; Hagos, A.; Quirk, S. "Fleximers". Design and synthesis of a new class of novel shape-modified nucleosides (1). *J. Org. Chem.* **2002**, *67*, 3365–3373. [[CrossRef](#)] [[PubMed](#)]
27. Chudinov, M.V. Nucleoside Analogs with Fleximer Nucleobase. *Chem. Heterocycl. Comp.* **2020**, *56*, 636–643. [[CrossRef](#)] [[PubMed](#)]
28. Seley-Radtke, K. Flexibility-Not just for yoga anymore! *Antivir. Chem. Chemother.* **2018**, *26*, 2040206618756788. [[CrossRef](#)] [[PubMed](#)]
29. Bag, S.S.; Talukdar, S.; Das, S.K.; Pradhan, M.K.; Mukherjee, S. Donor/acceptor chromophores-decorated triazolyl unnatural nucleosides: Synthesis, photophysical properties and study of interaction with BSA. *Org. Biomol. Chem.* **2016**, *22*, 5088–5108. [[CrossRef](#)]
30. Dziuba, D.; Postupalenko, V.Y.; Spadafora, M.; Klymchenko, A.S.; Guérineau, V.; Mély, Y.; Benhida, R.; Burger, A. A universal nucleoside with strong two-band switchable fluorescence and sensitivity to the environment for investigating DNA interactions. *J. Am. Chem. Soc.* **2012**, *134*, 10209–10213. [[CrossRef](#)]
31. Litvinov, V.P. Advances in the chemistry of naphthyridines. *Adv. Heterocycl. Chem.* **2006**, *91*, 189–300.
32. Darakshan; Parvin, T. Domino reaction for the synthesis of pyrazole/isoxazole fused naphthyridine derivatives Involving indole ring opening and double ring formation. *J. Org. Chem.* **2023**, *88*, 6847–6856. [[CrossRef](#)]
33. Wang, Y.; Lv, Z.; Chen, F.; Wang, X.; Gou, S. Discovery of 5-(3-chlorophenylamino)benzo[c][2,6]naphthyridine derivatives as highly selective CK2 inhibitors with potent cancer cell stemness inhibition. *J. Med. Chem.* **2021**, *64*, 5082–5098. [[CrossRef](#)]
34. Sun, Q.; Ren, R.; Wu, P.-P.; Zhuo, L.-S.; Dong, H.; Peng, H.-T.; Cao, Y.-F.; Luo, X.-G.; She, N.-F. A 2, 7-naphthyridine-based fluorescent turn-on probe for detection of biothiols in vitro and in vivo. *Dyes Pigm.* **2020**, *182*, 108702. [[CrossRef](#)]
35. Che, C.-M.; Wan, C.-W.; Ho, K.-Y.; Zhou, Z.-Y. Strongly luminescent metal-organic compounds: Spectroscopic properties and crystal structure of substituted 1,8-naphthyridine and its zinc (II) complex. *New. J. Chem.* **2001**, *25*, 63–65. [[CrossRef](#)]
36. Hikishima, S.; Minakawa, N.; Kuramoto, K.; Fujisawa, Y.; Ogawa, M.; Matsuda, A. Synthesis of 1,8-naphthyridine C-nucleosides and their base-pairing properties in oligodeoxynucleotides: Thermally stable naphthyridine:imidazopyridopyrimidine base-pairing motifs. *Angew. Chem. Int. Ed.* **2005**, *44*, 596–598. [[CrossRef](#)]
37. Oliveras, J.M.; Puig de la Bellacasa, R.; Estrada-Tejedor, R.; Teixidó, J.; Borrell, J.I. 1,6-Naphthyridin-2(1H)-ones: Synthesis and Biomedical Applications. *Pharmaceuticals* **2021**, *14*, 1029. [[CrossRef](#)] [[PubMed](#)]
38. Eldrup, A.B.; Christensen, C.; Haaima, G.; Nielsen, P.E. Substituted 1,8-Naphthyridin-2(1H)-ones are Superior to thymine in the recognition of adenine in duplex as well as triplex structures. *J. Am. Chem. Soc.* **2002**, *124*, 3254–3262. [[CrossRef](#)] [[PubMed](#)]

39. Lawson, C.; Füchtbauer, A.; Wranne, M.; Giraud, T.; Floyd, T.; Dumat, B.; Andersen, N.; El-Sagheer, A.; Brown, T.; Gradén, H.; et al. Synthesis, oligonucleotide incorporation and fluorescence properties in DNA of a bicyclic thymine analogue. *Sci. Rep.* **2018**, *8*, 13970. [[CrossRef](#)] [[PubMed](#)]
40. Samaan, G.N.; Wyllie, M.K.; Cizmic, J.M.; Needham, L.M.; Nobis, D.; Ngo, K.; Andersen, S.; Magennis, S.W.; Lee, S.F.; Purse, B.W. Single-molecule fluorescence detection of a tricyclic nucleoside analogue. *Chem. Sci.* **2020**, *12*, 2623–2628. [[CrossRef](#)] [[PubMed](#)]
41. Beghennou, A.; Gontard, G.; Dossmann, H.; Passador, K.; Thorimbert, S.; Corcé, V.; Botuha, C. 1,6-Naphthyridin-7(6H)-ones: Synthesis and optical properties. *Org. Biomol. Chem.* **2023**, *21*, 2976–2982. [[CrossRef](#)] [[PubMed](#)]
42. Fantoni, N.Z.; El-Sagheer, A.H.; Brown, T.A. Hitchhiker's guide to click-chemistry with nucleic acids. *Chem. Rev.* **2021**, *121*, 7122–7154. [[CrossRef](#)]
43. Agrahari, A.K.; Bose, P.; Jaiswal, M.K.; Rajkhowa, S.; Singh, A.S.; Hotha, S.; Mishra, N.; Tiwari, V.K. Cu(I)-Catalyzed Click Chemistry in Glycoscience and Their Diverse Applications. *Chem. Rev.* **2021**, *121*, 7638–7956. [[CrossRef](#)]
44. Amblard, F.; Sari, O.; Boucle, S.; Khalil, A.; Schinazi, R.F. Modifications of Nucleosides, Nucleotides, and Nucleic Acids using Huisgen's [3+2] Azide–Alkyne Cycloaddition: Opening Pandora's Box. In *Click Reactions in Organic Synthesis*; Chandrasekaran, S., Ed.; Wiley: Hoboken, NJ, USA, 2016.
45. Ermolat'ev, D.; Mehta, V.; Van der Eycken, E. Synthesis of Furo[2,3-b]pyrazine Nucleoside Analogues with 1,2,3-Triazole Linkage. *QSAR Comb. Sci.* **2007**, *26*, 1266–1273. [[CrossRef](#)]
46. Passays, J.; Rubay, C.; Marcélis, L.; Elias, B. Synthesis and Photophysical Properties of Triazolyl Ir^{III} Nucleosides. *Eur. J. Inorg. Chem.* **2017**, *3*, 623–629. [[CrossRef](#)]
47. Pandey, N.; Dwivedi, P.; Jyoti, S.M.; Kumar, D.; Tiwari, V.K.; Mishra, B.B. Click Chemistry Inspired Synthesis of Hydroxyanthracene Triazolyl Glycoconjugates. *ACS Omega* **2022**, *7*, 37112–37121. [[CrossRef](#)]
48. Singh, S.K.; Kumar, S.; Yadav, M.S.; Tiwari, V.K. Pyridyl Glycosyl Triazole/CuI-Mediated Domino/Tandem Synthesis of Quinazolinones. *J. Org. Chem.* **2022**, *87*, 15389–15402. [[CrossRef](#)] [[PubMed](#)]
49. Štimac, A.; Jože, K. An improved preparation of 2,3,5-tri-O-acyl-β-d-ribofuranosyl azides by the Lewis acid-catalysed reaction of β-D-ribofuranosyl acetates and trimethylsilyl azide: An example of concomitant formation of the α anomer by trimethylsilyl triflate catalysis. *Carbohydr. Res.* **1992**, *232*, 359–365. [[CrossRef](#)]
50. Luche, J.-L.; Gemal, A.L. Efficient synthesis of acetals catalyzed by rare earth chlorides. *J. Chem. Soc. Chem. Commun.* **1978**, *22*, 976–977. [[CrossRef](#)]
51. Brunel, D.; Dumur, F. Recent advances in organic dyes and fluorophores comprising a 1,2,3-triazole moiety. *New J. Chem.* **2020**, *44*, 3546–3561. [[CrossRef](#)]
52. Liese, D.; Haberhauer, G. Rotations in Excited ICT States—Fluorescence and its Microenvironmental Sensitivity. *Isr. J. Chem.* **2018**, *58*, 813–826. [[CrossRef](#)]
53. Nisic, F.; Speciale, G.; Bernardi, A. Stereoselective synthesis of α- and β-glycofuranosyl amides by traceless ligation of glycofuranosyl azides. *Chem. Eur. J.* **2012**, *18*, 6895–6906. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.