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Synthesis and Evaluation of the Biological Profile of Novel Analogues of Nucleosides and of Potential Mimetics of Sugar Phosphates and Nucleotides

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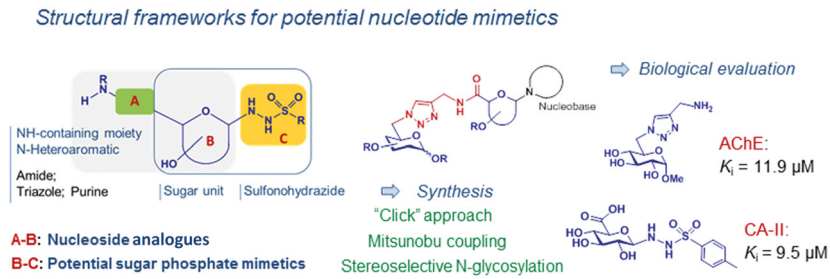

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Abstract The synthesis of purine/triazole 6'-isonucleosides and of glucuronic acid/glucuronamide-derived *N*-glycosylsulfonohydrazides through efficient and stereo- or regioselective methodologies is described. Their structures were envisaged to mimic nucleosides, sugar phosphates, or nucleotides, and were expected to provide potential inhibitors of therapeutically relevant enzymes, the active sites of which could potentially bind their structural fragments or functional groups. Such enzymes include cholinesterases, carbonic anhydrase II (CA-II) and cyclin-dependent kinase 2 (CDK-2). A (triazolyl)methyl amide-linked disaccharide nucleoside, based on a new prospective structural framework for analogues of nucleoside diphosphate sugars, was synthesized. The synthetic strategies employed unprotected or partially protected carbohydrate derivatives as precursors, including ribose, glucuronic acid, glucuronolactone, and glycopyranosides and relied on stereoselective N-glycosylation, regioselective Mitsunobu coupling and 'click chemistry' approaches. Some 6'-isonucleosides and triazole-containing glycoderivatives displayed moderate selective acetylcholinesterase inhibitory activities. The best inhibitor was an aminomethyltriazole 6'-isonucleoside with a K_i value of 11.9 µM. *N*-Glucuronylsulfonohydrazide showed good inhibition of CA-II (K_i = 9.5 µM). Molecular docking of the most active compounds into the effected enzymes showed interactions with key amino acid residues for substrate recognition. In addition, the tested compounds did not show toxicity to normal cells.

Key words nucleosides, nucleotides, carbohydrates, mimetics, cholinesterases

Nucleotides and nucleosides are essential components of cells and they play key roles in several fundamental biological processes including DNA and RNA synthesis, cell division, and metabolism.

Nuno M. Xavier (born November 1982 in Vila Real, Portugal) graduated in chemistry from the University of Lisbon in 2005. He received a dual Ph.D. degree in organic chemistry from the University of Lisbon and from the National Institute of Applied Sciences of Lyon in 2011, where he worked in the field of glycochemistry under the supervision of Professor A. P. Rauter and Dr. Y. Queneau, respectively. During his Ph.D. studies, he was also a visiting research student in the group of Professor J. Thiem at the University of Hamburg for four months. He joined Professor P. Kosma's glycochemistry group at the University of Natural Resources and Life Sciences of Vienna for one year as a postdoctoral member and worked on the synthesis of new heptose and sugar phosphate derivatives toward novel antibacterial drug candidates. He then returned to the University of Lisbon as postdoctoral fellow until the end of 2013, whereupon he was awarded an Investigator Starting Grant from the Portuguese Foundation for Science and Technology (FCT). Since then he has been a researcher (*FCT Investigator*) at the Faculty of Sciences, University of Lisbon. His research interests and activities, reported in 23 publications and discussed in various communications in several international scientific meetings and symposia, are centred on the development of synthetic strategies for carbohydrate-containing molecules of potential therapeutic interest. He was awarded with the Scientific Award of the *Groupe Lyonnais des Glycosciences* (France), a Young Scientist Award from the IUPAC/43rd IUPAC World Chemistry Congress and the Young Investigator Award in Glycosciences from the Alberta Ingenuity Centre for Carbohydrate Science (Canada). He is a member of IUPAC.

The development of modified analogues as well as mimetics of nucleotides/nucleosides is an attractive approach in drug discovery of compounds that aim to interfere with such physiological pathways, which are uncontrolled in diseases such as cancer or viral infections.[1] These molecules may act as antimetabolites, through incorporation into DNA or RNA or by inhibition of enzymes involved in nucleotide metabolism and nucleic acid synthesis such as DNA polymerase or ribonucleotide reductase. Antimetabolites constitute a class of anticancer and antiviral agents[1,2] that include structures such as pyrimidine[3] and purine nucleosides.[4] Nucleobase analogues are also suggested to behave as antimetabolites through diffusion into cells and subsequent conversion into analogues of nucleotides by enzymes that are involved in the metabolism of natural purines and pyrimidines.[3c] In addition to their potential to interfere in nucleic acid synthesis, nucleoside and nucleotide analogues can be exploited for targeting other nucleoside/nucleotide-dependent enzymes such as glycosyltransferases, ATPases, GTPases, phosphodiesterases or methyltransferases, which are valuable therapeutic targets for a number of diseases.[5] One of the most important targeted classes of nucleotide-dependent enzymes, particularly in cancer therapy, are kinases, which are ATP-dependent and responsible for protein phosphorylation.[6] Among them, cyclin-dependent kinases (CDKs) are directly involved in the regulation and in driving the cell cycle, and due to their frequent overactivation or overexpression in tumor cells,[7] CDK inhibition has emerged as a potential anticancer therapeutic strategy.[8] Some CDK inhibitors have demonstrated potent antitumor efficacy[8b,9] and mostly encompass small molecules that are structurally based on aromatic or heteroaromatic motifs.[9,10] Nucleobase analogues, including purine and pyrimidine derivatives, such as (*R*)-roscovitine[11] and the pyrazolopirymidine dinaciclib,[11,12] have attracted by far the highest attention as CDK inhibitors, among other heterocyclic derivatives such as thiazoles, pyrazoles, indoles or flavones.

In addition to antitumor and antiviral properties, the reported bioactivities exhibited by nucleoside and nucleotide analogues also include antimicrobial[13] and cholinesterase (ChE) inhibitory effects.[14] ChEs hydrolyse acetylcholine and are major drug targets for Alzheimer's disease.[15] Purine nucleosides of D-glucuronic acid derivatives have been shown to be effective and selective inhibitors of acetylcholinesterase,[14a] whereas mannosyl purine nucleosides have been described as potent selective butyrylcholinesterase inhibitors.[14b]

This biological profile encourages the search for efficient and straightforward approaches leading to new nucleoside/nucleotide-like derivatives. We report herein on the synthesis of new nucleoside analogues, namely 6'-isonucleosides, sugar derivatives containing uncharged surrogates for a phosphate moiety such as a sulfonohydrazide functionality, as well as more elaborated sugar-containing molecules comprised of an N-heteroaromatic unit and a phosphate/diphosphate isostere as potential nucleotide mimetics.

Biological evaluation of such compounds has focused on enzymes of therapeutic relevance such as ChEs and carbonic anhydrase II (CA-II). The latter is one of the CA isoforms that catalyzes the reversible hydration of carbon dioxide and it plays a crucial role in the regulation of the intraocular pressure. Its inhibition thus constitutes a therapeutic approach towards glaucoma.[16] Sulfonamide derivatives are among established inhibitors such as the clinically used acetazolamide.[17] Glycosylsulfonamides were also reported to inhibit various isoforms of CAs,[18] which prompted us to test the activity of sulfonohydrazide derivatives on CA-II.

As a nucleotide-dependent enzyme, CDK-2 was selected for inclusion in the biological assays, because it has become a privileged target for antitumor therapy among CDKs.[19] Its inhibition is suggested to induce selective cytotoxic effects on tumor cells through an associated persistence of a transcription factor (E2F) activity, the level of which is intolerable in transformed cells but still acceptable in normal cells.[8a]

Results on the bioactivity assessment of the new compounds, i.e., their enzyme inhibitory effects as well as their cytotoxicity on a panel of tumor and normal cells, are presented herein. For the active molecules, their possible interactions with the enzymes were inspected by docking simulations.

The general framework of one type of structure envisaged comprises a sugar moiety, a functional mimic of the phosphate group, and an NH-containing moiety such as an amide or an N-heteroaromatic unit (Figure 1). Molecules combining two structural elements of the skeleton, having a sugar unit and a phosphate isostere or possessing a sugar moiety and an N-heteroaromatic system, were also planned as mimetics of sugar phosphates and as nucleoside analogues, respectively.

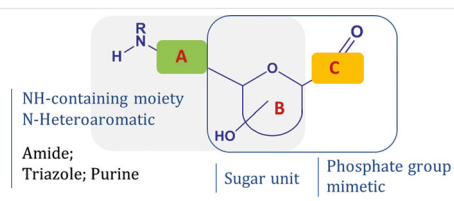
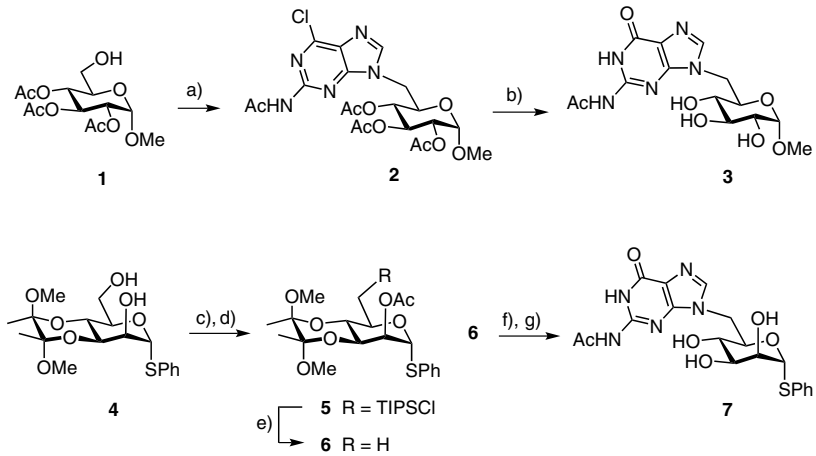


Figure 1 General skeleton of potential nucleoside mimetics (**A**–**B**), sugar phosphate mimetics (**B**–**C**), and nucleotide mimetics (**A**–**B**–**C**)

In this study we concentrated on isonucleosides as analogues of nucleosides. These regioisomers of nucleosides, in which the nucleobase is linked to the sugar moiety at a non-anomeric position, have been reported to display significant biological properties including antiviral[20] and anticancer activities.[21] In particular, based on *in silico* analysis, adenine 3'-isonucleosides showed the potential to inhibit



Scheme 1 *Reagents and conditions*: (a) PPh_3, DEAD, 2-NHAc-6-Cl-purine, r.t., 16 h, 80%; (b) TFA (80% aq), 60 °C, 16 h, 57%; (c) TIPSCl, py, cat. DMAP, r.t., 16 h; (d) Ac_2O, py, r.t., 16 h, 95%, two steps; (e) TBAF, THF, r.t., 1 h, 56%; (f) PPh_3, DEAD, 2-NHAc-6-Cl-purine, THF, reflux, 16 h; (g) TFA (80% aq), 60 °C, 16 h, 41%, two steps.

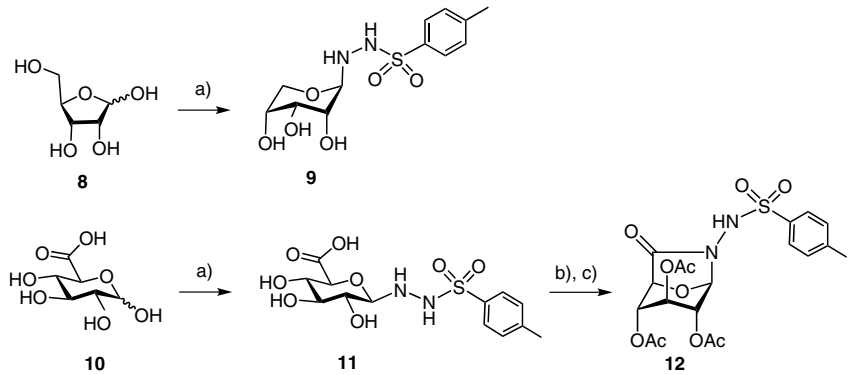
kinases,[22] whereas 2'-isonucleoside triphosphates showed the ability to be recognized by DNA polymerases.[23] Moreover, these nucleoside analogues are likely to present better stability towards enzymatic hydrolysis than their natural counterparts. In the isonucleosides reported, the nucleobase is linked at either C-2 or C-3 to the sugar backbone, especially at furanose units.[20c,21b,24] It was therefore appropriate to consider other positions of the sugar moiety for the coupling of a nucleobase towards new types of isonucleosides. Hence, the synthesis of 6'-isonucleosides, namely glycopyranos-6-yl purines, was undertaken. Their construction was accomplished through Mitsunobu reaction of *gluco*- and *manno*-configured glycosides containing a free hydroxyl group at C-6 with 2-acetamido-6-chloropurine (Scheme 1).

Thus, methyl 2,3,4-*O*-acetyl-α-D-glucopyranoside (**1**), available in three steps from methyl α-D-glucopyranoside, was reacted with 2-acetamido-6-chloropurine in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine to give the N^9-glucosylpurine **2** as the only formed regioisomer in 80% yield.[25] Evidence for the N9-C6' linkage was provided by HMBC experiments, which showed a key correlation between the sugar H-6' and C-4 of the purine moiety. Treatment of **2** with aqueous trifluoroacetic acid (TFA) at 60 °C effected both deacetylation at the sugar ring and hydrolysis at the chloro-containing 6-acetamidopurine motif, leading to the *N*-acetylguanine moiety **3**. The guanine system of **3** was inferred by the significant differences in the chemical shifts in the signals of the nucleobase moiety between **2** and **3** in their ^{13}C NMR spectra. Whereas C-6 of isonucleoside **3** (δ = 157.5 ppm) is deshielded relative to that of **2** (δ = 151.5 ppm), the signal for C-5 appears at higher field (δ = 120.4 ppm) than that of **2** (δ = 127.6 ppm). HRMS analysis further supported the assignment of the identity of **3**, showing the corresponding peak for the $[M + Na]^+$ molecular ion and the loss of the Cl-associated isotopic pattern.

An isonucleoside comprising a mannose-derived backbone was then synthesized. A suitable 6-monodeprotected mannosyl precursor was prepared from the 3,4-*O*-butane 2',3'-diacetal derivative of phenyl 1-thiomannoside (**4**), which could be accessed from mannose in four steps.[26] Selective protection of **4** at the primary hydroxyl group using triisopropylsilyl chloride (TIPSCl) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in pyridine and subsequent acetylation of the intermediate silyl ether, afforded fully protected thiomannoside **5**. Desilylation of **5** with tetra-*N*-butylammonium fluoride (TBAF) gave **6**, the coupling of which with 2-acetamide-6-chloropurine using the DEAD/triphenylphosphine system and further acid hydrolysis (aq TFA), employing similar conditions as with **2**, furnished the N^9-linked mannosylguanine **7**.

As potential mimetic structures of sugar phosphates, *N*-glycosylsulfonohydrazides were chosen. The sulfonohydrazide moiety was predicted to be a surrogate for the phosphate group due to its polar nature and propensity for hydrogen bonding interactions with biological targets. The anomeric sulfonohydrazide of ribose, the sugar unit present in ATP (which is the natural nucleotide substrate of enzymes such as kinases) as well as that of glucuronic acid were envisaged as target molecules.

The synthesis of *N*-ribosylsulfonohydrazide and of the glucuronic acid counterpart was carried out by treatment of ribose **8** and glucuronic acid **10** (Scheme 2), respectively, with tosylhydrazide in the presence of acetic acid at 40 °C; a methodology first reported for partially O-protected monosaccharides and more recently applied to fully unprotected sugars.[27,28] The reaction likely proceeds via the acyclic tosylhydrazone intermediate, which evolves to the more sta-



Scheme 2 *Reagents and conditions*: (a) $TsNHNH_2$, cat. AcOH, DMF, 40 °C, 48 h, quantitative (**9**), 67% (**11**); (b) Ac_2O, py, r.t., 5 min; (c) propargyl amine, CH_2Cl_2, two steps.

ble cyclic glycosylhydrazine isomer by intramolecular attack of the hydroxyl group at C-5 to the sp^2 carbon of the hydrazone. The β-*N*-glycosyl derivatives **9** and **11** were regio- and stereoselectively obtained in quantitative and in 67% yields, respectively.

The $^1C_4$ conformation adopted by **9** was revealed by its ^1H NMR spectrum, particularly through the coupling constants between H-4 and both of the protons at C-5 ($J_{H\text{-}4,H\text{-}5a}$ and $J_{H\text{-}4,H\text{-}5b}$ of 4.2 and 2.0 Hz), indicating the absence of a H-4–H-5a or H-5b *trans*-diaxial relationship as would be expected for a $^4C_1$ conformation. The β-anomeric configuration of **9** was established from the NOESY spectrum, in which H-1 only presents correlations with H-5b and H-2. Moreover, the small coupling constant between H-1 and H-2 ($J_{1,2}$ = 1.2 Hz) further confirmed the assigned conformation and anomeric configuration.

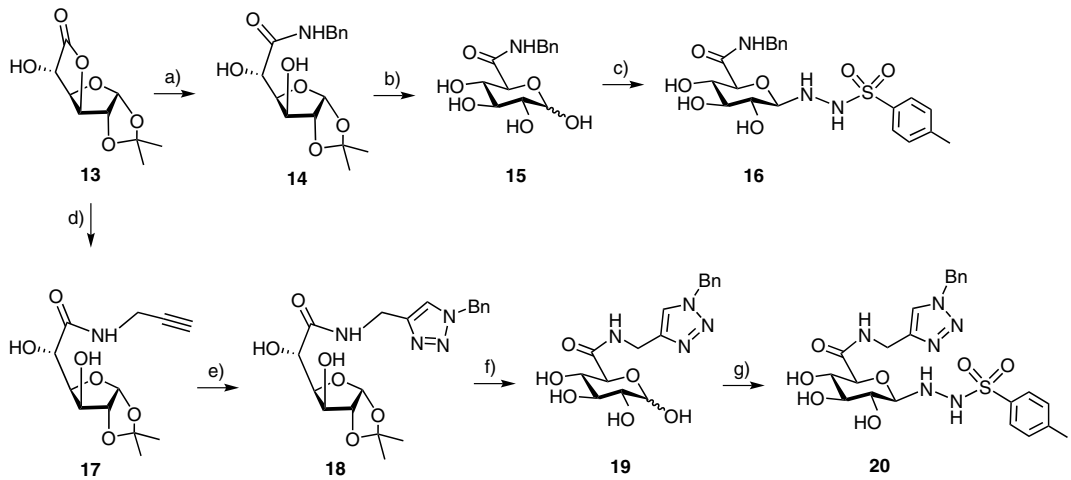
The *N*-glucuronylsulfonohydrazide **11** adopted a $^4C_1$ conformation, as judged by the coupling constants between H-2–H3, H3–H4, and H4–H5, whereas the large $J_{1,2}$ value (8.8 Hz) was clearly indicative of a β-anomeric configuration, supported by the NOESY correlations observed between H-1 and protons H-3 and H-5.

The sole formation of the β-anomers **9** and **11** may arise from their stabilization through the *exo*-anomeric effect, resulting from donation of electron density from the nitrogen lone pair to the endocyclic C1–O bond ($n_{N1}\rightarrow\sigma^*_{C1-O5}$); an orbital interaction that occurs commonly in *N*-glycosyl compounds.[29] In the case of ribosyl sulfonamide **9** and despite the fact that the sulfonamide function is in an axial orientation, the *anti*-relationship between H-1 and N*H* (since $J_{NH,H\text{-}1}$ = 10.9 Hz) is consistent with an *anti*-periplanar orientation between the nitrogen nonbonding orbital and the C1–O bond, which allows the expression of this electronic effect.

Acetylation of **11** with acetic anhydride/pyridine followed by treatment with *N*-propargylamine, furnished the bicyclic glucuronolactam-based sulfonohydrazide **12**. The ^1H NMR spectrum of **12** presented broad singlets for all protons of the pyranose ring, thus confirming its $^1C_4$ conformation and the HMBC correlation between the carbonyl carbon of the lactam functionality (δ = 167.0 ppm) with H-1, which appeared at a rather high chemical shift value (δ = 5.62 ppm), was in accordance with the presence of a urono-1,6-lactam.

Based on the structure of **11**, access to sulfonohydrazidyl glucuronamide derivatives bearing aromatic or N-heteroaromatic moieties, as molecules lying within the proposed general skeleton for nucleotide mimetics (Figure 1), was further targeted.

Glucofuranurono-6,3-lactone (**13**; Scheme 3) was the precursor for the introduction of the aromatic moieties at C-6 of the glycosyl unit through opening of the lactone moiety with *N*-benzylamine or with *N*-propargylamine. The *N*-benzyl glucofuranuronamide **14**[14a] was subjected to acid-mediated hydrolysis (aq TFA) to remove the 1,2-*O*- isopropylidene functionality and cause subsequent intramolecular ring closure to the pyranose form, to give **15** in quantitative yield. Glycosylation of tosylhydrazide with **15** led to the *N*-benzyl sulfonohydrazidyl β-glucuronamide **16** in 46% yield. The *N*-propargyl glucofuranuronamide **17**[30] was subjected to a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition by treatment with benzyl azide in the presence of the CuI/Amberlyst A-21 catalytic system,[31] leading to the expected *N*-benzyl triazole derivative **18** in 87% yield. Cleavage of the acetonide functionality of **18** by aqueous TFA and accompanying ring expansion afforded *N*-benzyltriazolylmethyl glucopyranuronamide (**19**), which was converted into the corresponding anomeric tosylhydrazinyl derivative **20** by using similar N-glycosylation conditions to those used for **15**.[32] Similar to that observed for the synthesis of **9** and **11**, only β-anomers **16** and **20** were obtained, as was confirmed by their $J_{1,2}$ values (7.9 Hz) in their ^1H NMR spectra as well as by the NOE correlations between H-1, H-3, and H-5. Of particular note is the use of simple, mild and efficient coupling methods in the synthesis of **16** and **20** that fulfill the requirements of 'click' chemistry processes.[33]

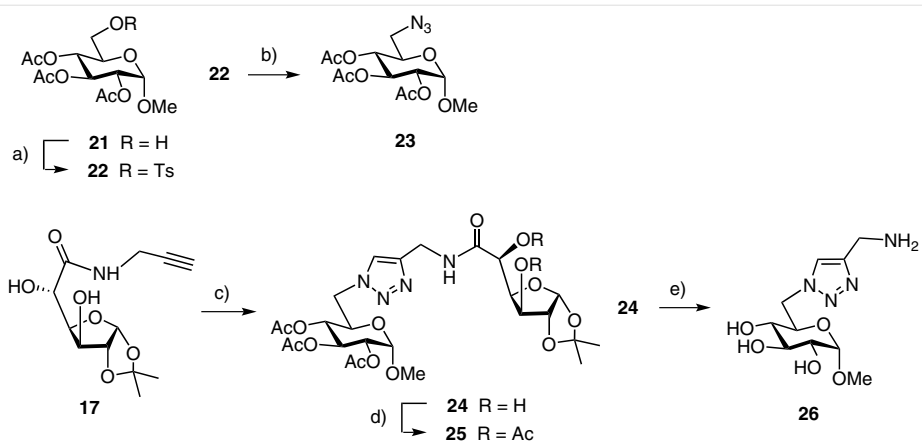


Scheme 3 *Reagents and conditions*: (a) $BnNH_2$, CH_2Cl_2, r.t., 16 h, quant.[14a]; (b) TFA (80% aq), r.t., 1.5 h, quant. (α/β = 1:0.60); (c) $TsNHNH_2$, cat. AcOH, DMF, 40 °C, 48 h, 46%; (d) propargylamine, CH_2Cl_2, r.t., 16 h; (e) BnN_3, CuI/Amberlyst A-21 (cat.), CH_2Cl_2, r.t., 2 h, 87%; (f) TFA (80% aq), r.t., 1 h, quant., α/β ratio, 1:0.75; (g) $TsNHNH_2$, cat. AcOH, DMF, 40 °C, 48 h, 45%.

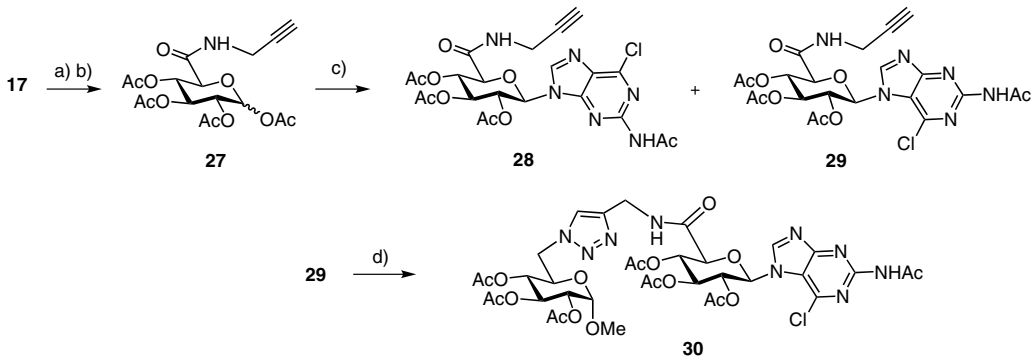
In addition to the Cu(I)-catalyzed cycloaddition leading to **18**, the formation of glucuronamide intermediates **14** and **17** by lactone ring opening with amines and the stereoselective coupling of lactols **15** and **19** with tosylhydrazide also merit the 'click' reaction terminology.

The *N*-propargyl glucuronamide **17** was also a precursor to the (triazolyl)methyl amide-linked disaccharide **24**, which is an analogue of **18** in which the benzyl group is replaced by a monosaccharide moiety (Scheme 4). Hence, the triacetylated methyl 6-azido-glucopyranoside **23**, which was synthesized by tosylation of methyl 2,3,4-*O*-acetyl-α-D-glucopyranoside (**21**) followed by nucleophilic replacement with sodium azide, was subjected to 'click' cycloaddition with **17** using the CuI/Amberlyst A-21 catalytic system to give **24** in 74% yield.[34] Acetylation of **24** with acetic anhydride and pyridine gave **25** in quantitative yield. Treatment of **24** with aqueous TFA (80%) at 60 °C effected both deacetylation and hydrolysis at the amide function, providing quantitatively the triazole isonucleoside **26**, an aminomethyl triazole analogue of the 6'-isonucleoside **3**. Access to **26** constitutes a successful application of a coupling–decoupling pathway in which 'click' reactions are used to assemble the different molecular units.[35]

The structural framework of **24** motivated attempts to access to a nucleoside derivative with a structure resembling that of nucleotide sugars; namely, the nucleoside diphosphate sugars (Figure 2,**A**), with the (triazolyl)methyl amide fragment being envisaged as a potential metabolically and hydrolytically stable neutral surrogate of the diphosphate moiety (Figure 2,**B**).



Scheme 4 *Reagents and conditions*: (a) TsCl, py, r.t., 16 h, 91%; (b) NaN_3, DMF, 80 °C, 16 h, 83%; (c) **23**, CuI/Amberlyst A21, CH_2Cl_2, r.t., 16 h, 74%; (d) Ac_2O, py, r.t., 1 h, quant.; (e) TFA (80% aq), 60 °C, 2 days.



Scheme 5 *Reagents and conditions*: (a) TFA (70% aq), 40 °C, 30 min; (b) Ac_2O, py, r.t., 30 min, 87%, two steps, α/β ratio: 1:0.5; (c) silylated 2-NHAc-6-Cl-purine, TMSOTf, MeCN, 65 °C, MW, max. 150 W, 1.5 h, 30% (**28**) and 29% (**29**); (d) **23**, CuI/Amberlyste A21, CH_2Cl_2, r.t., 16 h, 43%.

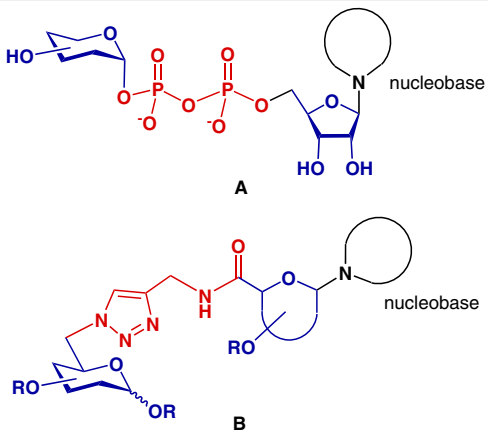


Figure 2 (Triazolyl)methyl amide-linked disaccharide nucleosides (**B**) as potential mimetics of nucleoside diphosphate sugars (**A**)

The ability of the triazole motif to act as a mimetic for the phosphate group has already been shown in the mimicry of glycosyl phosphate derivatives.[36] Moreover, the negatively charged diphosphate moiety is thus replaced by a less polar and neutral system of similar length and space, rendering the molecule more capable of cell penetration, which is important for the targeting of an intracellular enzyme. As potential mimetics of nucleotide sugars, molecules based on (triazolyl)methyl amide-linked disaccharide nucleosides may act as potential inhibitors of enzymes such as glycosyltransferases, which act on the biosynthesis of glycans and glycoconjugates and are potential drug targets against cancer, inflammation, and infection diseases.[37]

Access to structures based on the envisaged skeleton was based on a convergent methodology (Scheme 5). A nucleoside containing the *N*-propargyl moiety was synthesized starting from **17**, that underwent acid-mediated hydrolysis and further acetylation to afford the peracetylated *N*-propargyl glucuronamide **27** as the glycosyl donor. The latter was converted into N^9- and N^7-linked purinyl nucleosides **28** and **29**, which formed in a 1:1 ratio, by reaction with silylated 2-acetamido-6-chloropurine in the presence of trimethylsily trifluoromethanesulfonate (TMSOTf). The regiochemistry of the nucleosidic linkage in **28**–**29** was unambiguously assigned based on NMR data; particularly through HMBC experiments that, in the case of the N^9 nucleoside **28**, showed a correlation between the anomeric proton (H-1') and C-4 of the purine. Diagnostic features of the ^1H spectra of **28** and **29** are the significant chemical shift differences of H-1 and H-8 that are deshielded in the N^7 isomer. The N^7-nucleoside was then coupled with the previously synthesized 6-azido glucoside **23** under the aforementioned Cu(I)-catalyzed cycloaddition conditions to furnish **30** in moderate yield.

The 6′-isonucleosides **2**, **3**, **7**, and **26**, glycosyl sulfonohydrazides **9** and **11**, sulfonohydrazidyl glucuronamide derivatives **16** and **20**, (triazolyl)methyl amide-linked disaccharides **24** and **25**, and related nucleoside derivative **30** were subjected to enzyme inhibition and to cytotoxicity studies.

Cholinesterase assays were performed with acetylcholinesterase (AChE) from *Electrophorus electricus* and butyrylcholinesterase (BChE) from equine serum. Galanthamine hydrobromide, a clinically used ChE inhibitor, was used as standard. The significant results are presented in Table 1.

Among the purine/guanine isonucleosides, only the phenylthio mannopyranosid-6-yl guanine **7** showed significant effects on ChEs, showing selective and moderate inhibition of AChE (K_i = 18.8 µM). The aminomethyltriazole isonucleoside **26** was the best ChE inhibitor of the series, with a K_i value of 11.9 µM for AChE. With respect to the glycosylsulfonohydrazides, the most active compounds were the *N*-benzyltriazolylmethyl sulfonohydrazidyl glucuronamide **20**, which showed moderate inhibition of AChE (K_i = 31.0 µM) and glucuronolactam sulfonohydrazide **12**, which was the only compound to display significant inhibitory effects on BChE. Selectivity to AChE was also exhibited by the (triazolyl)methyl amide-linked disaccharide **24** with moderate inhibition (K_i = 26.7 µM).

Molecular docking of the most active compound (**26**) into the crystal structure of AChE was performed using GOLD 5.2 software[38] and AChE coordinates from the avail-

able PDB structure 4BDT.[39] Molecular interactions were inspected with the MOE 2012.13 package[40] (Figure 3). Interactions of **26** with amino acid residues located at the enzyme active site as well within the peripheral anionic site were displayed. In the active site, the quaternary amonium moiety is involved in cation-π interactions with His447 of the esteratic subsite and also interacts with Glu202 of the anionic subsite. The triazole ring has π-stacking contacts with the indole system of Trp86, which is a critical amino acid residue for substrate binding located at the anionic subsite.[41] The sugar moiety is positioned towards the peripheral anionic site of the enzyme, with interactions with Asp74 through two hydroxyl groups, one of them also contacting Tyr337 from the anionic subsite.

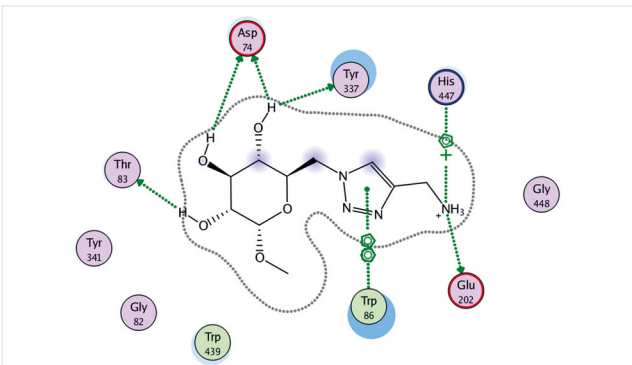


Figure 3 2D Schematic molecular interactions of the GOLD docking pose for compound **26** in AChE. Picture prepared with MOE 2012.13 software.

Evaluation of the abilities of the compounds to inhibit carbonic anhydrase II (CA-II, from bovine erythrocytes), using acetazolamide as standard inhibitor, revealed that only glucuronyl sulfonohydrazide (**11**) was an effective inhibitor, with a K_i value of 9.5 µM (Table 2). The *N*-benzyl glucuronamide counterpart **16** showed only 11% inhibition (at 50 µM) whereas the remaining *N*-glycosylsulfonohydrazides exhibited virtually no inhibitory effect.

Table 1 Significant Results on the Cholinesterase Inhibition Assays

Compound	AChE	BChE
	K_i (µM) (% inhibition)[a] [K_i' (µM)] (type of inhibition)	
Galantamine HBr	0.5 ± 0.0 (competitive)	9.4 ± 0.7 (competitive)
7	18.8 ± 2.0 (competitive)	> 100 (7%)
26	11.9 ± 1.6 (competitive)	> 100 (14%)
12	> 100 (31%)	51.4 ± 5.7 [147.3 ± 8.9] (mixed-type)
20	31.0 ± 9.3 [241.5 ± 42.5] (mixed-type)	> 100 (18%)
24	26.7 ± 2.2 [109.4 ± 17.6] (mixed-type)	> 100 (13%)

[a] % Inhibition at 50 µM

Table 2 Significant Results on the Carbonic Aanhydrase II Inhibition Assays

Compounds	K_i (µM) (% Inhibition)[a] [K_i' (µM)] (type of inhibition)
Acetazolamide	0.1 ± 0.0 (competitive)
11	9.5 ± 0.9 [49.6 ± 1.3] (mixed-type)
16	> 50 (11%)

[a] % Inhibition at 50 µM.

These results suggest that the carboxyl function in **11** is crucial for CA inhibition, since the presence of a sulfonohydrazide moiety in these molecules does not confer, by itself, CA inhibitory ability. In the case of known primary sulfonamide CA inhibitors, their binding to CA is driven by the coordination of the deprotonated sulfonamide function to the enzyme-bound Zn^{2+} ion through the sulfonamide nitrogen.[42] The glycosyl sulfonohydrazides are probably not as prone to coordination to the Zn^{2+} cation through a sulfonohydrazide nitrogen atom via deprotonation, as glycosyl derivatives comprising a primary sulfonamide function are,[18a] and hence the presence of a more acidic functionality will increase their binding affinities.

This assumption is supported by molecular docking studies of the binding mode of *N*-glucuronylsulfonohydrazide (**11**) to the active site of CA-II, using available PDB structure 2X7T[43] (Figure 4). The carboxyl function in its deprotonated form coordinates the Zn^{2+} and also interacts through a hydrogen bond with Thr198, a key residue for the catalytic activity of the enzyme, since it interacts with the Zn^{2+}-coordinated water, enhancing its nucleophilicity for further attack to the substrate (CO_2).[16] Most of the known sulfonamide CA-II inhibitors also interact with this residue through a sulfonyl oxygen atom.

The compounds were also screened for their inhibitory action towards recombinant CDK-2/cyclin E. However none of the molecules showed significant inhibition of this complex at concentrations below 100 µM.

All the compounds were tested for their cytotoxicity against breast adenocarcinoma cell line, MCF-7, and a chronic myeloid leukemia cell line, K562 and six of them, namely **2**, **3**, **26**, **11**, **20**, and **24**, selected among each group of molecules synthesized, were further tested on other cancer cell lines: G361 (melanoma), HeLa (cervix carcinoma), HCT116 (colon carcinoma), CEM (T-cell leukemia), and THP-1 (monocytic myeloid leukemia), and on nonmalig-

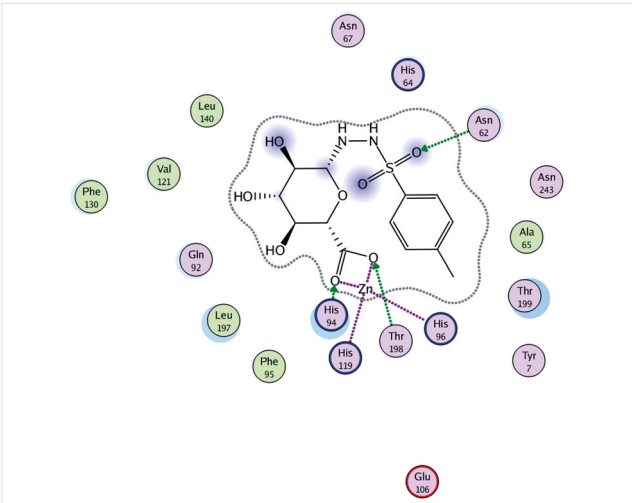


Figure 4 2D schematic of molecular interactions of the GOLD docking pose for **11** in the CA-II active site. Picture prepared with MOE 2012.13 software.

nant BJ fibroblasts and murine embryonic fibroblasts NIH 3T3. No cytotoxicity effect of the compounds was detected at concentrations below 100 µM on MCF7 and K562 cell lines, whereas additional tests on other cell lines still did not reveal significant antiproliferative activity on tumor cells or cytotoxicity to healthy cells, at concentrations below 50 µM (compound **20**) or at below 200 µM (compounds **2**, **3**, **26**, **11**, and **24**).

In summary, in this contribution, novel molecules constructed on carbohydrate platforms; namely 6'-isonucleosides, *N*-glycosylsulfonohydrazides derived from glucuronic acid, and a (triazolyl)methyl amide-linked disaccharide nucleoside, were synthesized as analogues and potential mimetics of nucleosides, glycosyl phosphates, and nucleotides. Sulfonohydrazide and (triazolyl)methyl amide systems were proposed as novel bioisostere moieties for phosphate or diphosphate functionalities contained in nucleotides. A new structural skeleton for potential mimetics of nucleoside diphosphate sugars was proposed and a synthesis of a molecule based on such a framework was accomplished. These results are expected to motivate the synthesis of structurally related compounds that could provide potential new glycosyltransferase inhibitors.

The potential bioactivity of the synthesized compounds was subsequently demonstrated by the moderate activities to AChE exhibited by some isonucleosides and triazole glycoderivatives, with the aminomethyl triazole isonucleoside **26** being the best acetylcholinesterase inhibitor (K_i = 11.9 µM). Moreover, *N*-glucuronylsulfonohydrazide **7**, synthesized in one step from D-glucuronic acid, displayed good inhibition of carbonic anhydrase II (K_i = 9.5 µM).

The ability of the catalytic sites of such enzymes to bind nucleoside analogues or molecules possessing functional groups/motifs mimicking the structural fragments of nucleotides was therefore shown. However, the compounds did not exhibit significant CDK-2 inhibitory effects and cytotoxicity to tumor cells. Considering the structures of the known CDK-2 inhibitors, mostly based on flattened and heteroaromatic systems, the high conformational freedom of the synthesized molecules, arising particularly from the carbohydrate unit, may be a reason for the lack of activity. Hence, more constrained structures, obtained through modifications at the sugar ring, may contribute to effective binding as well as to target selectivity. Further investigations on this topic will address these aspects.

The lack of toxicity of the tested molecules to healthy cells will prompt future structural optimization and synthesis of analogues to attain improved bioactivities towards the therapeutic targets on which efficacy was detected.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1560591.

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(25) **Synthesis of 2-Acetamide-6-chloro-9-(methyl 2,3,4-*O*-acetyl-6-deoxy-α-D-glucopyranosid-6-yl)purine (2) through Mitsunobu Reaction:** To a solution of methyl 2,3,4-*O*-acetyl-α-D-glucopyranoside (**1**; 100 mg, 0.31 mmol) in THF (5 mL) under nitrogen, PPh_3 (163 mg, 0.62 mmol), diethyl azodicarboxylate (DEAD; 0.62 mmol, 0.1 mL) and 2-acetamido-6-chloropurine (132 mg, 0.62 mmol) were sequentially added. The mixture was stirred at r.r. under nitrogen for 16 h. The solvent was evaporated and the residue was subjected to column chromatography on silica gel (EtOAc–petroleum ether, 1:1 to 1:9) to afford **2** (128 mg, 80%) as a white solid. $[\alpha]_D^{20}$ +15 (*c* = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (s, 1 H, NH), 8.13 (s, 1 H, H-8), 5.46 (t, *J* = 9.4 Hz, 1 H, H-3'), 4.96 (d, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1'), 4.84–4.72 (m, $J_{2',3'}$ = 10.2 Hz, 2 H, H-2', H-4'), 4.45–4.29 (m, 2 H, H-6'a, H-6'b), 4.11 (ddd, 1 H, H-5'), 3.17 (s, 3 H, OCH_3), 2.51 (s, 3 H, CH_3, NHAc), 2.12, 2.06, 1.99 (3 × s, 9 H, CH_3, OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 170.1, 170.1 (*C*O, NHAc, *C*O, Ac), 152.8 (C-4), 152.2, 151.5 (C-2, C-6), 145.5 (C-8), 127.6 (C-5), 96.9 (C-1'), 70.6 (C-2'), 69.7 (C-3'), 69.6 (C-4'), 67.5 (C-5'), 55.8 (OCH_3), 43.9 (C-6'), 25.3 (*C*H_3, NHAc), 20.9, 20.8, 20.7 (3 × *C*H_3, OAc). HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{20}H_{24}ClN_5O_9$: 536.1155; found: 536.1152.
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(32) **Synthesis of *N*-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl 1-Deoxy-1-(2-tosylhydrazin-1-yl)-β-D-glucopyranuronamide (20) through N-Glycosylation:** To a solution of *N*-(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl-α,β-D-glucopyranuronamide (**19**; 250 mg. 0.69 mmol) in DMF (1.5 mL), *p*-toluenesulfonyl hydrazide (145 mg, 0.78 mmol, 1.1 equiv) and glacial acetic acid (4 μL, 0.07 mmol, 0.1 equiv) was added. The reaction mixture was allowed to stand at 40 °C without stirring for 48 h, then the solvent was evaporated under vacuum. Diethyl ether (20 mL) was added to the residue and the mixture was vigorously stirred for 24 h. The mixture was filtered and the white solid was washed with diethyl ether, dichloromethane, and cold methanol to give pure **20** (165 mg, 45%) as a white solid; mp 154–155.7 °C; $[\alpha]_D^{20}$ –2 (*c* = 0.4, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.86 (s, 1 H, H-9), 7.77 (d, *J* = 8.1 Hz, 2 H, H_a, Ts), 7.43–7.27 (m, 7 H, H_b, Ts, Ph), 5.56 (s, 2 H, CH_2, Bn), 4.50, 4.45 (2 × d, *J* = 15.7 Hz, AB system, CH_2-7), 3.85 (d, $J_{1,2}$ = 7.9 Hz, 1 H, H-1), 3.65 (d, $J_{4,5}$ = 8.5 Hz, 1 H, H-5), 3.49–3.36 (m, 2 H, H-2, H-3, H-4), 2.43 (s, 3 H, Me, Ts). ^{13}C NMR (100 MHz, CD_3OD): δ = 172.3 (*C*O), 145.2 (Cq, Ts), 137.3 (2 × Cq, Ts, Ph), 130.7, 130.0, 129.6, 129.2, 129.0 (*C*H, Ts, Ph), 91.8 (C-1), 77.6 (C-3), 76.9 (C-5), 73.5, 71.0 (C-2, C-4), 55.0 (*C*H_2, Bn), 35.2 (C-7), 21.5 (*C*H_3, Ts). HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{23}H_{28}N_6O_7S$: 533.1813; found: 533.1827.
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(34) **Synthesis of *N*-[1-(Methyl 2,3,4-*O*-acetyl-6-deoxy-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl-1,2-*O*-isopropylidene-α-D-glucofuranuronamide (34) through CuI/Amberlyst A21-Catalyzed Cycloaddition:** To a solution of *N*-propargyl 1,2-*O*-isopropylidene-α-D-glucofuranuronamide (**17**; 180 mg, mg, 0.66 mmol) in dichloromethane (7 mL),

methyl 2,3,4-*O*-acetyl-6-azido-6-deoxy-α-D-glucopyranoside (**23**; 0.66 mmol, 229 mg) and CuI/Amberlyste A21 (126 mg) were added. The suspension was stirred overnight at r.t., then the catalyst was filtered off and the solvent was evaporated. The residue was subjected to column chromatography on silica gel (ethyl acetate to ethyl acetate–methanol, 9.5:0.5) to give triazole-linked disaccharide **24** (304 mg, 74%) as a white solid.

Data for 24: mp 211–213 °C; $[\alpha]_D^{20}$ = +25 (*c* = 0.3, CH_3OH). ^1H NMR (400 MHz, MeOD): δ = 7.92 (s, 1 H, H-9), 5.93 (d, $J_{1',2'}$ = 3.4 Hz, 1 H, H-1), 5.40 (t, $J_{2',3'} = J_{3',4'}$ = 9.7 Hz, 1 H, H-3'), 5.01–4.80 (m, $J_{1',2'}$ = 3.7 Hz, $J_{2',3'}$ = 9.7 Hz, 3 H, H-1', H-2', H-4'), 4.62 (dd, part A of ABX system, $J_{5',6'a}$ = 2.0 Hz, $J_{6'a,6'b}$ = 14.2 Hz, 1 H, H-6'a), 4.57–4.46 (m, 4 H, H-2, H-6'b, H-7a, H-7b), 4.38 (d, $J_{4,5}$ = 6.3 Hz, 1 H, H-5), 4.25 (dd, $J_{3,4}$ = 2.1 Hz, 1 H, H-4), 4.22–4.12 (m, 1 H, H-3, H-5'), 3.14 (s, 3 H, Me), 2.07, 2.02, 1.98 (3 × s, 3 × 3 H, 3 × CH_3, Ac), 1.46 (s, 3 H, CH_3, *i*-Pr), 1.32 (s, 3 H, CH_3, *i*-Pr). ^{13}C NMR (100 MHz, MeOD): δ = 171.7, 171.5, 171.4 (3 × CO, Ac), 146.9 (C-8), 125.4 (C-9), 113.0 (Cq, *i*-Pr), 106.4 (C-1), 97.9 (C-1'), 86.5 (C-2), 82.4 (C-4), 75.8 (C-3), 71.9 (C-3'), 71.4, 71.3 (C-2', C-4'), 71.0 (C-5), 69.0 (C-5'), 55.9 (CH_3, OMe), 51.7 (C-6'), 35.7 (C-7), 27.1, 26.4 (2 × CH_3, *i*-Pr), 20.6, 20.6, 20.4 (3 × CH_3, Ac). HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{25}H_{36}N_4O_{14}$: 617.2301; found: 617.2304..

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