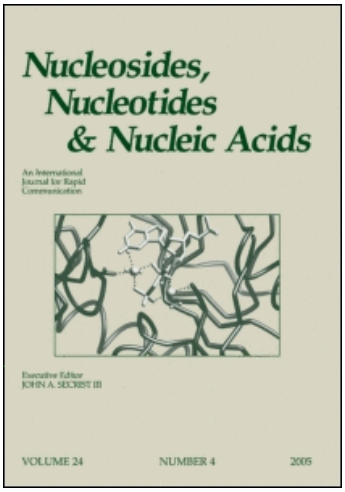

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β-1,2,3-Triazolyl-Nucleosides as Nicotinamide Riboside Mimics

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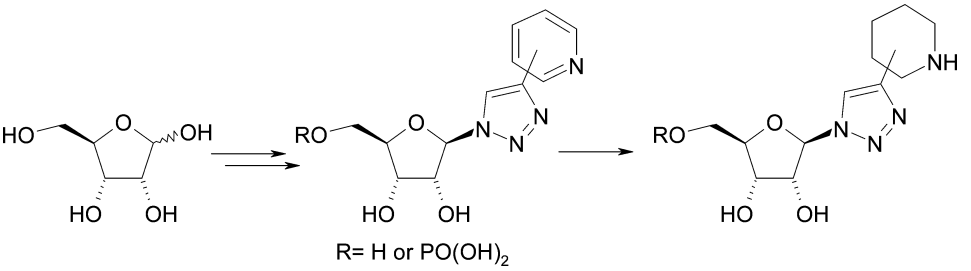

□ *The synthesis of a series of pyridine- and piperidine-substituted 1,2,3-triazolides linked to a riboside moiety is described. The presence of a triazolide substituent on the pyridine moiety permitted the facile reduction of the latter under mild hydrogenation conditions. These analogues were modelled as to define their similarity to nicotinamide riboside and quantify their ability to bind NAD-dependent protein deacetylases.*

Keywords Nucleoside; nucleotide; sirtuin; inhibition

One family of protein deacetylases catalyzes the removal of the acetate moiety from acetylated lysine residues on proteins, such as histones, using nicotinamide adenine dinucleotide (NAD) as a co-substrate.[1] Upon catalysis, in addition to the deacetylated protein being released, NAD is converted into 2″/3″-*O*-acetylated-adenosine diphosphate ribose and

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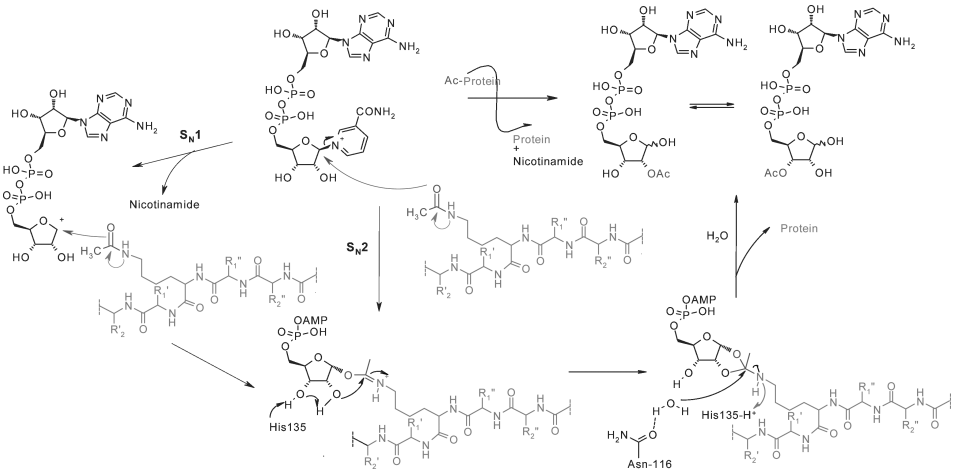


SCHEME 1 Currently proposed mechanisms of deacetylation catalyzed by NAD-dependent protein deacetylases.

nicotinamide. In cells, these deacetylases are regulated by endogenous nicotinamide which acts as a non-competitive enzyme inhibitor.[2,3] Considering that regulation of such NAD-dependent deacetylases is implicated in numerous biological processes including life-span expansion and p53 expression in eukaryotes,[4] identifying structural and chemical features that molecules should possess in order to attain potent inhibition is the first step to developing a robust program toward drug discovery targeting such enzymes.[5,6]

Two mechanisms can be proposed to explain the early steps of the NAD-dependent enzyme catalyzed deacetylation (Scheme 1). The first proposed pathway results in the formation of a stabilized oxycarbocation reaction intermediate, hence is defined as the S_N1 mechanism. Such intermediate has been observed in enzymatic reactions involving a nicotinamide-riboside bond cleavage.[7,8] The second predicted pathway involves a transition state intermediate resulting from the nucleophilic attack of the carbonyl group from the acetyl-lysine moiety and the simultaneous breakage of the nicotinamide bound via an S_N2 mechanism.[9] As a starting point in our search for chemical scaffolds that could lead to NAD-dependent deacetylase inhibition, we decided to investigate the possibilities of mimicking the increased distance between the nicotinamide and the C1″-carbon of the nicotinamide riboside of NAD occurring for either of these mechanisms. While many nucleoside analogues, mimicking both the adenosine and the nicotinamide moieties, have been synthesized thus far, compounds simulating the elongation of the nucleosidic bond between the C1″ of the northern riboside and the nitrogen of the pyridine ring, have been under less scrutiny.[10, 11] Developing an effective synthetic programme of work that would allow the identification of inhibitors of NAD-dependent

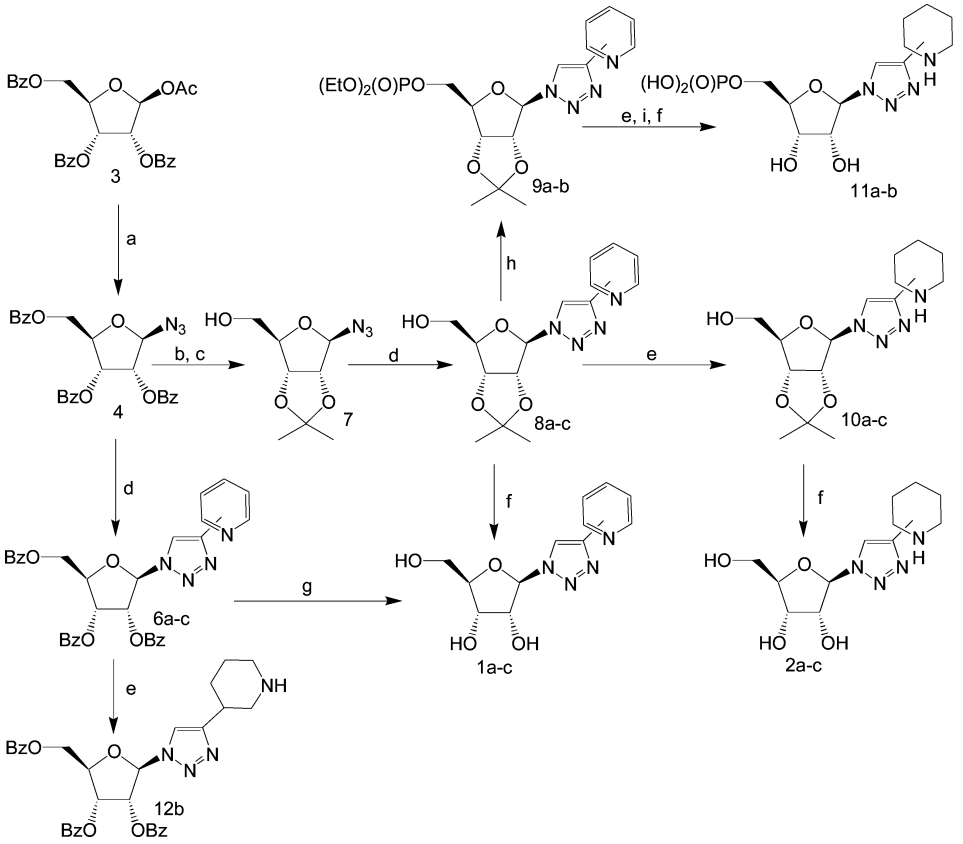
TABLE 1 Optimization of the 1,3-dipolar cycloaddition reaction conditions

Product	n-ethynylpyridine	Reaction conditions	Yield
6a	**5a**: n = 2	A	41%
		B	–
		C	–
6b	**5b**: n = 3	A	85%
		B	47%
		C	<5%
6c	**5c**: n = 4	A	15%
		B	0%
		C	0%
8a	**5a**: n = 2	A	-
		B	31%
8b	**5b**: n = 3	A	79%
		B	50%
8c	**5c**: n = 4	A	–
		B	12%

A: $CuSO_4$ (0.2 eq.), sodium ascorbate (0.4 eq.), *t*BuOH/H_2O, 40°C.
B: $CuSO_4$ (0.1 eq.), sodium ascorbate (1.0 eq.), H_2O, 100°C.
C: CuI (1.5 eq.), DBU (15 eq.), toluene, 90°C.

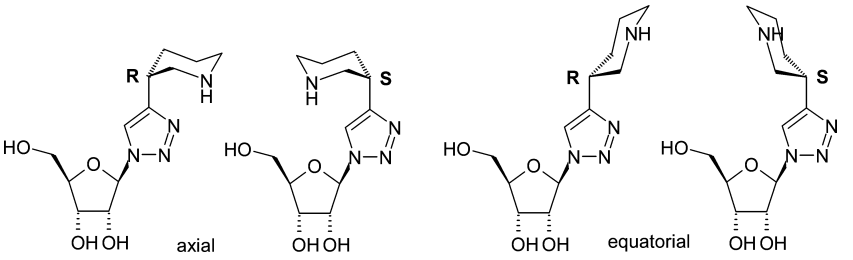
deacetylases requires a reliable computational process which allows for the early elimination of unsuitable synthetic targets. To this end, a small series of modified nucleosides and nucleotides have been synthesized and evaluated as putative inhibitors of yHst2, a yeast NAD-dependent histone deacetylase, while a wider range of analogues were screened in silico against various yHSt2-crystal structures for comparison. Nucleosidic analogues **1a-c** (Scheme 2) possess a pyridine bounded to the riboside ring via a 1,2,3-triazolide moiety, thus providing additional distance between the riboside C1″ carbon and pyridine ring. Triazolide linkers were chosen for their ease of accessibility as well as their ability to enter conjugation with other aromatic moieties such as pyridine. We thought that as pyridine possessed an electron-withdrawing ability, the actual electron density over the triazolide ring would potentially match that of the electron density observed during the breaking of the ribose-nicotinamide glycosidic bond. Further modifications of these triazolide-containing nucleoside mimics offered the piperidine derivatives **2a-c** (Scheme 2). Finally, a versatile synthetic sequence allowed access to the nucleotide parents.

We aimed at gaining easy access to fully and partially deprotected nucleoside analogues to facilitate further chemical modifications such as selective 5′-*O*-phosphorylation. We thus prepared the triazolide adducts via two distinct synthetic sequences. The fully deprotected nucleoside pyridine mimic series **1a-c** was easily obtained in three steps from the commercially available *β*-1-*O*-acetyl-2,3,5-tri-*O*-benzoate riboside **3**[12] by simple Lewis acid-catalyzed substitution of acetyl group with azide which afforded *β*-1-azido-2,3,5-tri-*O*-benzoate riboside **4**. Direct triazolide formation from **4**



SCHEME 2 Synthesis of pyridine and piperidine containing 1,2,3-triazolide adducts.

and the appropriately subtituted alkyne-pyridines **5a-c** (Scheme 2) yielded the fully benzoylated nucleoside analogues **6a-c** which, once treated with methanolic sodium methoxide, yielded the triazolide nucleoside mimics **1a-c**. The acetonide-protected pyridine 1,2,3-triazolides **8a-c** were obtained from the corresponding azido-riboside **7** and the appropriate alkyne-pyridines **5a-c** (Scheme 2). Only 1,4-disubstituted triazolides were products of these reactions. Optimization of the reaction conditions (Table 1),[13,14] such as variation of the type of catalyst, the catalyst ratio, the pH and the solvent mixture, permitted access to the pyridine triazolides in yields ranging from 10% to 85% (Table 1). Low yields were particularly consistent with 4-ethynylpyridine, **5c**. This low conversion could be related to the nature of the reagent itself; 4-ethynylpyridine was purchased as a chloride salt, unstable once neutralized. The reaction conditions (100°C for extended period of time) were possibly too rigorous for this particular pyridine-based reagent. Additionally, the triazolide products **6a-c** and **8a-c** displayed extremely poor solubility in both organic and hydrophilic solvents including water and methanol, which rendered their extraction and purification



SCHEME 3 Possible chirality and conformation adopted by **2b.**

difficult. Finally, DBU-catalyzed reactions were unsuitable for the formation of the ester protected triazolides as rapid partial debenzoylation occurred upon work-up. Phosphorylation and access to the protected nucleotides **9a-b** was easily achieved using a chlorodiethylphosphite/tBuOOH combination. The palladium-catalyzed reduction of **8a-c** to yield **10a-c** was carried out in the presence of formic acid under hydrogen atmosphere at balloon pressure. Unlike the reduction of pyridines, where high hydrogen pressure is required, triazolide-substituted pyridines were very rapidly reduced under these mild conditions. It must also be noted that only one diastereoisomeric piperidine derivative was obtained in each case and that the reduction was quantitative. Attempts were made at identifying the stereochemistry at the newly generated chiral center and the overall conformation of the piperidine ring (Scheme 3). Unfortunately, NOE experiments were inconclusive and none of the derivatives were crystallizable. Subsequent removal of the isopropylidene group offered the fully deprotected pyridine **1a-c** and piperidine **2a-c** substituted triazolides. A similar sequence that also included a TMSBr-catalyzed deprotection of the **9a-b** phosphate triesters yielded the piperidine nucleotide derivative **11a-b**. It is noticeable that the partially protected piperidines, including the benzoylated triazolide **12b**, are ideal synthetic intermediates for further synthetic manipulations and access to libraries of modified nucleosides.

a) $TMSN_3$, $AlCl_3$, CH_3CN, 95%, b) MeONa, MeOH, 90%, c) acetone, H_2SO_4, 91% d) n-ethynylpyridine **5a-c**, cycloaddition conditions A-C; e) Pd/C, MeOH, HCOOH (10% v/v), H_2, quant.; f) TFA/H_2O, 1/1, v/v, 95–99%; g) MeOH, MeONa, quant.; h) $(EtO)_2PCl$, Et_3N, tBuOOH, 90%; i) TMSBr, DCM, rt, quant.

We then examined these novel triazolides in silico. First, we wished to establish which of the triazolide series (**1a-c** versus **2a-c)** best mimicked electronically the early stage of the catalysis, that is, the nicotinamide-riboside bond breakage. Second, in order to establish a binding affinity pattern, we wished to model each template in the binding site of yHst2 co-crystallized with carba-NAD (substrate analogue) (PDB: 2OD2),[2] with an S_N1-based cationic reaction intermediate mimic (adenosine 5′-diphosphate (hydroxymethyl) pyrrolidinediol, ADP-HPD) (PDB: 2OD7),[2]

with nicotinamide (in the absence (PDB: 1YC5)[15] or the presence of ADP-HPD (PDB: 2OD9)[2]) or with adenosine diphosphate ribose in the absence of nicotinamide (PDB: 1SZD).[16]

To study the electronic character and the electronic similarity of the triazolyl-nucleoside analogues **1a-c** and **2a-c** to nicotinamide riboside, geometrical optimizations were performed at B3LYP/6–31G* level with Gaussian03 program (Gaussian Inc., USA; see Chart 1). Comparing the LUMO orbitals (Figure 1A) of nicotinamide riboside to that of **1a-c** and **2a-c**, we observed that the LUMOs of nicotinamide riboside and of the piperidine derivatives **2a-c**, were overwhelmingly localized on the C1″ carbon of the ribose ring and the nicotinamide or triazolide ring. This indicated that the C1″ carbons of ribosides **2a-c** were of similar electrophilicity as that of the C1″ carbon of nicotinamide riboside. In contrast, the LUMO orbital distributions of pyridine derivatives **1a-c** on the C1″ carbon was found to be zero. From the analysis of the HOMO orbitals of the piperidine triazolide derivatives (Figure 1; **2a-c**), we observed that the triazolide ring made little contribution towards the HOMO orbitals, while the HOMO-1 orbitals were mostly located on the triazolide ring. On comparing the orbital energies of the triazolide compounds, we found that the energy gap between HOMO and HOMO-1 orbitals was not noticeable, which made possible electron transfer from the second highest occupied orbital. In contrast, for the pyridine derivatives (**1a-c**), electron transfer occurred from the HOMO orbitals (Figure 1). Overall, this indicated that nicotinamide riboside shared more electronic similarities with the piperidine triazole-ribosides than with the pyridine triazole-ribosides.

A docking study using the Glide program (Schrodinger Inc.) was then performed to establish whether the **2a-c** series could favorably fit into the active site(s) of yHst2 enzymes. Energy minimization for **2a-c** at B3LYP/6-31G* using Gaussian shows that **2c** is the most energy-favorable species among all the piperidine derivatives and the energy difference between two diastereoisomers for **2a** and **2b** can be ignored, which is further confirmed by single point energy calculation at larger basis set B3LYP/6-311+G** and solvation effect implied by CPCM solvation model (Supplementary Table).

Nicotinamide and nicotinamide riboside were first docked into the carba-NAD-binding site of the resolved yHst structures (PDB: 2OD2).[2] Results show that both nicotinamide and its riboside derivative bind in the C-pocket with the same orientation as the nicotinamide carbasugar part of the co-crystallized carba-NAD (Figure 2A and 2B). The triazolyl-nucleosides **1a-c** and **2a-c** and the phosphorylated species **11a-c** were then docked into the yHst2-carba-NAD-binding pocket. Analogous to nicotinamide riboside, the piperidine derivatives were also found to bind preferentially in the conserved C-pocket. In the crystal structure of yHst2 complexed with carba-NAD or free nicotinamide, the amide group and carboxyl group of the carboxamide of nicotinamide form favorable H-bond interactions

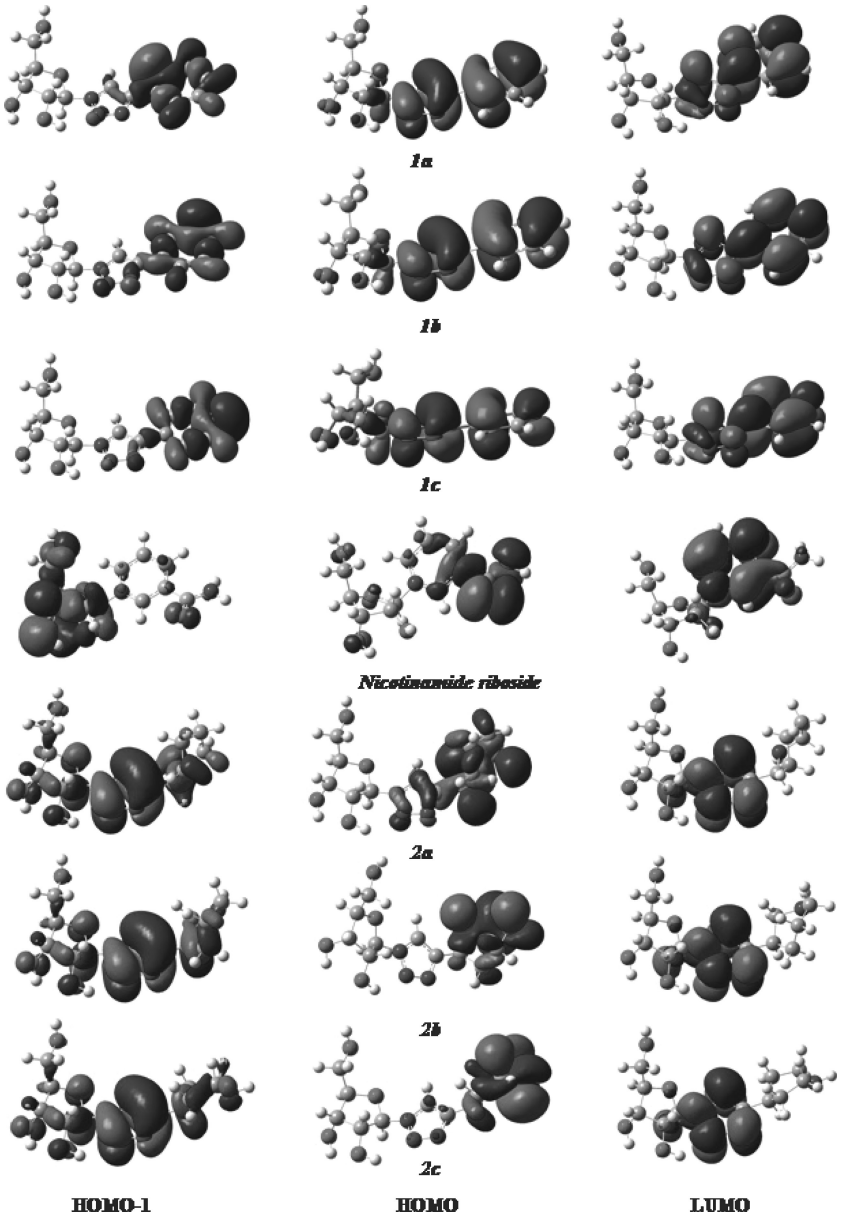
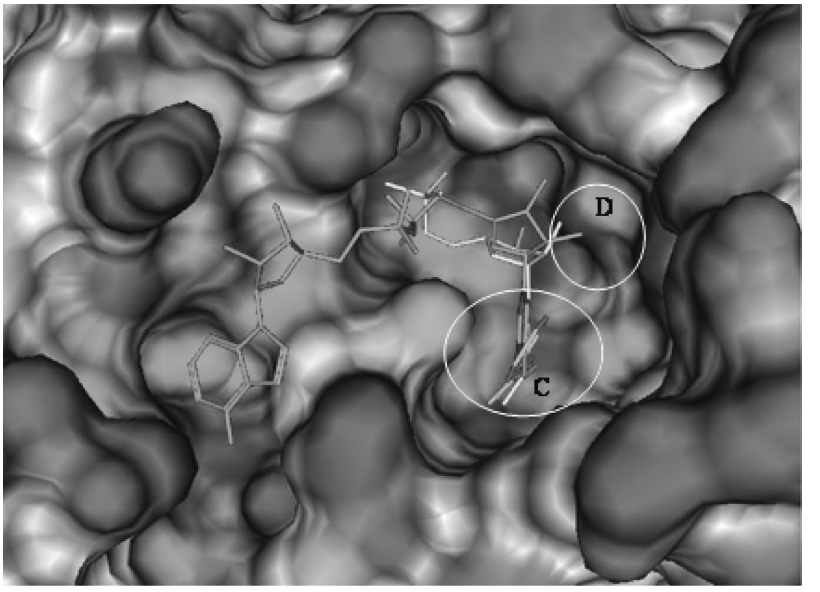
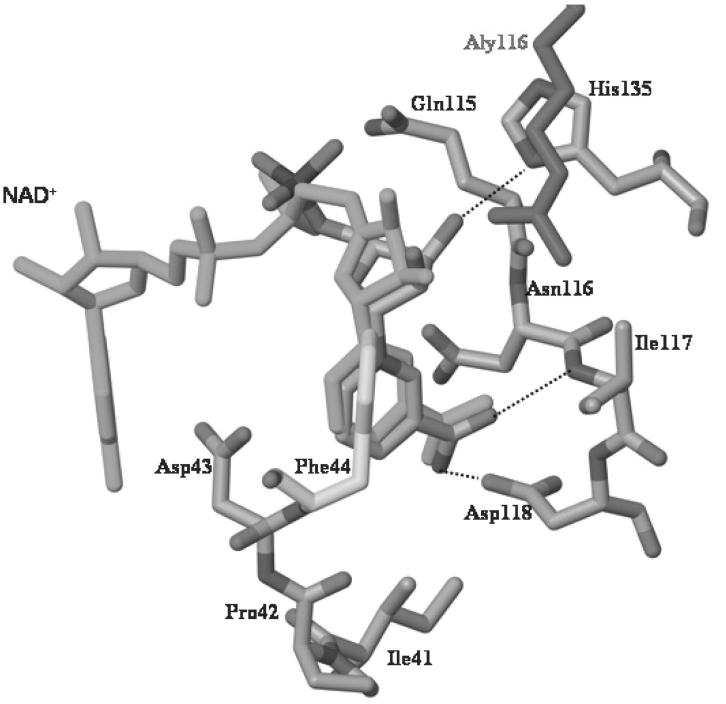


FIGURE 1 Frontier orbitals for triazolide compounds **1a-c** and **2a-c** and nicotinamide riboside.

with the carboxyl oxygen of Asp118 and the backbone nitrogen of Ile117, respectively. Docking the series **2a-c** showed that either the heterocyclic nitrogen or the carbon atoms of the piperidine ring could approach the carboxyl oxygen of Asp118 and the backbone nitrogen of Ile117 (Figure 3) of the C-pocket.



(a)



(b)

FIGURE 2 Docked poses of phosphorylated nicotinamide riboside in the pocket C of yHst2 (A) Conserved pockets C and D are shown by surface representation of yHst2. Docked pose phosphorylated nicotinamide riboside (yellow) overlap neatly with the northern ribose of NAD^+ (orange). Nicotinamide (blue) is also docked into the C pocket. (B) Interaction of phosphorylated nicotinamide riboside (colored by element) with conserved residues in pocket C. NAD is shown in green; Phe44 (cyan) forms hydrophobic contact with the nicotinamide pyridine ring.

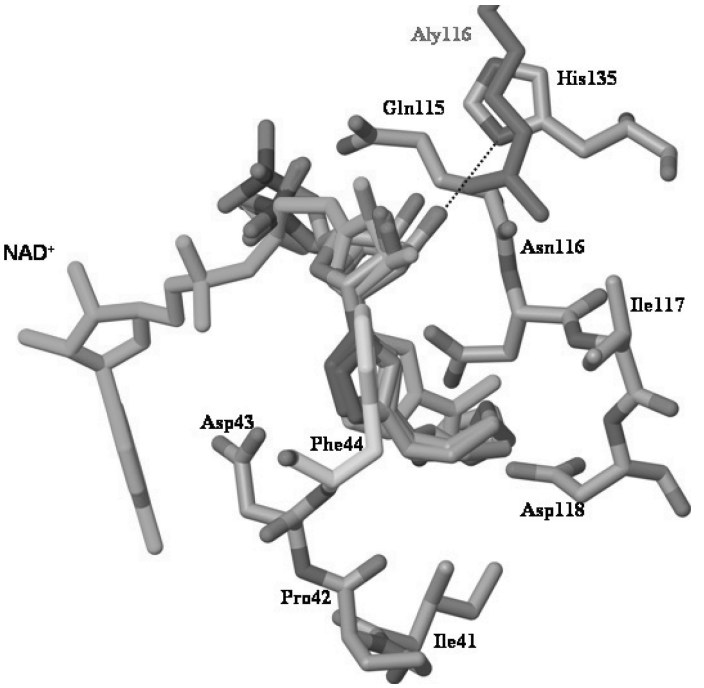


FIGURE 3 Docked poses of **11a-c** in yHst2 pocket C. Interaction of **11a-c** (colored by element) with conserved residues in pocket C. NAD^+ is shown in green, and acetyl-lysine is in magenta. Phe44 (cyan) forms hydrophobic contact with the triazolide ring.

A good overlap was also observed between the triazolide ring binding to the C-pocket and that of the pyridine ring of carba-NAD, indicating that the favorable hydrophobic interaction with Phe44. Figure 4 shows the interactions of **11a-c** with the conserved C-pocket of the NAD-binding site. Compound **11b** presented the highest docking binding affinity with yHst2, forming H-bond interactions between the piperidine-NH and the carboxyl oxygen of Asp118. The piperidine **11c** offered similar binding affinity as **11b,** but weaker H-bond interactions between the piperidine NH and the backbone amido-nitrogen of Ile117 was observed. Finally, the piperidine nitrogen in compound **11a** was too far to form favorable electrostatic interactions with the surrounding amino acids (Table 2). As expected, **2a-c** ranked lower than its phosphorylated parent series **11a-c** (Table 2) emphasising the importance of the phosphate moiety and its interactions within the Rossmann fold.

Modeling with structures 1SZD and 2OD7, showed a preference for a nicotinamide docked into the D-pocket while for all docking experiments, the nucleosides **2a-c** and nucleotides **11a-c** showed high scoring in the C-pocket, except when nicotinamide was present in the D-pocket (PDB: 2OD9). Under these conditions, none of the piperidine derivatives docked

TABLE 2 Docking scores of **2a-c** and **11a-c**

Target	1SZC	2OD2	2OD9	1SZD	2OD7	2OD9[a]
Nicotinamide riboside mononucleotide	**−7.44 C**[b]	−6.82 **C**	−6.83 **C**	−6.94 **C**	−6.44 **C**	**−6.67 A**
11b	−6.68 **C**	**−6.91 C**	**−7.29 C**	−6.82 **C**	−6.80 **C**	−5.62 **A**
11c	−6.87 **C**	−6.67 **C**	−7.05 **C**	−6.64 **C**	−6.65 **C**	−6.09 **A**
11a	−6.10 **C**	−6.63 **C**	−6.37 **C**	−6.19 **C**	−6.76 **C**	−6.40 **A**
Nicotinamide riboside	−6.96 **C**	−6.56 **C**	−6.68 **C**	**−7.01 C**	**−7.15 C**	−5.46 **C**
2b	−6.23 **C**	−5.94 **C**	−5.98 **C**	−5.90 **C**	−6.15 **C**	−5.69 **A**
2c	−5.94 **C**[c]	−5.44 **C**	−6.02 **C**	−5.56 **C**	−5.66 **C**	−5.68 **A**
2a	−5.92 **C**	−5.56 **C**	−5.73 **C**	−5.98 **C**	−5.20 **C**	−5.56 **A**
Nicotinamide	−5.11 **C**	−5.08 **C**	−5.06 **C**	−4.95 **D**	−4.98 **D**	−5.93 **C**
ADP-ribose	−8.63	−9.94	−8.43	−8.30	−9.83	−8.77

[a]With a nicotinamide molecule in the D site

[b]Capital A, C or D in bold represents that the named compound is docked in the adenosine monophosphate binding fold or the C or D pockets, respectively.

[c]Cell in red indicates that the docked pose flapped in the pocket.

in the C-pocket but instead docked in the adenosine monophosphate binding site. These docking experiments also suggested that **11a-c** showed great binding similarities to that of the substrate mimic carba-NAD, thus indicating that unfortunately they were more likely to be substrate analogues than transition state mimetics.

Finally, ADP-ribose, which has been shown to be a 100-fold more potent an inhibitor than nicotinamide,[16] was also docked in the various enzyme complexes. As expected, it unanimously showed best docked scores compared to the rest of the compounds examined. Therefore, it can be used as threshold to identify potential nucleotide-based inhibitors using respective

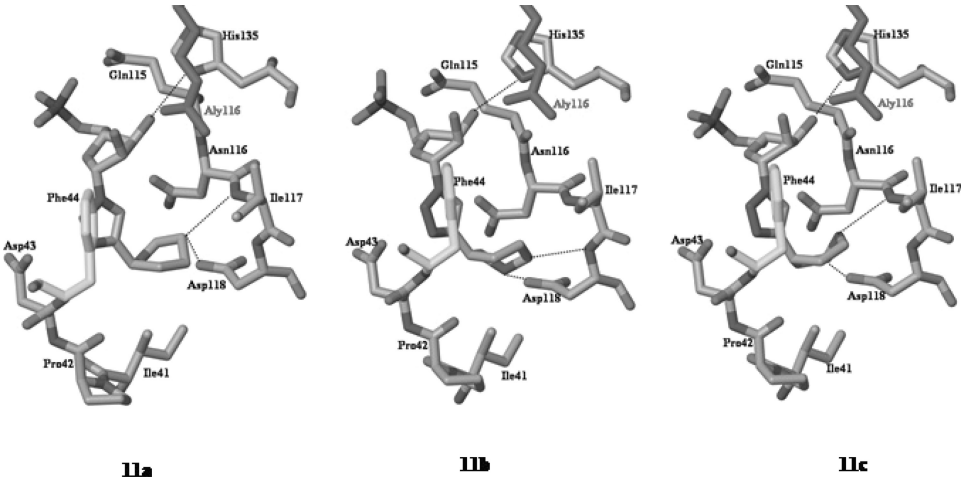


FIGURE 4 Interaction of **11a-c** with conserved amino acids in pocket C of yHst2.

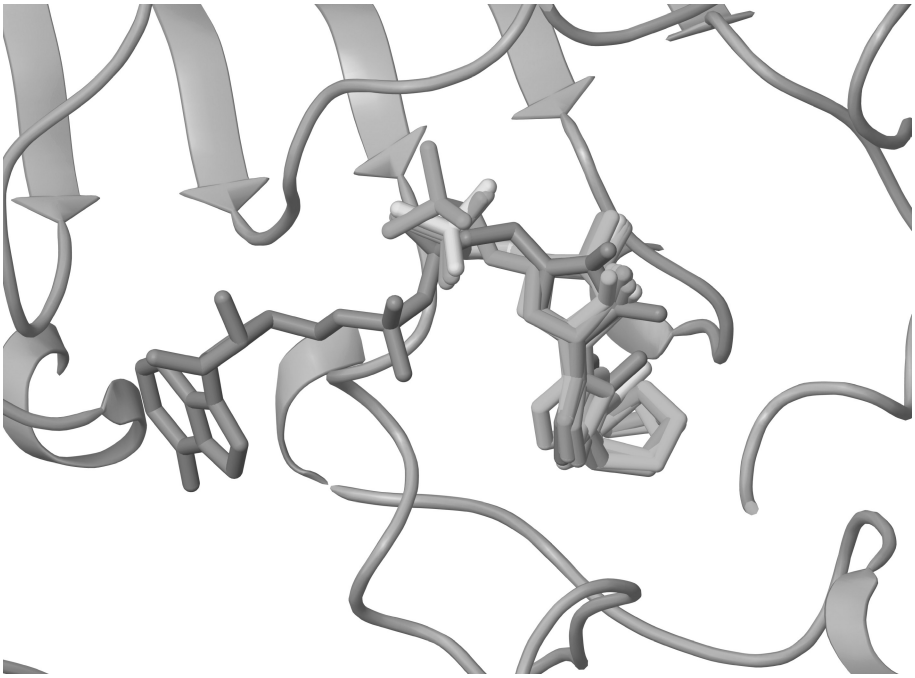


FIGURE 5 Docked poses of **2a-c** (cyan) and **11a-c** (green) in pocket C. NAD is in pink. Docked pose of the phosphorylated nicotinamide riboside is shown in yellow.

protein complex structure (2OD2 for screening of substrate-based templates and 2OD7 for screening of intermediate-based templates).

In order to relate these empirical results to actual inhibition of yHst2, compounds **1a**, **2a, 11a,** and **11b** were evaluated for activity. It was felt that preliminary evaluation of these few compounds would offer sufficient information with regard to the validity of the overall approach to such inhibitor design. A standard inhibition assay (1 μM yHst2 (1-294), 160 μM NAD^+, 100 uM acetyl-lysine [saturating concentrations of substrates], reaction time 15 min) was thus used.[2,16] Only the phosphorylated derivative **11a** displayed some level of inhibitory activity with an IC_{50}~0.5 mM, and was at least five-fold less potent than nicotinamide.[2] It was surprising to find out that **11b** inhibited yHst2 at even higher concentrations (mM). This observation did not match the calculations. However, it is worth noticing that the inhibitory activity of **11b** was potentiated by the presence of nicotinamide but yet remained poor, IC_{50}~5 mM. This modulation was not observed for **11a,** thus indicating that these two very similar compounds do not bind yHst2 in a similar manner. As proposed earlier, it is possible that **11b** binds the adenosine monophosphate binding pocket once nicotinamide is in use. Alternatively, it is possible that **11b** adopted a non-productive conformation in solution where the amino moiety and the phosphate moiety form a

tight ion pair thus restricting free rotation around the glycosidic linkage. Considering that most of the compounds synthesized scored higher than nicotinamide in the docking experiments and yet they were found to be either non-inhibitors or poor-ones, it will be difficult to reliably use this method of screening substrate analogues and predict potent yHst2 inhibitors.

In conclusion, we have developed a versatile synthetic sequence which involved the facile access to triazolide substituted piperidine that gives access to partially protected pyridine- and piperidine-containing nucleoside analogues. These routes are easily adapted to the synthesis of the nucleotide parents. Surprisingly, the piperidine series were found to share high similarities with nicotinamide riboside through frontier molecular orbital analysis. Docking experiments predicted that the piperidine derivatives would bind preferentially the C-pocket of yHst2, yet unfortunately, none of the compounds synthesized offered any relevant level of enzyme inhibition. Overall, the combined studies show that in silico prediction of the inhibition of the sirtuin enzymes by highly flexible substrate-like molecules like those here reported can be deceptive, and that virtual screening proved to be unhelpful with regards to substrate-based sirtuin inhibitor design.

COMPUTATIONAL EXPERIMENTAL

All of the computational study was carried out on Intel Xeon 5130 × 2 workstations.

Theoretical Calculation

The B3LYP functional and the 6-31G* basis set were used in Gaussian 03(Gaussian. Inc) for geometry optimizations of the structures listed in Chart 1. The frontier molecular orbitals were plotted by GaussView3.0.

CHART 1

Energy calculation for 2a-2c for diastereoisomers of piperidine derivatives

Chiral center [1]	B3LPY/6–31G*	B3LYP/6–31G* // B3LPY/6–311+G**	CPCM// B3LPY/6–31G*
(*S*)-2a-piperidine	−989.1267473(4.64)[2]	−989.431678 (4.90)	−989.1582874 (5.80)
(*R*)-2a-piperidine	−989.1244254(6.09)	−989.4296841(6.15)	−989.1556881 (7.43)
(*S*)-2b-piperidine	−989.122422 (7.35)	−989.4277702 (7.35)	−989.1550031 (7.86)
(*R*)-2b-piperidine	−989.1231952 (6.86)	−989.4286943 (6.78)	−989.1561039& (7.17)
boat-2c-piperidine	−989.1227848 (7.12)	−989.4283452 (6.99)	−989.1558797 (7.31)
chair-2c-piperidine	−989.134134 (0)	−989.4394915 (0)	−989.1675289 (0)

Note: The chirality at the point of attachment to the triazole (with chirality at the sugar remaining the same). The values in bracket are relative energies (in kcal/mol) relative to 2c in chair conformation.

Docking

Docking studies were performed using the program Glide (version 4.5) (Schrodinger, Inc. http://www.schrodinger.com/) through Mastro graphic interface for triazolyl-nucleosides and their phosphorylated derivatives. Conformational flexibility was considered by extensive conformational a search implemented in Glide. Best docked poses were minimized with the OPLS forcefield and scored using the GlideScore scoring function.

Protein Structures Used in Docking Studies

The available crystal structure of SIR2 complex can be classified as those in complex with a free nicotinamide inhibitor (1YC5), a substrate (1SZC: Sir2/Carba-Nicotinamide-Adenine-Dinucleotide, 2OD2), an intermediate (2OD9: yHst2/ADP-HPD + Nicotinamide), or a product (1SZD: APR, 2OD7).

Experimental

General: The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$, D_2O, CD_3OD or $(CD_3)_2SO$ on a Bruker AC 300 MHz spectrometer or a Bruker AMX 500 spectrometer. HMQC and ^{1}H-^{1}H COSY experiments were performed on a Bruker AMX 500 spectrometer. TMS (0 ppm, ^{1}H NMR), $CDCl_3$ (77 ppm, ^{13}C NMR) and triethyl phosphate (0.2 ppm, ^{31}P NMR) were used as internal references. The chemical shifts (δ) are reported in p.p.m. (parts per million) and the coupling constants (J values) are recorded in Hz. The hydrogen and carbon assignments were made when 2D experiments (^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC experiments) allowed it. Mass spectra were obtained on a LCT premier Micromass Technologies Waters (ASEP Belfast).

Benzoic acid (2R,3R,4R,5R)-3,4-dibenzoyl-5-azido-tetrahydro-furan-2-ylmethyl ester 4[12] (azido-2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl). Aluminium trichloride (336 mg, 2.5 mmol) and azidotrimethylsilane (2.65 cm^3, 20 mmol) were added to a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside 3 (1.0 g, 2.0 mmol) in dry CH_3CN (12 cm^3) under magnetic stirring. The resulting reaction mixture was stirred for 2 hours. The crude reaction mixture was then concentrated under reduced pressure. The residue was then partitioned between water (50 cm^3) and $CHCl_3$ (50 cm^3). The aqueous layer was extracted with $CHCl_3$ (50 cm^3 × 3). The combined organic layers were then dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude azido derivative as colorless oil (0.97g, 99%).

δ_H (500 MHz, $CDCl_3$) δ 8.20–7.89 (6H, m, Ar), 7.62–7.32 (9H, m, Ar), 5.85 (1H, dd, J 4.9, 6.5, H-3), 5.68 (1H, d, J_{1-2} 1.6, H-1), 5.58 (1H, dd, J_{1-2} 1.6, J_{2-3} 4.8, H-2), 4.81- 4.75 (2H, m, H-4; H-5), 4.56 (1H, dd, J_{4-5} 5.4, $J_{5-5'}$

13.2, H-5′); δ_C (125 MHz, $CDCl_3$) δ 166.2 (C = O), 165.2 (C = O), 165.1 (C = O), 133.7 (Ar), 133.6 (Ar), 133.2 (Ar), 129.8 (Ar), 129.7 (Ar), 129.5 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (2, Ar), 93.3 (C-1), 79.9, 75.3, 71.5, 63.8 (C-5); HRMS (ES) Calcd for $C_{26}H_{21}N_3O_7Na$ ($M+Na^+$) 510.1277 found 510.1257.

(2R,3R,4S,5R)-2-Azido-5-hydroxymethyl-tetrahydro-furan-3,4-diol 13.[17] To a suspension of the azido precursor (benzoic acid (2R,3R, 4R,5R)-3,4-dibenzoyl-5-azido-tetrahydro-furan-2-ylmethyl ester 4, 1.99 mmol, 0.97g) in MeOH (20 cm^3), was added sodium methoxide (0.2 mmol) and the resulting reaction mixture was stirred overnight. The crude reaction mixture was neutralized with Dowex (H^+), filtered and concentrated to dryness. The residue was then partitioned between H_2O and $CHCl_3$. The aqueous layer was washed with $CHCl_3$ and then freezed-dried to afford the crude product as an oil (0.3 g, 86%).

δ_H (500 MHz, D_2O) 5.21 (1H, d, J_{1-2} 1.2, H-1), 4.09 (1H, dd, J_{2-3} 4.7, J_{3-4} 6.9, H-3), 3.94 (1H, appdt, J_{4-5} 3.0, J_{3-4} 6.3, H-4), 3.88 (1H, dd, J_{1-2} 1.2, J_{2-3} 4.5, H-2), 3.74 (1H, dd, J_{4-5} 3.0, $J_{5-5'}$ 12.5, H-5), 3.56 (1H, dd, $J_{4-5'}$ 5.8, $J_{5-5'}$ 12.5, H-5′); δ_C (125 MHz, D_2O) 94.9 (C-1), 83.8 (C-4), 74.9 (C-2), 70.5 (C-3), 62.2 (C-5).

((3aR,4R,6R)-6-Azido-2,2-dimethyl-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl) methanol 7. To an acetone solution (100 cm^3) of the fully deprotected azido-sugar **13** was added $H_2SO_{4(conc.)}$ (5 drops) and the resulting reaction mixture was stirred overnight. The reaction was then diluted with $CHCl_3$ (100 cm^3) and neutralized with $NaHCO_{3,\,sat.}$ (50 cm^3). The aqueous layer was extracted with $CHCl_3$ (3 × 50 cm^3 portions). The combined organic extracts were dried ($MgSO_4$), filtered concentrated under reduced pressure to afford the crude product as yellow oil. Purification by flash chromatography (75/25→50/50, PE/EtOAc, v/v) afforded the pure product as a clear oil 1.44 g (91%).

δ_H (500 MHz, $CDCl_3$) δ 5.52 (1H, s, H-1), 4.74 (1, dd, J_{1-2} 1.0, J_{2-3} 6.0, H-2), 4.50 (1H, d, J_{2-3} 5.9, H-3), 4.23 (1H, dt, J_{3-4} 0.9, J_{4-5} 4.8, H-4), 3.73 (1H, dd, J_{4-5} 4.3, $J_{5-5'}$ 12.2, H-5), 3.66 (1H, dd, $J_{4-5'}$ 5.2, $J_{5-5'}$ 12.2, H-5′), 2.48 (1H, br s, OH), 1.47 (3H, s), 1.29 (3H, s); δ_C (125 MHz, $CDCl_3$) δ 112.9 ($C(CH_3)_2$), 97.8 (C-1), 88.4 (C-4), 85.8 (C-3), 81.6 (C-2), 63.4 (C-5), 26.4 (CH_3), 24.8 (CH_3); HRMS (ES) Calcd for $C_8H_{12}N_3O_4$ (M-H-) 214.0828 found 214.0835.

Typical Procedure for Triazole Formation (8a-c)

To a stirred solution of isopropylidene protected sugar azide **7** (0.5M; typically; 105 mg) and n-ethynyl-pyridine (0.5M) in H_2O/*t*BuOH (1/1, v/v) was added a solution of $CuSO_4 \times 5H_2O$ (0.2 eq.) and sodium ascorbate (0.4 eq.) in water. The resulting reaction mixture was stirred overnight at 40°C. The crude reaction mixture was extracted with three equal amounts of EtOAc, combined, dried ($MgSO_4$), filtered and evaporated to afford

the crude triazolide derivative. The residue was then purified by flash chromatography (100/0 → 95/5, $CHCl_3$/EtOH, v/v), to afford the pure product.

[(3aR,4R,6R,6aR)-2,2-Dimethyl-6-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-methanol 8a. (0.08g; 41%) δ_H (500 MHz, $CDCl_3$) δ 8.43 (1H, ddd, J 0.9, 1.7, 4.9), 8.40 (1H, s, C*H* = C), 8.00 (1H, dt, J 1.0, 8.0), 7.69 (1H, dd, J 1.8, 7.7), 7.17 (1H, ddd, J 1.2, 4.9, 7.5), 6.19 (1H, d, J_{1-2} 1.9, H-1), 5.33 (1H, dd, J_{1-2} 1.9, J_{2-3} 5.9, H-2), 4.99 (1H, dd, J_{3-4} 1.7, J_{2-3} 5.9, H-3), 4.53 (1H, ddd, J_{3-4} 1.9, J_{4-5} 3.3, $J_{4-5'}$ 5.1, H-4), 3.78 (1H, dd, J_{4-5} 3.4, $J_{5-5'}$ 12.3, H-5), 3.67 (1H, dd, $J_{4-5'}$ 5.2, $J_{5-5'}$ 12.3, H-5′), 1.57 (3H, s), 1.35 (3H, s); δ_C (125 MHz, $CDCl_3$) δ 149.5 (Ar), 149.1 (Ar), 147.9 (Ar), 137.0 (Ar), 123.0 (Ar), 122.2 (Ar), 120.3 (CH = *C*), 113.6 (*C*$(CH_3)_2$), 94.9 (C-1), 89.1 (C-4), 85.3 (C-2), 81.8 (C-3), 62.8 (C-5), 26.9 (C(*C*$H_3)_2$), 25.1 (C(*C*$H_3)_2$); HRMS (ES) Calcd for $C_{15}H_{19}N_4O_4$ ($M+H^+$) 319.1406 found 319.1410.

[(3aR,4R,6R,6aR)-2,2-Dimethyl-6-(4-pyridin-3-yl-[1,2,3]triazol-1-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-methanol 8b. (0.11g; 85%) δ_H (500 MHz, DMSO) δ 9.05 (1H, appd, J 2.2), 8.85 (1H, s, C*H* = C), 8.56 (1H, dd, J 1.6, 4.8), 8.23 (1H, appdt, J 2.0, 7.9), 7.50 (1H, dd, J 4.8, 7.5), 6.31 (1H, d, J_{1-2} 1.8, H-1), 5.40 (1H, dd, J_{1-2} 1.9, J_{2-3} 6.0, H-2), 4.95 (1H, dd, J_{3-4} 1.9, J_{2-3} 6.0, H-3), 4.29 (1H, ddd, J_{3-4} 1.9, 5.7, 6.0, H-4), (H-5 and H-5′ under water signal), 1.53 (3H, s), 1.35 (3H, s); δ_C (125 MHz, DMSO) δ 149.1 (Ar), 146.4, 143.9, 132.6, 126.3, 124.1, 121.5 (CH = *C*), 112.8 (*C*$(CH_3)_2$), 93.4 (C-1), 88.0 (C-4), 84.0 (C-2), 81.6 (C-3), 61.2 (C-5), 26.7 (C(*C*$H_3)_2$), 25.0 (C(*C*$H_3)_2$); HRMS (ES) Calcd for $C_{15}H_{19}N_4O_4$ ($M+H^+$) 319.1406 found 319.1428.

[(3aR,4R,6R,6aR)-2,2-Dimethyl-6-(4-pyridin-4-yl-[1,2,3]triazol-1-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-methanol 8c. (0.02 g; 15%) δ_H (500 MHz, DMSO) δ 8.96 (1H, s, C*H* = C), 8.64 (2H, dd, J 1.6, 4.5), 7.83 (2H, dd, J 1.6, 4.5), 6.32 (1H, d, J_{1-2} 1.8, H-1), 5.41 (1H, dd, $J_{1-2 \text{ or } 3-4}$ 1.8, $J_{2-3 \text{ or } 3-4}$ 6.0, H-2/H-3), 5.06 (1H, br s, OH), 4.94 (1H, dd, $J_{1-2 \text{ or } 3-4}$ 1.8, $J_{2-3 \text{ or } 3-4}$ 6.0, H-2/H-3), 4.30 (1H, ddd, J J_{3-4} 1.8, J_{4-5} 5.8, $J_{4-5'}$ 5.9, H-4), (H-5 and H-5′ under water signal), 1.52 (3H, s), 1.35 (3H, s); δ_C (125 MHz, DMSO) δ 150.4, 144.3, 137.5, 122.8, 119.6 (CH = *C*), 112.8 (*C*$(CH_3)_2$), 93.5 (C-1), 88.1 (C-4), 84.0 (C-2), 81.6 (C-3), 61.2 (C-5), 26.7 (C(*C*$H_3)_2$), 25.0 (C(*C*$H_3)_2$); HRMS (ES) Calcd for $C_{15}H_{19}N_4O_4$ ($M+H^+$) 319.1406 found 319.1402.

Typical Procedure for Pyridine Reduction (10a-c)

Pd/C (10% wt) was added to a solution of triazolide diluted in MeOH/HCOOH (9/1, v/v). The resulting suspension was stirred vigorously under an atmosphere of H_2 overnight. The crude reaction mixture was filtered through a pad of celite, which was thoroughly rinsed with MeOH.

The combined filtrates were evaporated to dryness, coevaporated twice with toluene to afford the crude piperidine derivative quantitatively.

[(4R,6R)-2,2-Dimethyl-6-(4-piperidin-2-yl-[1,2,3]triazol-1-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-methanol 10a. δ_H (500 MHz, D_2O) δ 8.26 (1H, s, C*H* = C), 6.35 (1H, s, H-1), 5.53 (1H, d, *J* 6.0), 5.01 (1H, d, *J* 6.0), 4.52–4.47 (2H, m), 3.59 (1H, dd, *J* 4.8, 12.1), 3.48–3.45 (2H, m), 3.17–3.13 (1H, m), 2.23–2.20 (1H, m), 2.01–1.92 (3H, m), 1.74–1.65 (2H, m), 1.59 (3H, s), 1.40 (3H, s); δ_C (125 MHz, D_2O) δ 144.7 (CH = *C*), 124.0 (*C*H = C), 115.0 ($C(CH_3)_2$), 93.9 (C-1), 88.6 (C-4), 84.4 (C-2), 81.7 (C-3), 61.7 (C-5), 52.2, 43.3, 26.2 (2), 24.6, 22.0, 21.9; HRMS (ES) Calcd for $C_{15}H_{25}N_4O_4$ ($M+H^+$) 325.1876 found 325.1880.

[(4R,6R)-2,2-Dimethyl-6-(4-piperidin-4-yl-[1,2,3]triazol-1-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-methanol 10b. δ_H (500 MHz, DMSO) δ 8.12 (1H, s br, C*H* = C), 6.20 (1H, s br, H-1), 5.30 (1H, appd, J_{2-3} 5.3, H-2), 4.90 (1H, appd, $J_{1-2 \text{ and } 3-4}$ 5.7, H-3), 4.21 (1H, appt, $J_{3-4,\ 4-5 \text{ and } 4-5'}$ 5.6, H-4), 3.34 (1H, appd, J 5.1), 3.20–3.15 (2H, m), 2.95–2.83 (3H, m), 2.00–1.97 (2H, m), 1.69–1.65 (2H, m), 1.51 (3H, s), 1.33 (3H, s); δ_C (125 MHz, DMSO) δ 148.1 (CH = *C*), 121.7 (*C*H = C), 113.6 ($C(CH_3)_2$), 93.7 (C-1), 88.5 (C-4), 84.8 (C-2), 82.5 (C-3), 62.0 (C-5), 44.4, 32.3, 30.5, 27.6, 25.9; HRMS (ES) Calcd for $C_{15}H_{25}N_4O_4$ ($M+H^+$) 325.1876 found 325.1880.

[(3aR,4R)-2,2-Dimethyl-6-(4-piperidin-3-yl-[1,2,3]triazol-1-yl)-tetrahydro-furo [3,4-d] [1,3]dioxol-4-yl]-methanol 10c. δ_H (500 MHz, MeOD) δ 8.19 (1H, s, C*H* = C), 6.23 (1H, s, H-1), 5.29 (1H, appd, J_{2-3} 4.4, H-2), 4.92 (1H, appd, $J_{1-2 \text{ and } 3-4}$ 4.0, H-3), 4.23 (1H, s br), 3.36–3.34 (2H, m), 3.17–3.07 (1H, m), 2.81–2.70 (1H, m), 2.06–2.02 (1H, m), 2.01–1.92 (1H, m), 1.77–1.61 (3H, m), 1.52 (3H, s), 1.34 (3H, s); δ_C (125 MHz, MeOD) δ 148.0 (CH = *C*), 121.8 (*C*H = C), 113.9 ($C(CH_3)_2$), 94.7 (C-1) 88.8 (C-4), 85.1 (C-2), 82.2 (C-3), 62.1 (C-5), 44.2, 31.4, 28.5, 26.4, 24.6, 22.0; HRMS (ES) Calcd for $C_{15}H_{25}N_4O_4$ ($M+H^+$) 325.1876 found 325.1866.

General Procedure for the Phosphorylation (9a-b)

Dry Et_3N (3.0 eq.) was added to a stirred solution of 2,3-*O*-protected triazolide (**8a-b;** typically 0.032 g; 0.1mmol) in dry DCM (5 ml); after 5 minutes, diethylchlorophosphite (1.5 eq.) was added; and the resulting reaction mixture was then stirred for 2 hours. The phosphite triester was oxidized with *tert*-butylhydroperoxide (2.0 eq.). After 20 minutes under stirring the crude reaction mixture was concentrated to dryness. The crude residue was purified by flash chromatography (100/0→100/5 $CHCl_3$/EtOH, v/v) to afford the pure phosphate triester.

Phosphoric acid (3aR,4R,6R)-2,2-dimethyl-6-(4-pyridin-2-yl-[1,2,3] triazol-1-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-4-ylmethyl ester diethyl ester 9a. (0.04 g; 90%) δ_H (500 MHz, $CDCl_3$) δ 8.57 (1H, ddd, *J* 0.9, 1.7, 4.9), 8.33 (1H, s, C*H* = C), 8.14 (1H, dt, *J* 1.0, 7.7), 7.77 (1H, dt, *J* 1.8, 7.7), 7.23 (1H, ddd, *J* 1.1, 4.8, 7.5), 6.20 (1H, d, J_{1-2} 1.3, H-1), 5.53 (1H, dd,

J_{1-2} 1.3, J_{2-3} 6.0, H-2), 5.01 (1H, dd, J_{3-4} 2.2, J_{2-3} 6.0, H-3), 4.56 (1H, dt, J_{3-4} 2.1, J_{4-5} 5.6, H-4), 4.05–3.96 (6H, m, CH_2CH$_3$×2, H-5 and H-5′), 1.59 (3H, s, C(CH_3)$_2$), 1.39 (3H, s, C(CH_3)$_2$), 1.24 (6H, ddt, J 1.0, 4.8, 7.1, CH_3CH$_2$×2); δ_C (125 MHz, $CDCl_3$) δ 149.8, 149.5, 148.7, 136.9, 123.1, 121.9, 120.1, 114.0, 93.8 (C-1), 86.7 (d, J 7.9, C-4), 84.5 (C-2), 81.7 (C-3), 66.2 (d, J 5.6, C-5), 64.1 (d, J 5.7, CH$_2$CH$_3$), 26.8 (C(CH_3)$_2$), 25.2 (C(CH_3)$_2$), 16.0 (d, J 7.4, CH$_2$$CH_3$×2); δ_P (121 MHz, $CDCl_3$) 0.15 (s); HRMS (ES) Calcd for $C_{19}H_{28}N_4O_7P$ ($M+H^+$) 455.1696 found 455.1690.

Phosphoric acid (3aR,4R,6R)-2,2-dimethyl-6-(4-pyridin-3-yl-[1,2,3] triazol-1-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-4-ylmethyl ester diethyl ester 9b. (0.04 g; 85%) δ_H (500 MHz, $CDCl_3$) δ 9.04 (1H, s, Ar), 8.57 (1H, d, J 4.2, Ar), 8.20 (1H, d, J 7.9, Ar), 8.17 (1H, s, CH = C), 7.35 (1H, dd, J 4.8, 7.9, Ar), 6.23 (1H, d, J_{1-2} 1.3, H-1), 5.46 (1H, dd, J_{1-2} 1.3, J_{2-3} 6.0, H-2), 4.99 (1H, dd, J_{3-4} 2.0, J_{2-3} 6.0, H-3), 4.57–4.55 (1H, m, H-4), 4.11–3.97 (6H, m, CH_2CH$_3$×2, H-5 and H-5′), 1.59 (3H, s, C(CH_3)$_2$), 1.39 (3H, s, C(CH_3)$_2$), 1.22 (6H, dt, J 0.7, 7.1, CH_3CH$_2$×2); δ_C (125 MHz, $CDCl_3$) δ 149.4, 147.0, 145.1, 133.1, 123.8, 119.7, 114.1, 94.1 (C-1), 86.6 (d, J 8.0, C-4), 86.5 (C-2), 81.4 (C-3), 66.3 (d, J 5.7, C-5), 64.1 (d, J 5.7, CH$_2$CH$_3$×2), 26.9 (C(CH_3)$_2$), 25.1 (C(CH_3)$_2$), 16.0 (d, J 6.5, CH$_2$$CH_3$×2); δ_P (121 MHz, $CDCl_3$) 0.02 (s); HRMS (ES) Calcd. for $C_{19}H_{28}N_4O_7P$ ($M+H^+$) 455.1696 found 455.1693.

Phosphoric acid mono-[(2R,3S,5R)-3,4-dihydroxy-5-(4-piperidin-2-yl-[1,2,3]triazol-1-yl)-tetrahydro-furan-2-ylmethyl] ester 11a. The phosphate triester 9a (0.08 mmol) was reduced using the procedure for pyridine reduction as described for **10a-c** to afford quantitatively phosphoric acid (3aR,4R,6R)-2,2-dimethyl-6-(4-piperidin-2-yl-[1,2,3]triazol-1-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-4-ylmethyl ester diethyl ester **14a** as colorless oil (δ_H (500 MHz, MeOD) δ 8.34 (1H, s, CH = C), 6.37 (1H, br s, H-1), 5.49 (1H, appd, $J_{1-2\text{ and }2-3}$ 5.7, H-2), 4.56–4.54 (2H, m), 4.13–4.08 (6H, m, CH_2CH$_3$×2, H-5, H-5′), 3.52–3.48 (1H, m), 3.25–3.22 (1H, m), 2.29–2.25 (1H, m), 2.11–1.97 (3H, m), 1.86–1.79 (2H, m), 1.61 (3H, s, C(CH_3)), 1.43 (3H, s, C(CH_3)), 1.33 (6H, appt, J 6.9, CH$_2$CH_3×2); δ_C (125 MHz, MeOD) δ 145.6, 124.6, 115.2, 95.1 (C-1), 88.1 (d, J 7.7, C-4), 85.8 (C-2), 82.9 (C-3), 68.1, (d, J 5.6, C-5), 65.8 (d, J 6.0, CH_2CH$_3$), 53.2, 46.0, 29.8, 27.2, 25.4, 23.1, 16.4 (d, J 6.4, CH$_2$CH_3); δ_P (121 MHz, MeOD) -0.7; HRMS (ES) Calcd for $C_{19}H_{34}N_4O_7P$ ($M+H^+$) 461.2165 found 461.2152). The crude reaction mixture was dissolved in dry DCM and bromotrimethylsilane (6 eq.) was then added. The reaction mixture was monitored by TLC. After complete conversion the reaction mixture was concentrated to dryness. The crude residue was then resuspended in water and trifluoroacetic acid was added. The resulting reaction mixture was stirred for 30 minutes. The crude reaction mixture was evaporated to dryness, coevaporated twice with toluene to afford the crude deprotected triazolide **11a**.

δ_H (500 MHz, D_2O) δ 8.29 (0.7H, s, C*H* = C), 8.19 (0.3H, br s, H-1), 6.10 (1H, s br), 4.36–4.25 (2H, m), 4.04–4.00 (4H, m), 3.80 (1H, m), 3.43–3.39 (1H, m), 3.14–3.17 (1H, m), 2.18–1.63 (8H, m δ_C (125 MHz, D_2O) δ 144.6, 144.3, 123.8, 123.7, 123.0, 92.8, 92.5, 84.5, 83.3, 83.2, 74.4, 73.8, 69.5, 69.2, 65.9, 65.0 (d, *J* 5.9), 51.2, 51.1 (2), 44.3, 27.4, 27.3, 20.9, 15.0 (2); δ_P (121 MHz, D_2O) 0.3; HRMS (ES) Calcd for $C_{12}H_{20}N_4O_7P$ ($M\text{-}H^+$) 363.1070 found 363.1078.

5-*O*-diethylphosphate-2,3-*O*-isopropylidene-1-*N*-(4′-piperidin-3″-yl)-[1′,2′,3′]-triazol-β-D-ribofuranoside 14b. Pd/C (10 %) was added to a solution of 2,3-*O*-protected triazolide **9b** (0.6 g, 1.32 mmol) in MeOH/HCOOH (9/1, v/v). The reaction mixture was stirred under an atmosphere of H_2 for 2 days. The reaction mixture was filtered through an AUTOTOP syringe filter (Whatman) and the solvent was evaporated to dryness. The residue was coevaporated with toluene to remove residual HCOOH and then it was dissolved in water and washed with $CHCl_3$. The aqueous layer was freeze-dried to give pure intermediate (0.30 g, 49%) as a white solid.

^{1}H NMR (DMSO): δ 8.04 (1H, s, C*H* = C), 6.28 (1H, s, H-1), 5.50 (1H, d, *J* 5.9 Hz, H-2), 5.06 (1H, dd, *J* 5.9, 1.9 Hz, H-3), 4.66–4.60 (1H, m, H-4), 4.20–3.79 (8H, m, H-5, CH_2CH$_3$), 3.56 (1H, d, *J* 12.1 Hz), 3.39 (1H, d, *J* 13.1 Hz), 3.30–3.18 (1H, m), 3.07 (1H, dd, *J* 12.0, 1.8 Hz), 2.99 (1H, dd, *J* 11.8, 2.4 Hz), 2.19–1.95 (2H, m), 1.86–1.68 (2H, m), 1.54 (3H, s, C(CH_3)$_2$), 1.36 (3H, s, C(CH_3)$_2$), 1.18 (6H, t, *J* 7.1 Hz, CH$_2$CH_3). ^{13}C NMR (DMSO): δ 148.2 (*C* = CH), 122.7 (*C*H = C), 115.0 (*C*(CH$_3$)$_2$), 94.1 (C-1), 86.5 (d, *J* 8.3 Hz, C-4), 84.5 (C-2), 81.4 (C-3), 67.5 (d, *J* 5.4 Hz, C-5), 66.0 (*C*H$_2$CH$_3$), 47.8, 44.4, 31.2, 28.2, 26.3 (d, *J* 6.9 Hz, C(*C*H$_3$)$_2$), 24.6 (C(*C*H$_3$)$_2$), 21.9, 16.0 (d, *J* 6.4 Hz, CH$_2$*C*H$_3$), 15.7 (d, *J* 6.3 Hz, CH$_2$*C*H$_3$). ^{31}P NMR (D_2O): δ-0.05. $[\alpha]_D^{20} = -38.7^\circ$ (DMSO); HRMS (ES) Calcd for $C_{19}H_{33}N_4O_7PCl$ ($M+Cl^-$) 495.1781, found 495.1786.

5-*O*-phosphate-1-*N*-(4′-piperidin-3″-yl)-[1′,2′,3′]-triazol-β-D-ribofuranoside 11b. TMSBr (275 μL, 2.08 mmol) was added to a solution of 2,3-*O*-protected triazolide intermediate **14b** (160 mg, 0.35 mmol) in dry DCM and the reaction mixture was stirred overnight. The reaction mixture was evaporated to dryness. The residue was dissolved in water and Et_3N was used to adjust pH to 7. The aqueous phase was freeze-dried to give pure product **11b** as a triethylamine salt.

^{1}H NMR (DMSO): δ 8.08 (1H, s, C*H* = C), 6.03 (1H, t, *J* 4.8 Hz, H-1), 4.54 (1H, dt, *J* 4.7, 1.5 Hz, H-2), 4.35 (1H, t, *J* 4.7 Hz, H-3), 4.26 (1H, dt, *J* 5.1, 2.7 Hz, H-4), 4.04 (1H, ddd, *J* 11.6, 4.8, 2.6 Hz, H-5), 3.99–3.95 (1H, m, H-5), 3.52–3.49 (1H, m), 3.32 (1H, td, *J* 6.8, 3.7 Hz), 3.21 (1H, tt, *J* 11.4, 3.9 Hz), 3.04 (1H, t, *J* 11.9 Hz), 2.93 (1H, dt, *J* 12.3, 3.2 Hz), 2.11–2.02 (1H, m), 1.96–1.88 (1H, m), 1.80–1.64 (2H, m). ^{13}C NMR (DMSO): δ 148.2 (*C* = CH), 121.5 (*C*H = C), 92.8 (C-1), 84.4 (d, *J* 8.5 Hz, C-4), 75.5 (C-2), 70.7 (C-3), 65.0 (d, *J* 2.8 Hz, C-5), 47.6, 44.3, 31.1, 27.9 (d, *J* 10.8 Hz), 21.8

^{31}P NMR (D_2O): δ1.00 $[\alpha]_D^{20} = -15.3^\circ$ (DMSO). HRMS (ES) Calcd for $C_{12}H_{21}N_4O_7P$ (M-H^+) 363.1075, found 363.1075.

General Procedure for Triazole Formation (6a-c)

Benzoic acid (2R,3R,4R,5R)-3,4-dibenzoyl-5-azido-tetrahydro-furan-2-ylmethyl ester **4** (100 mg, 1.0 eq) was suspended in water (10 cm^3). Sodium ascorbate (1.0 eq) and L-ascorbic acid (1.0 eq) were added to the suspension. n-Ethynyl-pyridine (1.0 eq) was added to the reaction mixture. $CuSO_4.5H_2O$ (0.1 eq) was added and the resulting reaction mixture was then heated at 100°C for 17 hours. The reaction was monitored by TLC (9/1 $CHCl_3$/EtOH, v/v). The reaction mixture was cooled to room temperature and extracted with EtOAc (30 cm^3 × 3). Combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude reaction product.

Benzoic acid (2R,3R,4R,5R)-3,4-dibenzoyl-5-(4-pyridin-2-yl-[1,2,3] triazol-1-yl)-tetrahydro-furan-2-ylmethyl ester 6a. (0.04 g, 31%) δ_H (500 MHz, $CDCl_3$) δ 8.56–8.53 (1H, m, Ar), 8.41 (1H, s, C*H* = C), 8.15–8.12 (1H, m, Ar), 8.04–7.93 (7H, m Ar), 7.80–7.74 (1H, m, Ar), 7.61–7.47 (4H, m, Ar), 7.44–7.33 (7H, m, Ar), 7.24–7.21 (1H, m, Ar), 6.50 (1H, d, J_{1-2} 3.2, H-1), 6.33 (1H, dd, J_{1-2} 3.3, J_{2-3} 5.0, H-2), 6.15 (1H, appt, J_{2-3} 5.7, H-3), 4.92 (1H, dd, J_{3-4} 4.2, J_{4-5} 9.8, H-4) 4.81 (1H, dd, J_{4-5} 3.75, $J_{5-5'}$ 12.5, H-5), 4.64 (1H, dd, J_{4-5} 4.65, $J_{5-5'}$ 12.2, H-5′); δ_C (125 MHz, $CDCl_3$) δ 166.1 (C = O), 165.1 (C = O), 165.0 (C = O), 149.7 (Ar), 149.4 (Ar), 148.8 (Ar), 136.8 (Ar), 133.8 (Ar), 133.6 (Ar), 133.2 (Ar), 129.9 (Ar), 129.8 (2, Ar), 129.3, 128.6, 128.5, 128.4, 123.0, 121.4, 120.4 (CH = *C*), 90.5 (C-1), 81.2 (C-4) 75.3 (C-2), 71.6 (C-3), 63.6 (C-5); HRMS (ES) calcd for $C_{33}H_{27}N_4O_7$ (M+H^+) 591.1880 found 591.1869

Benzoic acid (2R,3R,4R,5R)-3,4-dibenzoyl-5-(4-pyridin-3-yl-[1,2,3] triazol-1-yl)-tetrahydro-furan-2-ylmethyl ester 6b. (0.06 g, 50%) δ_H (500 MHz, $CDCl_3$) δ 8.77 (1H, s br), 8.49 (1H, s br), 7.98–7.88 (8H, m, Ar), 7.51–7.42 (3H, m, Ar), 7.34–7.29 (6H, m, Ar), 6.46 (1H, d, J_{1-2} 3.6, H-1), 6.24 (1H, dd, J_{1-2} 3.7, J_{2-3} 5.1, H-2), 6.09 (1H, t, J_{2-3} 5.35, H-3), 4.86–4.82 (2H, m, H-4 and H-5 or H-5′), 4.55–4.52 (1H, m, H-5 or H-5′), δ_C (125 MHz, $CDCl_3$) δ 166.0 (C = O), 165.1 (C = O), 165.0 (C = O), 149.3 (Ar), 146.9 (Ar), 145.2 (Ar), 133.9 (Ar), 133.7 (Ar), 133.5 (Ar), 133.0 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.1 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.3 (Ar), 118.9 (CH = *C*), 90.4 (C-1), 81.3 (C-4) 75.2 (C-2), 71.4 (C-3), 63.3 (C-5); HRMS (ES) calcd. for $C_{33}H_{27}N_4O7$ (M+H^+) 591.1880 found 591.1863

Benzoic acid (2R,3R,4R,5R)-3,4-dibenzoyl-5-(4-pyridin-4-yl-[1,2,3] triazol-1-yl)-tetrahydro-furan-2-ylmethyl ester 6c. (0.01 g, 12%) δ_H (500 MHz, $CDCl_3$) δ 8.10 (1H, s, C*H* = C), 8.06–7.96 (7H, m, Ar), 7.62–7.52 (4H, m, Ar), 7.45–7.38 (6H, m, Ar), 6.54 (1H, d, J_{1-2} 3.6, H-1), 6.29 (1H,

dd, J_{1-2} 3.3, J_{2-3} 5.1, H-2), 6.14 (1H, t, J_{2-3} 5.4, H-3), 4.97–4.91 (2H, m, H-4 and H-5 or H-5′), 4.60 (1H, dd, J 4.5, 13.2, H-5 or H-5′), δ_C (125 MHz, $CDCl_3$) δ 165.8 (C = O), 164.9 (C = O), 164.0 (C = O), 133.7 (Ar), 133.6 (Ar), 133.3 (Ar), 129.7 (Ar), 129.6 (Ar), 129.4 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 119.7 (CH = *C*), 90.3 (C-1), 81.2 (C-4), 75.0 (C-2), 71.1 (C-3), 63.0 (C-5); HRMS (ES) calcd for $C_{33}H_{27}N_4O_7$ ($M+H^+$) 591.1880 found 591.1824.

Typical Procedure for Acetonide Removal (1a-c and 2a-c)

Trifluoroacetic acid was added to a solution/suspension of triazolide in water. The resulting reaction mixture was stirred for 30 minutes. The crude reaction mixture was evaporated to dryness, co-evaporated twice with toluene to afford the crude deprotected triazolide derivative as the trifluoroacetate salt quantitatively.

(2R,5R)-2-Hydroxymethyl-5-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-tetrahydro-furan-3,4-diol 1a. δ_H (500 MHz, D_2O) δ 8.99 (1H, s, C*H* = C), 8.72 (1H, d, J 5.6), 8.61 (1H, appdt J 1.2, 6.1), 8.35 (1H, d, J 8.1), 7.96 (1H, appt, J 6.7), 6.22 (1H, d, J_{1-2} 3.8, H-1), 4.74 (1H, dd, J_{1-2} 3.9, J_{2-3} 5.8, H-2), 4.43 (1H, t, $J_{2-3 \text{ and } 3-4}$ 5.2, H-3), 4.25 (1H, dt, J_{4-5} 3.2, J_{3-4} 5.1, H-4), 3.84 (1H, dd, J_{4-5} 3.2, $J_{5-5'}$ 12.7, H-5), 3.72 (1H, dd, $J_{4-5'}$ 4.9, $J_{5-5'}$ 12.7, H-5′); δ_C (125 MHz, D_2O) δ 147.9, 141.6, 126.5, 126.1, 125.1, 93.0 (C-1), 86.0 (C-4), 75.5 (C-2), 70.6 (C-3), 61.4 (C-5); HRMS (ES) Calcd for $C_{12}H_{14}N_4O_4Na$ ($M+Na^+$) 301.0913 found 301.0914.

(2R,5R)-2-Hydroxymethyl-5-(4-pyridin-3-yl-[1,2,3]triazol-1-yl)-tetrahydro-furan-3,4-diol 1b. δ_H (500 MHz, D_2O) δ 9.14 (1H, s), 8.87 (1H, ddd, J 1.4, 2.0, 8.3), 8.72 (1H, s, C*H* = C), 8.69 (1H, d, J 5.7), 8.08 (1H, dd, J 6.1, 8.0), 6.13 (1H, d, J 3.9, H-1), 4.66 (1H, appd, J 4.5, H-2), 4.38 (1H, t, J 5.2, H-3), 4.19 (1H, dt, J 3.3, 5.1, H-4), 3.79 (1H, dd, J 3.2, 12.7, H-5), 3.67 (1H, dd, J 5.0, 12.7, H-5′); δ_C (125 MHz, D_2O) δ 143.5, 141.8, 140.7, 138.5, 130.5, 128.2, 123.6, 92.8 (C-1), 85.9 (C-4), 75.4 (C-2), 70.6 (C-3), 61.5 (C-5); HRMS (ES) Calcd for $C_{12}H_{15}N_4O_4$ ($M+H^+$) 279.1093 found 279.1092.

(2R,5R)-2-Hydroxymethyl-5-(4-pyridin-4-yl-[1,2,3]triazol-1-yl)-tetrahydro-furan-3,4-diol 1c. δ_H (500 MHz, D_2O) δ 8.96 (1H, s, C*H* = C), 8.74 (2H, appd, J 7.0), 8.38 (2H, appd J 7.0), 6.19 (1H, d, J_{1-2} 3.8, H-1), 4.42 (1H, t, $J_{1-2 \text{ and } 2-3}$ 5.2, H-3), 4.23 (1H, dt, J_{4-5} 3.2, J_{3-4} 5.1, H-4), 3.83 (1H, dd, J_{4-5} 3.2, $J_{5-5'}$ 12.7, H-5), 3.71 (1H, dd, $J_{4-5'}$ 5.0, $J_{5-5'}$ 12.7, H-5′); δ_C (125 MHz, D_2O) δ 147.4, 142.1, 126.2, 123.2, 93.0 (C-1), 86.0 (C-4), 75.5 (C-2), 70.6 (C-3), 61.5 (C-5); HRMS (ES) Calcd for $C_{12}H_{15}N_4O_4$ ($M+H^+$) 279.1093 found 279.1085.

(2R,5R)-2-Hydroxymethyl-5-(4-piperidin-2-yl-[1,2,3]triazol-1-yl)-tetra-hydro-furan-3,4-diol 2[a]. δ_H (500 MHz, D_2O) δ 8.17 (1H, s, C*H* = C), 6.04 (1H, d, J_{1-2} 4.0, H-1), 4.55 (1H, appt $J_{1-2 \text{ and } 2-3}$ 4.4, H-2), 4.39 (1H, dd, J

2.9, 11.8), 4.28 (1H, t, $J_{2-3\text{ and }3-4}$ 5.2, H-3), 4.10 (1H, dt, J_{4-5} 3.4, J_{3-4} 5.1, H-4), 3.70 (1H, dd, J_{4-5} 3.2, $J_{5-5'}$ 12.7, H-5), 3.58 (1H, dd, $J_{4-5'}$ 5.0, $J_{5-5'}$ 12.7, H-5′), 3.35 (1H, d, J 12.9), 3.04 (1H, dt, J 2.6, 12.5), 2.11–2.09 (1H, m), 1.93–1.80 (3H, m), 1.65–1.51 (2H, m); δ_C (125 MHz, D_2O) δ 163.2 (q, J 35.7 Hz, C = O), 144.5 (CH = *C*), 123.5 (*C*H = C), 116.7 (q, J 291.3 Hz), 92.6 (C-1), 85.8 (C-3), 75.3 (C-2), 70.6 (C-4), 61.5 (C-5), 52.2, 45.3, 28.4, 21.9; HRMS (ES) Calcd for $C_{12}H_{21}N_4O_4$ ($M+H^+$) 285.1563 found 285.1560.

(2R,5R)-2-Hydroxymethyl-5-(4-piperidin-3-yl-[1,2,3]triazol-1-yl)-tetrahydro-furan-3,4-diol 2b. δ_H (500 MHz, D_2O) δ 7.83 (1H, s, C*H* = C), 5.80 (1H, d, J_{1-2} 4.0, H-1), 4.34 (1H, appt $J_{1-2\text{and }2-3}$ 4.5, H-2), 4.09 (1H, t, $J_{2-3\text{ and }3-4}$ 5.1, H-3), 3.92 (1H, dt, J_{4-5} 3.3, J_{3-4} 5.0, H-4), 3.53 (1H, dd, J_{4-5} 3.2, $J_{5-5'}$ 12.7, H-5), 3.41 (1H, dd, $J_{4-5'}$ 5.0, $J_{5-5'}$ 12.7, H-5′), 3.21 (1H, appd, J 12.6), 3.13 (1H, appd, J 12.7), 3.04–2.99 (1H, m), 2.83 (1H, t, J 12.1), 2.76–2.71 (1H, m), 1.89–1.86 (1H, m), 1.75–1.71 (1H, m), 1.60–1.43 (2H, m); δ_C (125 MHz, D_2O) δ 162.9 (q, J 35.7 Hz, C = O), 147.9 (CH = *C*), 122.3 (*C*H = C), 116. (q, J 291.3 Hz), 92.6 (C-1), 85.7 (C-3), 75.3 (C-2), 70.6 (C-4), 61.5 (C-5), 47.7, 44.4, 31.1, 28.0, 21.8; HRMS (ES) Calcd for $C_{12}H_{21}N_4O_4$ ($M+H^+$) 285.1563 found 285.1561.

(2R,5R)-2-Hydroxymethyl-5-(4-piperidin-4-yl-[1,2,3]triazol-1-yl)-tetrahydro-furan-3,4-diol 2c. δ_H (500 MHz, D_2O) 8.01 (1H, s, C*H* = C), 6.01 (1H, d, J_{1-2} 3.8, H-1), 4.56 (1H, appt $J_{1-2\text{and }2-3}$ 4.3, H-2), 4.31 (1H, t, $J_{2-3\text{ and }3-4}$ 4.9, H-3), 4.13 (1H, dd, J_{3-4} 4.3, $J_{4-5'}$ 7.4, H-4), 3.74 (1H, dd, J_{4-5} 2.5, $J_{5-5'}$ 12.7, H-5), 3.62 (1H, dd, $J_{4-5'}$ 4.8, $J_{5-5'}$ 12.7, H-5′), 3.40 (2H, appd, J 12.9), 3.11–3.04 (3H, m), 2.17 (2H, appd, J 14.1), 1.86–1.79 (2H, m), 1.65–1.51 (2H, m, H-γ'', H-δ''); δ_C (125 MHz, D_2O) δ 163.2 (q, J 36.4 Hz, C = O), 150.4 (CH = *C*), 121.8 (*C*H = C), 116.8 (q, J 291.0 Hz), 92.6 (C-1), 85.7 (C-3), 75.3 (C-2), 70.6 (C-4), 61.5 (C-5), 43.9, 30.6, 28.1; HRMS (ES) Calcd for $C_{12}H_{21}N_4O_4$ ($M+H^+$) 285.1563 found 285.1562.

Benzoic acid (2R,3R,4R,5R)-3,4-dibenzoyl-5-(4-piperidin-3-yl-[1,2,3]triazol-1-yl)-tetrahydro-furan-2-ylmethyl ester 12b. Pd/C (10% wt) was added to a solution of benzoic acid (2R,3R,4R,5R)-3,4-dibenzoyl-5-(4-pyridin-3-yl-[1,2,3]triazol-1-yl)-tetrahydro-furan-2-ylmethyl ester **6b** diluted in MeOH. The resulting suspension was stirred vigorously under an atmosphere of H_2 overnight. The crude reaction mixture was filtered through a pad of celite, which was thoroughly rinsed with MeOH. The combined filtrates were evaporated to dryness, coevaporated twice with toluene to afford the crude piperidine derivative, quantitatively. Further purification by flash chromatography (9/1, $CHCl_3$/EtOH, v/v) afforded the product as a clear oil (32.8 mg, 32%).

δ_H (500 MHz, $CDCl_3$) δ 7.97–7.95 (2H,m, Ar), 7.90–7.86 (4H, m, Ar), 7.66 (1H, s br, C*H* = C), 7.52–7.45 (3H, m, Ar), 7.39–7.36 (2H, m, Ar), 7.31–7.27 (4H, m, Ar), 7.29–7.19 (1H, m, Ar), 6.37–6.35 (1H, m,

H-1), 6.17–6.16 (1H,m), 6.07 (1H, t, *J* 5.0), 4.80–4.74 (2H, m), 4.54–4.48 (1H, m), 3.61–3.56 (1H, m), 3.43–3.35 (2H, m), 2.97–2.92 (1H, m), 2.87–2.80 (1H, m), 2.01–1.97 (2H, m), 1.87–1.81 (1H, m); δ_C (125 MHz, $CDCl_3$) δ 166.0, 165.1, 165.0, 147.6, 133.8, 133.6 (2), 133.5, 129.9, 129.8, 129.3, 129.2, 128.6, 128.5 (2), 128.4, 120.3 (2), 90.3 (C-1), 81.2, 81.1, 75.1, 71.7, 71.6, 63.7, 63.6, 47.7 (2), 44.0, 30.5, 28.5, 21.7; HRMS (ES) calcd for $C_{33}H_{33}N_4O_7$ (M^+ H^+) 597.2349 found 597.2353.

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