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Synthesis and Evaluation of the First Generation of Glycosylated Nucleoside Analogues as Potential Inhibitors of the Base J Metabolism in Kinetoplastid Parasites.

Océane Monfret^[a], Dan Liu^[a], Manon Louis^[a], Sihem Rebei^[a], Dominique Urban^[a], Yann Bourdreux^[a], Elisabeth Mouray^[b], Philippe Grellier^[b], Gilles Doisneau^{*[a]}, Dominique Guianvarc'h^{*[a]}

[a] Dr. O. Monfret, Dr. D. Liu, Dr. M. Louis, S. Rebei, Dr. D. Urban, Dr. Y. Bourdreux, Dr. G. Doisneau, Prof. Dr. D. Guianvarc'h
Université Paris-Saclay, CNRS
Institut de Chimie Moléculaire et des Matériaux d'Orsay, UMR CNRS 8182, 91405, Orsay, France

[b] Dr. E. Mouray, Prof. Dr. P. Grellier
MCAM, UMR 7245 CNRS, Muséum National d'Histoire Naturelle, CNRS, CP52,
57 rue Cuvier, Paris 75005, France

E-mail: gilles.doisneau@universite-paris-saclay.fr, dominique.guianvarch@universite-paris-saclay.fr

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Abstract: Leishmaniasis and trypanosomiasis are severe parasitic infections with significant impacts on veterinary and public health, particularly affecting the poorest populations. The therapeutic arsenal against these neglected tropical diseases is limited, and the emergence of resistance to available medicines complicates treatment. These kinetoplastid parasites share a unique DNA modification, *i.e.* β -D-glucosyl-5-hydroxymethyluracil (base J), which appears to play an essential role in parasite development and survival through an original epigenetic mechanism, allowing adaptation to environmental changes. To date, the regulatory pathway of this unique nucleobase has not been explored as a potential therapeutic target. In this work, we report the synthesis of several families of base J analogues and assess their potential against different kinetoplastid species. Some compounds have shown antiparasitic activity and no toxicity toward human primary fibroblasts, revealing promising new strategies for antiparasitic therapy.

Introduction

Leishmaniasis and trypanosomiasis are severe vector-borne parasitic diseases caused by Kinetoplastidae parasites—*Leishmania* transmitted by phlebotomine sandflies, *Trypanosoma brucei* by tsetse flies in sub-Saharan Africa, and *Trypanosoma cruzi* by triatomine bugs in Latin America—that can be lethal and have major veterinary and public health impacts, particularly among the poorest populations, with an estimated 700,000 to 1 million new cases annually.^[1] To date, the therapeutic arsenal against these neglected tropical diseases remains limited, as available drugs are often expensive, require prolonged treatment durations, and exhibit considerable toxicity and severe side effects, while the emergence of clinical resistance further compromises treatment efficacy.^{[2],[3],[4]} In addition, in the context of global climate changes, leishmaniasis is considered at risk of emerging in temperate countries.^{[5],[6],[7]} This situation requires an urgent need for the development of new therapeutics against kinetoplastid parasites that are less toxic, affordable and effective. Identifying new therapeutic targets based on original mechanisms of action constitutes an important issue.^[8]

During their life cycle, kinetoplastids must adapt to important environmental changes they encounter

during their transfer from one host to another. This adaptation requires the development of highly efficient gene regulation mechanisms to reprogram parasite gene expression, ensuring survival and growth within the new host. Although still in its infancy, numerous publications in recent years have highlighted the importance of epigenetic regulation in these parasites.^{[9],[10],[11]} Molecular characterization of these epigenetic modifications and their underlying mechanisms could open new therapeutic perspectives for the development of next-generation antiparasitic drugs.^[12] For example, histone deacetylase inhibitors are currently under investigation for various parasite infections, since some histone deacetylases play crucial roles in modulating parasite gene expression, ensuring survival under various conditions.^{[13],[14],[15]} Consequently, targeting parasite-specific epigenetic marks represents a promising strategy for identifying novel therapeutic targets to control these infections. The glycosylated nucleobase β -D-glucosyl-5-hydroxymethyluracil (glc-5hmU), also known as Base J, is a rare DNA modification first identified in 1993 in *Trypanosoma brucei* and conserved among kinetoplastids such as *T. cruzi* and *Leishmania* (Figure 1A).^{[16],[17]}

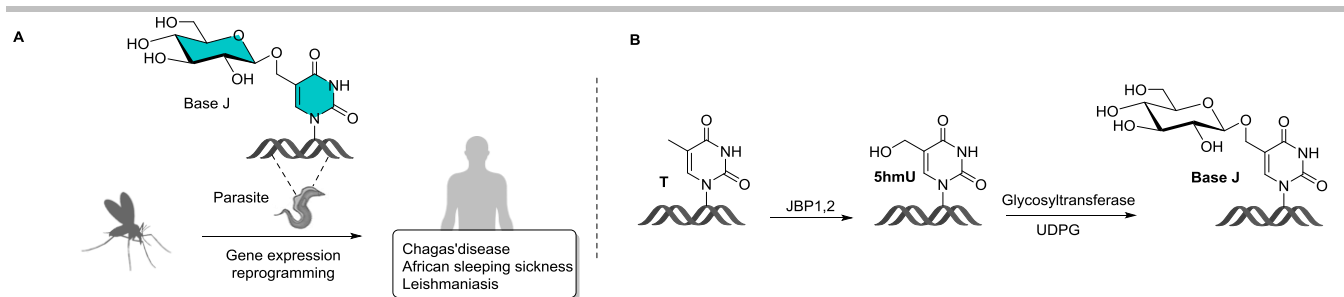


Figure 1. A. Structure of Base J found in Kinetoplastidae. B. Base J biosynthesis

Base J replaces 0.5 to 1% of thymines and is mainly found in the telomeric and sub-telomeric regions of kinetoplastid DNA. Its biosynthesis occurs in two steps.^{[18],[19]} First, a specific thymidine in DNA is oxidized into 5-hydroxymethyldeoxyuridine (5hmdU) by JBP1 and JBP2 (J binding proteins), which are Fe(II) dioxygenases dependent on 2-oxoglutarate. In the second step, 5hmdU is glycosylated by a glycosyltransferase (JGT) using uridine diphosphate glucose (UDPG) as the sugar donor (Figure 1B). In *T. brucei*, Base J is correlated with the regulation of variant surface glycoproteins (VSG) expression, enabling the parasite to escape from the host immune system through antigenic variation. Deletion of both JBP1 and JBP2 results in cells lacking Base J, without being lethal to *T. brucei* in culture. In *Leishmania*, Base J is mainly found in telomeric regions, is enriched at sites involved in RNA polymerase II initiation and termination, and should be involved in the efficient termination of transcription by Pol2.^{[20],[21]} The Base J metabolic pathway appears to be crucial for parasite survival, as JBP1 knockout is lethal for *Leishmania*.^[22]

Besides these studies on the biological role of Base J, no strategy has yet been developed to target this unique regulatory pathway with chemical compounds. In this study, our objective was to develop a first generation of Base J analogues capable of potentially interfering with its biological functions. We envisioned that nucleoside analogues derived from 5-hydroxymethyl-2'-deoxyuridine, diversely functionalized at the C5 position of the nucleobase - notably with glycoside moieties - could interfere with enzymes directly involved in Base J biosynthesis or with J-binding proteins responsible for recognizing this DNA modification.

Here, we report the synthesis of a collection of glycosylated or pseudo-glycosylated nucleoside analogues designed to mimic Base J. First, we developed a series of direct Base J analogues, consisting of variously protected or unprotected 5-hydroxymethyl-2'-deoxyuridines, in which the hydroxyl group of the allylic alcohol was glycosylated. The second series includes various triazole-modified thymidines obtained from 5-azidomethyl-2'-deoxyuridine and a variety of aryl alkynes or propargyl-O-pyranosides through the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC), allowing easy access to molecular diversity. The third series was also synthesized using CuAAC, but with the positions of the

alkyne and azide groups reversed. The design combined synthetic feasibility with a systematic variation of protecting groups to evaluate their influence on antiparasitic activity. Compounds were evaluated for their activities against the promastigote forms of *Leishmania tarentolae* (*Lt*) and *Leishmania infantum* (*Li*), and on the bloodstream form of *Trypanosoma brucei brucei* (*Tbb*). Additionally, their toxicity against human primary fibroblast cells was evaluated. Structure-activity relationships for antiparasitic activity are discussed and several promising compounds have been identified.

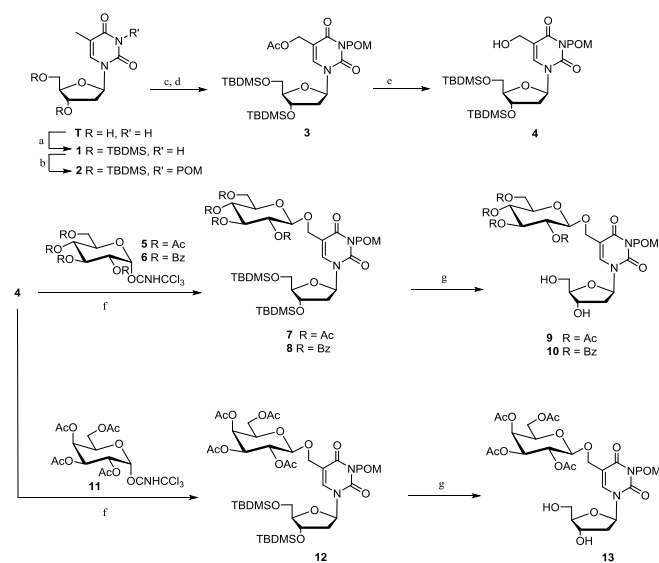
Results

Since targeting pathways involving Base J has never been explored, we adopted two complementary approaches. First, we designed direct analogues of Base J, *i.e.* glycosylated 5-hydroxymethyl-2'-deoxyuridine (5hmdU). In this series, we evaluated the effect of various protecting groups on the glucose moiety, the deoxyribose, and the N³ position of the nucleobase. Next, we envisioned generating a small collection of arylated or pseudo-glycosylated nucleoside analogues by developing convergent synthetic approaches from a common nucleoside precursor - either 5-azidomethyl-deoxyuridine or 5-alkynyloxymethyl-deoxyuridine - through CuAAC reaction. This strategy led to analogues, which are more resistant to enzymatic or acidic cleavage of the anomeric bond.

Synthesis of Base J and glycosyl analogues 7-13.

O-acetylated and O-benzoylated derivatives of Base J, **9** and **10**, were synthesized through the glycosylation of fully protected 5-hydroxymethyl-2'-deoxyuridine (5hmdU) **4** as acceptor, with trichloroacetimidates **5** and **6** according to a procedure previously reported by van Boom for the synthesis of Base J-containing oligonucleotides.^{[23],[24]} The synthesis of the protected 5hmdU **4** is described in Scheme 1 and begins with thymidine (T). Compound **1** (3',5'-di-O-TBDMS-thymidine) was obtained in quantitative yield by classical silylation of thymidine with TBDMSCI/imidazole in DMF.^[25] Then, the N³ position of the nucleobase was protected using chloromethyl pivalate (POMCl), yielding compound **2** in excellent yield (98%). Protecting the N³ position significantly improved the yields of subsequent bromination and

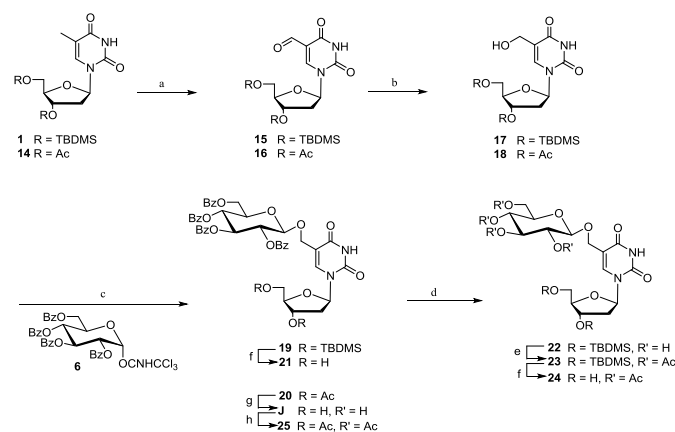
glycosylation steps, as previously reported.^[24] The key glycosyl acceptor **4** was synthesized through a three-step process. First, compound **2** was subjected to a radical bromination using azobisisobutyronitrile (AIBN) and *N*-bromosuccinimide (NBS) in benzene. The crude allylic bromide intermediate was directly engaged in the next step with cesium acetate in DMF, affording the allylic acetate compound **3** (55% for the two steps). Next, deprotection of the acetate group was performed with K_2CO_3 in methanol to produce the target compound **4** (50%). The *O*-acetylated and *O*-benzoylated α -D-glucopyranosyl trichloroacetimidates **5** and **6** were prepared according to established procedures.^[26] Compounds **7** and **8** were obtained by glycosylation of acceptor **4** using the corresponding α -trichloroacetimidate **5** or **6** in the presence of trimethylsilyl triflate (TMSOTf) as a promoter in yields of 39% and 91% respectively. The removal of silyl protecting groups was performed using $Et_3N \cdot HF$ in pyridine to give **9** and **10** (60% and 68% respectively). A Base J analogue **12** featuring a β -galactopyranosyl moiety was obtained following the same procedure by glycosylation of **4** using the corresponding α -trichloroacetimidate donor **11** and was obtained in a yield of 38%. The removal of silyl protecting group was performed using $Et_3N \cdot HF$ in pyridine to give **13** (75%) (Scheme 1).



Scheme 1. Reagents and conditions: (a) TBDMSCl (2.2 equiv.), imidazole (4.0 equiv.), DMF, rt, 16 h, quantitative; (b) POMCl (2 equiv.), K_2CO_3 (2.3 equiv.), DMF, rt, 16 h, 98%; (c) AIBN (0.15 equiv.), NBS (1.3 equiv.), benzene, 95°C, 45 min; (d) CsOAc (1.5 equiv.), DMF, rt, 1 h, 55% over 2 steps; (e) K_2CO_3 (0.1 equiv.), MeOH, rt, 16 h, 50%; (f) **5**, **6**, **11** (1.1 equiv.), TMSOTf (0.15 equiv.), molecular sieves powder 4Å, DCE, 0 °C to rt, 1 h, 38% (**7**), 90% (**8**), 38% (**12**); (g) $Et_3N \cdot HF$ (2.1 equiv.), pyridine, rt, 4-48 h, 60% (**9**), 68% (**10**), 75% (**13**)

Protected intermediates of Base J were synthesized without the POM protecting group following an alternative procedure to obtain the corresponding 3',5'-di-*O*-protected 5hmdU (Scheme 2). Since the bromination led to low yields when the N^3 is

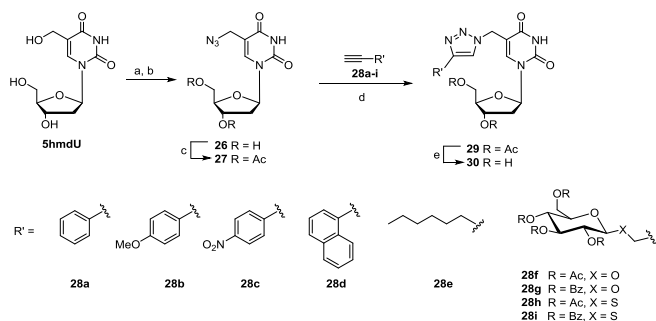
unprotected, we developed an alternative pathway that relies on the methyl oxidation of thymidine, followed by the reduction of the resulting aldehyde.^[27] The 3',5'-di-*O*-TBDMS-thymidine **1** and 3',5'-di-*O*-acetylthymidine **14**^[28,29] were oxidized with potassium persulfate in the presence of $CuSO_4$ and 2,6-lutidine in acetonitrile and water to give the corresponding protected 5-formyl-2'-deoxyuridine **15** and **16**. Then, aldehydes **15** and **16** were reduced with Luche's reagent, $CeCl_3/NaBH_4$ in MeOH, affording the allylic alcohols **17** and **18** with yields of 68% and 58% over two steps, respectively. Compounds **19** and **20** were obtained by glycosylation reactions of acceptors **17** and **18** with the α -trichloroacetimidate **6** in the presence of TMSOTf as a promoter (63% and 73%, respectively). The glycosylated compounds **19** and **20** were then subjected to deprotection/protection steps to obtain various compounds protected on either the deoxyribose or the glucose moiety. Removal of the silyl protecting group from **19** was performed using $Et_3N \cdot HF$ in pyridine to give **21** (71%). Treatment of **19** with K_2CO_3 in methanol gave compound **22** (95%). Acetylation of **22** was performed in the presence of acetic anhydride in pyridine, affording **23** in a quantitative yield. Finally, the silyl protecting group of **23** was removed using $Et_3N \cdot HF$ in pyridine to give **24** (91%). Base **J** was obtained from compound **20** by treatment with *t*-BuOK in methanol, and was isolated in 58% yield. An acetylated version of Base **J** was obtained after treatment with acetic anhydride in pyridine furnishing compound **25** in 95% yield.



Scheme 2. Reagents and conditions: (a) $K_2S_2O_8$ (2.0 equiv.), $CuSO_4 \cdot 5H_2O$ (0.4 equiv.), 2,6-lutidine (3.6 equiv.), ACN/H_2O (1/1, v/v), 65 °C, 2 h, 47% (**15**) or 16 h, 91% (**16**); (b) $CeCl_3 \cdot 7H_2O$ (3.0 equiv.), $NaBH_4$ (1.2 equiv.), MeOH, 0 °C \rightarrow rt, 35 min, 68% (**17**) or 58% (**18**); (c) **6** (1.1 equiv.), TMSOTf (0.15 equiv.), molecular sieves powder 4Å, DCE, 0 °C to rt, 1 h, 63% (**19**) or 73% (**20**); (d) K_2CO_3 (1.1 equiv.), MeOH, rt, 16 h, quantitative (**22**); (e) Ac_2O (6.0 equiv.), pyridine, rt, 16 h, 91% (**24**), 71% (**21**); (f) $Et_3N \cdot HF$ (2.1 equiv.), pyridine, rt, 16 h, 91% (**24**), 71% (**21**); (g) *t*BuOK (5.6 equiv.), MeOH, rt, 16 h, 58% (**J**); (h) Ac_2O (6.0 equiv.), pyridine, rt, 16 h, 95% (**25**).

Synthesis of triazole-modified thymidines from azide-modified thymidine. Due to the inherent lability of the glycosidic bond, we synthesized triazole derivatives (Scheme 3). In the first strategy, our

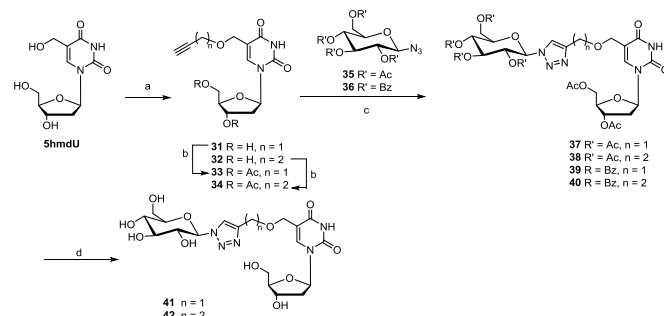
attention turned in the CuAAC involving azide-modified thymidine. Compound **26** was synthesized from 5-hydroxymethyl-2'-deoxyuridine (5hmdU) in two steps: i) chlorination by trimethylchlorosilane (TMSCl) in 1,4-dioxane, and ii) substitution with sodium azide.^{[27],[30]} The first reaction likely proceeds through silylation of the 5-hydroxymethyl group, followed by elimination of OTMS moiety, generating a resonance-stabilized C5 carbocation that is subsequently trapped by chloride to afford the 5-chloromethyl product. Acetylation of **26** was performed with acetic anhydride in pyridine, yielding 5-(azidomethyl)-3',5'-di-O-acetyl-2'-deoxyuridine **27** in quantitative yield.^[31] Azide **27** was then subjected to 1,3-dipolar cycloaddition with various alkyne-containing reagents **28a-i** in a water/DMF mixture, catalyzed by Cu(I) generated *in situ* from copper sulfate and sodium ascorbate. Alkynes **28a-e** are commercially available. Alkynes (**28f-g**) were synthesized according to reported procedures^{[32],[33]} while alkynyl S-glycoside derivatives (**28h-i**) were obtained by reacting the thiuronium salt derived from acetylated glucose with propargyl bromide (see supplementary information).^[34] The reaction furnished 5-alkyl, 5-aryl, 5-glycosyl-triazolmethyl derivatives of 2'-deoxyuridine **29a-i**. Finally, the compounds **29a**, **29f**, **29h** were treated with K₂CO₃ in methanol to afford the deprotected products **30a**, **30f**, **30h**.



Scheme 3. Reagents and conditions: (a) TMSCl (5.0 equiv.), dioxane, 3.5 h, 60 °C; (b) NaN₃ (12.0 equiv.), DMF, 1 h, 60 °C, 43%; (c) Ac₂O (6.0 equiv.), pyridine, rt, 4 h, quantitative; (d) R¹-alkyne **28a-i** (1.0 equiv.), CuSO₄ (0.5 equiv.), sodium ascorbate (1.0 equiv.), DMF, H₂O, rt, 3.5 h; (e) K₂CO₃ (0.1 equiv.), MeOH, rt, 72 h.

Synthesis of triazole-modified thymidines from alkyne-modified thymidine. According to a reverse strategy, CuAAC was considered between an alkyne-modified thymidine and glycosylazide (Scheme 4). Thus, 5hmdU was reacted with propargyl alcohol and 3-butyne-1-ol in the presence of Amberlyst 15 to furnish the alkyne-functionalized nucleosides **31** and **32** in good yields (72% and 89%, respectively). Subsequent treatment with acetic anhydride in pyridine afforded the alkyne-linked 3',5'-di-O-acetyl-2'-deoxyuridine **33** and **34** in 89% and 67% yields, respectively. Alkynes **33** and **34** were then subjected to 1,3-dipolar cycloaddition with β-D-glucosyl azides **35** and **36**, synthesized *via* the reaction of acetobromoglucose with sodium azide in dichloromethane followed by ester protecting group manipulation.^{[35],[36]} Cycloaddition

reactions in water and DMF under the catalysis of Cu(I) generated *in situ* from copper sulfate and sodium ascorbate, afforded 5-glycosyl-triazolmethyl derivatives of 2'-deoxyuridine **37-40** in good yields. Finally, compounds **37** and **38** were deprotected in the presence of K₂CO₃ in methanol to obtain compounds **41** and **42**.



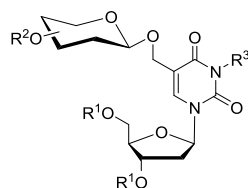
Scheme 4. Reagents and conditions: (a) Propargyl alcohol (25.0 equiv.) or 3-butyne-1-ol (25.0 equiv.), Amberlyst 15 (1g/mmol), rt, 24 h, 72% (**31**), 89% (**32**); (b) Ac₂O (7.0 equiv. or 50.0 equiv.), pyridine, rt, 48 h, 89% (**33**), 67% (**34**); (c) β-D-Glucosyl azide (1.0 equiv.), CuSO₄ (0.5 equiv.), sodium ascorbate (1.0 equiv.), DMF, H₂O, rt, 3.5 h, 62% (**37**), 76% (**38**), 72% (**39**), 71% (**40**); (d) K₂CO₃ (0.3 equiv.), MeOH, rt, 48 h, 99% (**41**), 97% (**42**).

The synthesized nucleoside analogues were evaluated *in vitro* for their antiparasitic activity against the promastigote forms of *Leishmania tarentolae* (*Lt*) and *Leishmania infantum*, (*Li*) and the bloodstream form of *Trypanosoma brucei brucei* (*Tbb*), the causative agent of African sleeping sickness in cattle. Cytotoxicity assays were conducted using the human primary fibroblast cell line AB943 to assess the potential toxic effects of the compounds on mammalian cells.

Structure-activity relationship of the glycosylated 5-hydroxymethyl-deoxyuridine derivatives. In this series, various intermediates of the synthesis of Base J were tested bearing different protecting groups. Structure-activity relationship information may be concluded from the results summarized in Table 1. Interestingly, most of the compounds are active against *Tbb*. SAR analysis was conducted on *Tbb* growth inhibition and was mainly supported by inhibitory data on *Lt* and *Li*.

First, the O-benzoylation of the glucose hydroxyl groups resulted in similar (**8** vs **7** and **19** vs **23**) or a much better anti-trypanosomal activity than O-acetylation (compare **10** vs **9**, **20** vs **25**, **21** vs **24**). In each case, the deprotection of the glucose moiety resulted in a dramatic decrease in activity up to a factor of about 100 (**22** vs **19**, **J** vs **21**, **22** vs **23**). Secondly, the nature of the sugar has an impact on the activity. Indeed, galactose provided an advantage over glucose (see **12** and **7** with IC₅₀ = 2 and 64.5 μM, respectively).

Table 1. *In vitro* antiparasitic activity and cytotoxicity of the glycosylated 5-hydroxymethyl-2'-deoxyuridine derivatives



Compound	Glycosyl core	R ₁	R ₂	R ₃	IC ₅₀ <i>Tbb</i> (μM)	IC ₅₀ <i>Lt</i> (μM)	IC ₅₀ <i>Li</i> (μM)	IC ₅₀ cell line AB943 (μM)
7	Glc	TBDMS	Ac	POM	64.5	>100	>100	>50
8	Glc	TBDMS	Bz	POM	58.5	84	nd	>100
9	Glc	H	Ac	POM	49	>100	>100	>100
10	Glc	H	Bz	POM	2.5	>100	>100	>100
12	Gal	TBDMS	Ac	POM	2	>100	>100	>100
13	Gal	H	Ac	POM	>50	nd	nd	>50
19	Glc	TBDMS	Bz	H	0.15	15	83	>50
20	Glc	Ac	Bz	H	2.5	57	>100	>50
21	Glc	H	Bz	H	4	50	54	13
22	Glc	TBDMS	H	H	17	68	>100	>50
23	Glc	TBDMS	Ac	H	0.1	4	15	16
25	Glc	Ac	Ac	H	65	71	>100	>50
24	Glc	H	Ac	H	>50	>100	>100	>50
J	Glc	H	H	H	>100	>100	>100	>100

Nd: not determined. All values are expressed as mean of at least two independent experiments.

Pentamidine was used as the positive control IC₅₀ 5 nM.

Regarding the deoxyribose protecting groups effect, the results are different depending on whether the nucleobase is protected or not. When the latter was protected on N³ by the POM group, the activity is better when the deoxyribose is deprotected (compare **9** vs **7** and **10** vs **8**), compound **10** displaying an interesting IC₅₀ of 2.5 μM. On the contrary, when the nucleobase was free, all compounds showed better activity when the deoxyribose alcohols were either silylated (compare **21** vs **19**, **23** vs **24** and **22** vs **J**) or acetylated (compare **25** vs **24** and **20** vs **21**). The silylated compounds showed significantly better activities than their acetylated analogues (compare **19** vs **20** and **23** vs **25**), a result that could be attributed to a better permeability of these compounds. Finally, the effect of the N³ nucleobase protection can be determined by comparing **7** vs **23**, **8** vs **19**, **9** vs **24** and **10** vs **21**, showing an unfavourable effect of the protecting group in the silylated series. Most compounds do not exhibit cytotoxicity except for **21** and **23**.

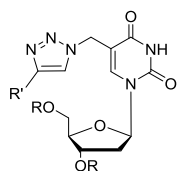
Structure–activity relationship of the 5-aryl-triazolylmethyl-deoxyuridine derivatives. We also tested the activity of aryl triazole-based analogues (Table 2). Among the acetylated compounds, all aryl-functionalized derivatives exhibited activity against *Tbb* (e.g., compounds **29a-d**). The variation of substituents on the phenyl ring revealed that para-substitution with an electron-withdrawing group enhanced activity by twofold (**29a** versus **29c**), while substitution with an electron-donating group reduced activity by half (**29a** versus **29b**). The introduction of a naphthyl moiety

increased activity compared to the phenyl group (**29a** versus **29d**). In contrast, the alkyl functionalized compound **29e** was inactive. The activity of 5-azidomethyl-2'-deoxyuridine **26** and its acetylated derivative **27** was also assessed. Compound **27** displayed activity with an IC₅₀ of 46 μM against *Tbb*, whereas the deprotected compound **26** was inactive. Similarly, the deprotected compound **30a** was inactive on *Tbb*. Importantly, none of the active compounds exhibited cytotoxicity.

Structure–activity relationship of the 5-glycosyl-triazolylmethyl-2'-deoxyuridine derivatives.

Glycosylated analogues were also evaluated either in its fully deprotected form or with protecting groups on the glucose (benzoyl or acetyl) and deoxyribose (acetyl) moieties. The results are summarized in Table 3. Only O-benzoylated compounds **29g** and **29i** showed activity with IC₅₀ values of 6 and 61 μM, respectively, while O-acetylation of the glucose in compounds **29f** and **29h** was detrimental, leading to complete loss of anti-trypanosomal activity. The increased activity of the O-benzoyl-modified glycosyl moiety and acetylated deoxyribose could partly be attributed to their higher lipophilicity, thus potentially enhancing passive membrane permeability. Interestingly, the isosteric sulfur analog **29i** of the compound **29g**, in which the glycosidic oxygen is replaced by a sulfur atom, resulted in a tenfold loss of activity (IC₅₀ = 61 and 6 μM respectively). Once again, none of the active compounds were cytotoxic.

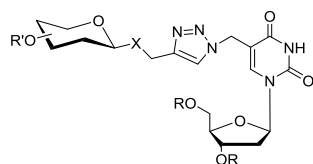
Table 2. *In vitro* antiparasitic activity and cytotoxicity of the 5-aryl-triazolylmethyl-2'-deoxyuridine derivatives



Compound	R	R'	IC ₅₀ <i>Tbb</i> (μM)	IC ₅₀ <i>Lt</i> (μM)	IC ₅₀ <i>Li</i> (μM)	IC ₅₀ cell line AB943 (μM)
27 ^[a]	Ac	-	46,5	>100	nd	>100
26 ^[a]	H	-	>100	>100	nd	>100
29a	Ac		27.5	>100	>100	>100
30a	H		>100	>100	nd	>100
29b	Ac		57	>100	nd	>100
29c	Ac		12	>100	>100	>100
29d	Ac		12	>100	>100	>100
29e	Ac		>100	>100	nd	>100

^[a] 5-azidomethyl-deoxyuridine. Nd : not determined. All values are expressed as mean of at least two independent experiments. Pentamidine was used as the positive control IC₅₀ 5 nM.

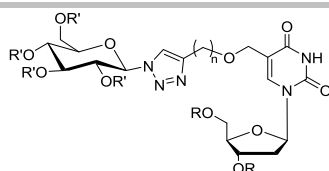
Table 3. *In vitro* antiparasitic activity and cytotoxicity of 5-glycosyl-triazolidomethyl-deoxyuridine derivatives



Compound	R	Glycosyl core	IC ₅₀ <i>Tbb</i> (μM)	IC ₅₀ <i>Lt</i> (μM)	IC ₅₀ <i>Li</i> (μM)	IC ₅₀ cell line AB943 (μM)
29f	Ac		>100	>100	nd	>100
29g	Ac		6	>100	>100	>100
30g	H		>100	>100	nd	>100
29h	Ac		>100	>100	nd	>100
29i	Ac		61	>100	nd	>100
30h	H		>100	>100	>100	>50

Nd : not determined. All values are expressed as mean of at least two independent experiments. Pentamidine was used as the positive control IC₅₀ 5 nM.

Table 4. *In vitro* antiparasitic activity and cytotoxicity of the triazolyl-methoxy and ethoxymethyl-2'-deoxyuridine derivatives.



Compound	R	Glycosyl core	n	IC ₅₀ <i>Tbb</i> (μM)	IC ₅₀ <i>Lt</i> (μM)	IC ₅₀ <i>Li</i> (μM)	IC ₅₀ cell line AB943 (μM)
31 ^[a]	H	-	1	>100	>100	nd	>100
32 ^[a]	H	-	2	>100	>100	>100	>100
33 ^[a]	Ac	-	1	>100	>100	nd	>100
34 ^[a]	Ac	-	2	>100	>100	>100	>100
37	Ac		1	>100	>100	nd	>100
39	Ac		1	34	>100	nd	>100
41	H		1	45	72	>100	>50
38	Ac		2	>100	>100	nd	>100
40	Ac		2	14	>100	>100	>100
42	H		2	68	>100	>100	>50

^[a] Alkyne-functionalized deoxyuridine. Nd: not determined. All values are expressed as mean of at least two independent experiments. Pentamidine was used as the positive control IC₅₀ 5 nM.

Structure–activity relationship of the triazolyl-methoxy and ethoxymethyl-deoxyuridine derivatives.

The antiparasitic activity of compounds in which the glycosidic bond is replaced by a triazole moiety was evaluated and the results are summarized in Table 4. Firstly, compounds **31-34** showed no activity, whereas **39, 40, 41** and **42** exhibited activities against *Tbb*, indicating that the glycosyl-triazolyl moiety plays a significant role in antiparasitic activity. Secondly, the *O*-acetylation of glucose in **37** and **38** resulted in a loss of anti-trypanosomal activity, whereas *O*-benzoylation in **39** and **40** proved beneficial with IC₅₀ values of 34 and 14 μM, respectively. The effect of benzoyl on the glucosyl moiety and acetyl on the deoxyribose one could partly be attributed to their lipophilicity, thus potentially increasing the passive membrane permeability. Third, the fully deprotected compounds **41** and **42** demonstrated activity against *Tbb* with IC₅₀ values of 45 and 68 μM, respectively. In this case, this suggests that cell uptake of these compounds may depend on

active transport mechanisms, possibly mediated by nucleoside or sugar-specific membrane transporters. Finally, the length of the carbon chain between the triazole and the allylic oxygen showed limited influence on activity. Interestingly, none of the active compounds exhibited cytotoxicity, highlighting their potential selectivity for parasite targets.

Discussion

In this study, we identified several glycosylated or pseudo-glycosylated nucleoside analogues that mimic Base J and exhibit anti-trypanosomal and anti-leishmanial activities. The key structure–activity relationship (SAR) insights upon *T. brucei brucei* are summarized in Figure 2.

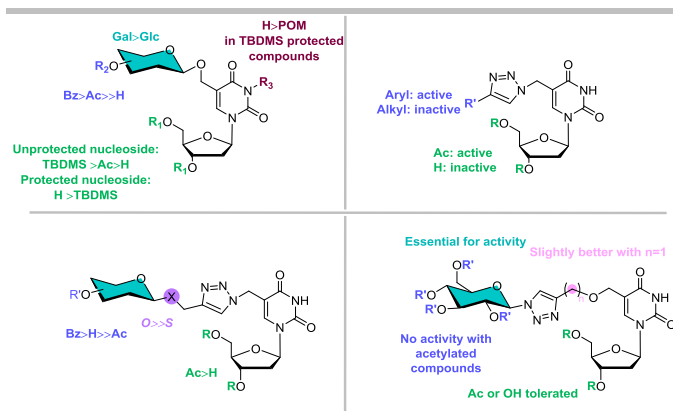


Figure 2. Structure–activity relationship (SAR) insights upon *Trypanosoma brucei brucei* of glycosylated or pseudo-glycosylated nucleoside analogues that mimic base J.

Several notable observations emerged: most fully deprotected nucleosides (**J**, **30a**, **30g**, **30h**) are inactive except for **41** and **42**. This is likely due to the substantial modifications at the 5-position of the pyrimidine ring, which may impair recognition and transport by the parasite's nucleoside transporters.^[37] Similarly, the glucose moiety is probably poorly recognized by hexose transporters.^[38,39] Consequently, the high polarity of these compounds likely hinders their ability to cross the parasite membranes *via* passive diffusion.

When the nucleosides are partially protected—either on the glucose unit or on the 2'-deoxyribose—an antiparasitic activity is often observed. Notably, the presence of a TBDMS (*tert*-butyldimethylsilyl) protecting group appears to significantly enhance activity. This kind of effect has previously been reported in antiviral and anticancer nucleoside analogues, where silyl ether masking groups were found to improve cellular uptake, likely due to increased lipophilicity and thus enhanced membrane permeability.^[40,41] The presence of a silyl group at the 5'-position suggests that these derivatives are unlikely to undergo intracellular phosphorylation and to act as chain terminators. Although the exact mechanism of action remains unclear, it has been proposed that some 5'-silylated nucleoside analogues may mimic the methyl donor SAM (*S*-adenosyl-*L*-methionine) cofactor binding to SAM-binding sites on methyltransferases.^[42]

In contrast, ester-protected analogues may facilitate passive diffusion and could subsequently be hydrolyzed by parasite esterases. Previous studies have demonstrated that *O*-acetylation of nucleoside analogues can dramatically improve biological activity—transforming inactive molecules into potent compounds—likely by enhancing uptake.^[43] We hypothesize that the esters may act as prodrugs, which are hydrolyzed within the cell by parasite enzymes. A similar strategy has been applied to pentamidine analogues, which were designed as esterase-activated prodrugs.^[44]

Finally, the mode of action of these compounds and their target remains to be elucidated. Figure 3 outlines the current working hypotheses. Base J analogues may interfere with base J-binding proteins either after incorporation into DNA or in their free nucleoside form. However, since deletion of proteins involved in base J synthesis (JBP1 and JBP2) does not seem to be lethal to *T. brucei brucei* in culture^[20], in contrast to the JBP1 knockout in *Leishmania*^[22], they may also alternatively affect other pathways, such as nucleoside metabolism (e.g., thymidylate synthase inhibition).

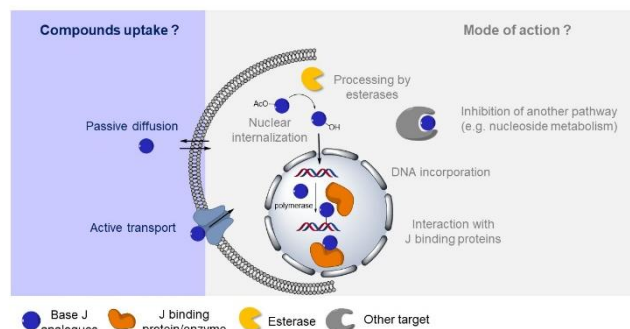


Figure 3. Proposed mechanisms for cellular uptake and the potential intracellular targets of Base J nucleoside analogues.

Conclusion

Several of the synthesized compounds have demonstrated promising antiparasitic activity in the micromolar range without exhibiting cytotoxicity on mammalian cell lines. These results highlight the potential of glycosylated or pseudo-glycosylated nucleosides as candidates for a new class of inhibitors, paving the way for innovative strategies in antiparasitic chemotherapy. This study represents the first attempt to target the Base J biosynthetic pathway, an interesting but underestimated therapeutic target in kinetoplastid parasites. Indeed, no compound targeting this unique DNA modification has been reported until now. This study is a first step in that direction with promising results even if, at this stage, the precise target remains to be defined. Furthermore, the mode of action of these compounds, which may act on distinct pathways, remains undefined. Some compounds may interfere directly with enzymes involved in Base J biosynthesis or recognition, while others could target thymidylate synthase or alternative pathways associated with nucleoside metabolism. Future studies will involve developing enzymatic assays to probe the interaction of these compounds with Base J-related enzymes, potentially clarifying their mechanism of action. In addition, the compounds should be evaluated in *in vitro* enzymatic assays on enzymes involved in the formation of the Base J in parasites, requiring the prior development of adapted assays. This provides a solid basis for exploring Base J as a novel therapeutic target and emphasizes the potential of nucleoside analogues in addressing medical needs

in parasitic diseases. Ongoing studies in our laboratory aim to refine this approach and contribute to the development of next-generation antiparasitic agents.

Finally, the glycosylated and pseudo-glycosylated nucleoside syntheses described here may find broader applications in the field of nucleic acid chemistry. Indeed, oligoglycoconjugates have shown promising potential in diverse areas such as biochip design, cell-surface recognition, and the intracellular delivery of oligonucleotides.^{[45,46],[47],[48],[49]} This work may therefore contribute to the development of increasingly potent sugar-modified nucleic acids for therapeutic, biotechnological, and synthetic biology applications.

Supporting Information

The authors have cited additional references within the Supporting Information.^[50, 59]

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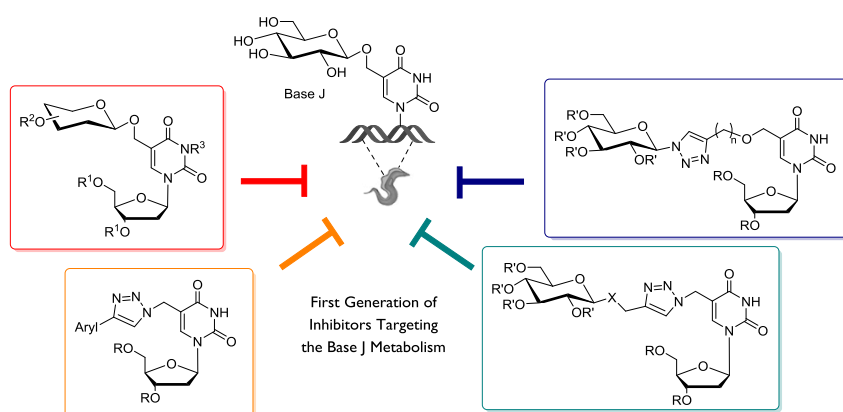
Keywords: glycosylated nucleosides • nucleoside analogues synthesis • Base J • antiparasitic activities • epidrugs

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Table of Contents



Novel analogues of the kinetoplastid-specific DNA base J unlock a new way to target parasite epigenetic regulation. Some compounds selectively inhibit *Leishmania* and *Trypanosoma* species without harming human cells, revealing a promising route toward innovative antiparasitic therapies.