



An Expedient Synthesis of Flexible Nucleosides through Enzymatic Glycosylation of Proximal and Distal Fleximer Bases

Sophie Vichier-Guerre, Therese Ku, Sylvie Pochet, Katherine Seley-Radtke

► To cite this version:

Sophie Vichier-Guerre, Therese Ku, Sylvie Pochet, Katherine Seley-Radtke. An Expedient Synthesis of Flexible Nucleosides through Enzymatic Glycosylation of Proximal and Distal Fleximer Bases. ChemBioChem, 2020, 21 (10), pp.1412-1417. 10.1002/cbic.201900714 . pasteur-03035362

HAL Id: pasteur-03035362

<https://pasteur.hal.science/pasteur-03035362>

Submitted on 11 Dec 2020

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

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



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

A EUROPEAN JOURNAL OF CHEMICAL BIOLOGY

CHEMBIOCHEM

SYNTHETIC BIOLOGY & BIO-NANOTECHNOLOGY

Accepted Article

Title: An expedient synthesis of flexible nucleosides through enzymatic glycosylation of proximal and distal fleximer bases.

Authors: Sophie Vichier-Guerre, Therese Ku, Sylvie Pochet, and Katherine L Seley-Radtke

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *ChemBioChem* 10.1002/cbic.201900714

Link to VoR: <http://dx.doi.org/10.1002/cbic.201900714>

WILEY-VCH

www.chembiochem.org

A Journal of



COMMUNICATION

An expedient synthesis of flexible nucleosides through enzymatic glycosylation of proximal and distal fleximer bases

Sophie Vichier-Guerre,^[a] Therese C. Ku,^[b] Sylvie Pochet,^{*,[a]} Katherine L. Seley-Radtke^{*,[b]}

[a] Dr. S. Vichier-Guerre, Dr. S. Pochet
Unité de Chimie et Biocatalyse
Institut Pasteur, CNRS UMR3523
28, rue du Dr Roux, F-75015 Paris, France
E-mail: sylvie.pochet@pasteur.fr

[b] Dr. T. C. Ku, Prof. K. Seley-Radtke
Department of Chemistry & Biochemistry
University of Maryland, Baltimore County
1000 Hilltop Circle, Baltimore, MD 21250, USA.
E-mail: kseley@umbc.edu title(s), Initial(s), Surname(s) of Author(s) including Corresponding Author(s)

Supporting information for this article is given via a link at the end of the document.

Abstract: The structurally unique “fleximer” nucleosides were originally designed to investigate how flexibility in the nucleobase could potentially affect receptor-ligand recognition and function. Recently they have shown low to sub-micromolar levels of activity against a number of viruses including coronaviruses, filoviruses and flaviviruses. The synthesis of *distal* fleximers in particular, has thus far been quite tedious and low yielding. As a potential solution to this issue, a series of *proximal* fleximer bases (flex-bases) were successfully coupled to both ribose and 2'-deoxyribose sugars using the *N*-deoxyribosyltransferase II of *Lactobacillus leichmannii* (LNDT) and *E. coli* purine nucleoside phosphorylase (PNP). To explore the range of this facile approach, transglycosylation experiments using a thieno-expanded tricyclic heterocyclic base, as well as several distal and proximal flex-bases were performed to determine whether the corresponding fleximer nucleosides could be obtained in this fashion, thus potentially significantly shortening the route to these biologically significant compounds. The results of those studies are reported herein.

The synthesis of nucleoside analogues has been classically achieved through various chemical methodologies and well-known coupling reactions.^[1-3] However, chemical synthesis typically involves difficult and time-consuming multistep processes. Moreover, suitable protection of various functional groups is typically required on the nucleos(t)ide sugars and/or on the heterocyclic bases, and subsequent deprotection steps often result in low overall yields. Another significant problem often encountered is the stereospecific control of configuration at the anomeric center (α versus β).^[2-4]

In contrast, enzymatic syntheses of nucleoside analogues do not typically require protecting groups and are highly stereospecific.^[5] Nucleoside phosphorylases (NPs) and *N*-deoxyribosyltransferases (NDTs) are the predominant classes of enzymes used in the synthesis of nucleosides by mediating the transglycosylation reaction of a nucleoside sugar to a free heterocyclic base.^[6-10]

NPs (EC 2.4.2.1) catalyse the reversible phosphorolysis of ribo- or 2'-deoxyribonucleosides to generate a free nucleobase and ribose- or 2-deoxyribose-1-phosphate in the presence of inorganic ortho-phosphate. Addition of a second nucleobase to the reaction mixture can promote the formation of a new nucleoside with the equilibrium in favor of nucleoside formation.^[11-12] NPs have been reported to accept modified bases as well as unnatural glycosyl donors.^[6-9,13-15]

NDTs (EC 2.4.2.6) catalyse the transfer of 2-deoxyribose between purine and pyrimidine bases.^[16] Two NDT-types have been defined based on their substrate specificity: NDT-I (also named PTD) specific for purines^[17] and NDT-II that accepts either purine or pyrimidine, with a strong preference for 2'-deoxyribosyl-pyrimidine as the donor substrate.^[18] Despite its relatively low acceptance for modified sugar moieties, NDT-II has served to synthesize some nucleoside analogues of biological interest.^[19,20] Interestingly, NDTs tolerate a wide range of modified nucleobases from azole derivatives^[21,22] to expanded-size purines^[23-25] with an increased regioselectivity as compared to PNP (i.e. N9 versus N7 for purines).^[6]

The fleximers were originally designed to investigate how flexibility in the nucleobase could affect receptor-ligand recognition and function.^[26-31] These interesting molecules have demonstrated several advantages over their analogous natural rigid purine nucleosides. For instance, the distal guanosine fleximer (Figure 1) was found to be an inhibitor of S-adenosyl-L-homocysteine hydrolase (SAHase), an adenosine-metabolizing enzyme.^[26]

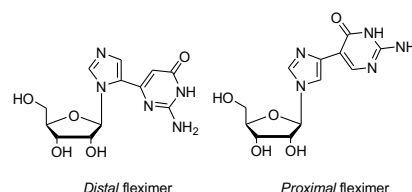


Figure 1. Seley-Radtke's guanosine fleximers.

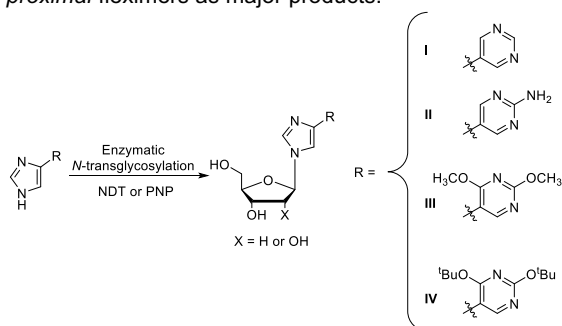
By rotation of the hemiaminal bond to place the heterocyclic moieties into a *syn*-like conformation rather than the thermodynamically favored anti-conformation, the flex-guanosine's base was able to reposition the amino group to mimic the adenosine nucleobase.^[26,32] Furthermore, the flex-guanosine triphosphate (Flex-GTP) was shown to be a superior substrate of human GDP-L-fucose pyrophosphorylase compared to the natural substrate guanosine triphosphate (GTP),^[33] likely due to the fleximer's ability to interact with amino acids in the active site not accessible by the natural substrate.^[34] This also allowed Flex-GTP to retain all activity when essential catalytic residues needed for GTP binding were mutated.^[33,34]

While the biological results for these compounds have been ground-breaking in some cases, their syntheses are nontrivial. To

COMMUNICATION

date, the only approach to realize the adenosine, guanosine and inosine *distal* fleximer nucleosides requires the construction of a tricyclic expanded purine nucleoside, which involves more than 7 steps to realize the base itself, with an additional 5-7 steps after the coupling reaction to convert the tricyclic nucleoside to the fleximer.^[28-29,35-41] Attempts to synthesize the tricyclic bases followed by coupling of the base to a ribose resulted only in the undesired N7 isomer, likely due to the sterics caused by the arched shape.^[29,41]

Previously, a series of imidazole nucleosides were successfully synthesized *via* transglycosylation methodologies.^[42,43] Of those, four products resembled the Seley-Radtke *proximal* fleximers (I-IV, Scheme). The flex-bases were synthesized using microwave-assisted Suzuki-Miyaura cross-coupling of 4(5)-iodimidazole and the boronic acid pyrimidine partners.^[44] Subsequent transglycosylation using *E. coli* PNP for ribonucleosides and *L*INDT for 2'-deoxyribonucleosides produced the corresponding *proximal* fleximers as major products.



Scheme 1. Enzymatic transglycosylation of *proximal* fleximers.^[42,43]

As a few *proximal* fleximer nucleosides have been realized *via* enzymatic transglycosylation, the expectation was that the same methodology could produce the desired *distal* and tricyclic nucleosides from their respective bases. Thus, a series of tricyclic^[41] and flex-bases^[45] (shown in Figure 2) were synthesized as previously reported and their transglycosylation products were studied.

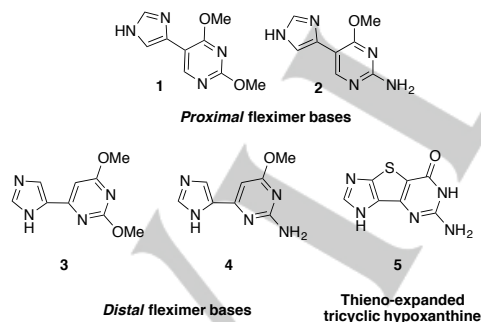


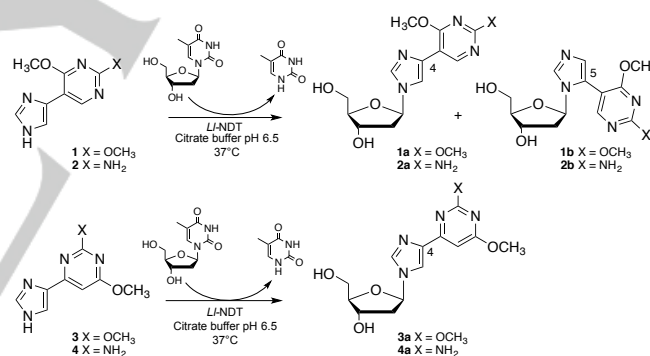
Figure 2. Flex- and tricyclic bases used for transglycosylation.

Enzymatic glycosylation mediated by *L*INDT

First, we tested the capability of NDT from *L*INDT to use these five unnatural nucleobases as substrate. Typically, the flex-base (1 μ mol) and thymidine used as the 2'-deoxyribose donor (4 μ mol) were incubated at 37°C in the presence of *L*INDT (different amounts of enzyme) in a 10 mM citrate buffer (0.1 mL) at the

optimum pH of 6.5. Due to the low water solubility of flex-bases, the transferase reactions were carried out in the presence of 5% v/v DMSO in the media.^[42] The rate of glycosylation (% conversion) was monitored by analytical reverse phase HPLC as a function of time and enzyme concentration (Table 1). The incubation temperature (37°C in place of 50°C) was selected in an attempt to optimize the enzyme activity and further isolate all the possible glycosylated products. Indeed, we previously showed that it is achievable to manage the regioselectivity of the transferase reaction (N1 versus N3) of some 4-substituted imidazole derivatives.^[43] All the glycosylation products were purified by reverse phase HPLC and their chemical structures, particularly the glycosylation site, were confirmed by NMR analysis.

As reported previously,^[42] the NDT-catalyzed transfer reaction between **1** and thymidine led to the simultaneous formation of N1 and N3 glycosylated products (**1a** and **1b**, Scheme 2) in a 1:1 ratio after 3 h at 37°C using NDT at 1.25 μ L/ μ mol acceptor (Table 1, entry 1). Nucleoside **1b** (i.e. C5 arylimidazole derivative) was progressively converted into the thermodynamically more stable N1 isomer **1a** (i.e. C4 arylimidazole nucleoside) (see Supporting Information, Figure S1A). By increasing enzyme concentration (from 1.25 to 10.0 μ L/ μ mol acceptor), the conversion was nearly complete, reaching a plateau at 90%, and nucleoside **1a** was formed as the sole product (Table 1, entries 7 & 8). A catalytic mechanism for *L*INDT-catalyzed glycosylation involving the formation of an oxocarbenium intermediate has recently been proposed.^[46]

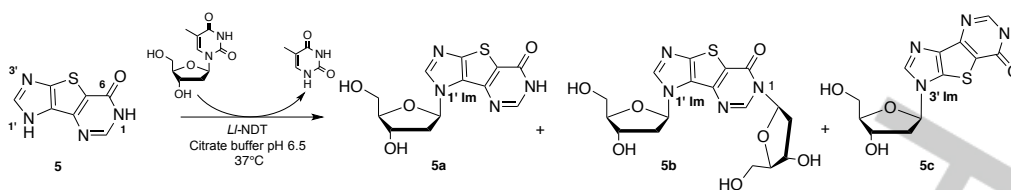


Scheme 2. NDT-catalyzed transglycosylation of flex-bases **1-4**.

A similar trend was observed in the case of flex-base **2** with the formation of both N1 and N3 glycosylated products (**2a** and **2b**, respectively) in a ratio depending on incubation time or enzyme concentration (Figure S1B). Compared to flex-base **1**, flex-base **2** appeared to be a better substrate since lower enzyme concentration was needed to achieve complete conversion (Table 1, entries 11-14). These results confirmed the easy accessibility of *proximal* 2'-deoxy-fleximers using trans-glycosylation reactions.

In the case of the flex-bases **3** and **4**, HPLC monitoring showed that the glycosylation reactions were more effective than in the case of **1** (Figure S2 and Table 1, entries 15-21). Only one isomer, the N-1 glycosylated product **3a** or **4a** was formed, even if the enzyme concentration was reduced 10-fold (entries 19-21). However, the target *distal* 2'-deoxynucleosides (glycosylation at the N3 position of the imidazole ring) were not detected under the conditions tested.

COMMUNICATION

Scheme 3. NDT-catalyzed transglycosylation of flex-base **5**.

Finally, we tested the tricyclic nucleobase **5** (Scheme 3) which contains, in addition to the imidazole ring N1 and N3, a nitrogen on the pyrimidine ring susceptible to be glycosylated, as previously observed in the transglycosylation of 5-(imidazol-4-yl)pyrimidine-2,4-(1*H*,3*H*)-dione.^[42] Heterocycle **5** was rapidly converted into glycosylated products under the conditions used (Table 1, entries 22-25), confirming that hindered nucleobases can serve as acceptors for NDT. HPLC monitoring of the reaction showed the formation of two main products **5a** and **5b**, and a third minor one **5c** (Figure 3).

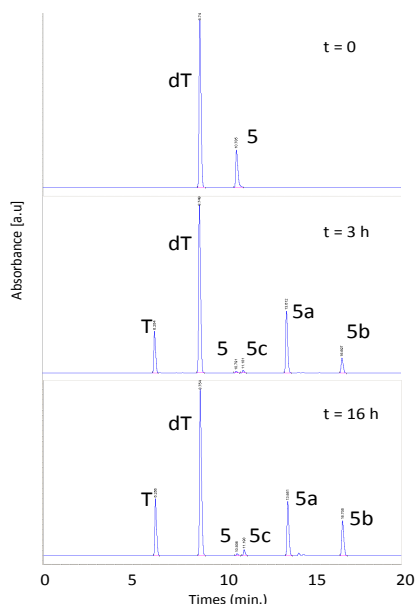


Figure 3. HPLC analysis of the transglycosylation reaction of **5** in the presence of thymidine (4 equiv) and NDT (0.63 $\mu\text{L}/\mu\text{mol}$ acceptor) at $t = 0, 3$ h and 12 h at 37°C . HPLC conditions: 0–20% linear gradient of acetonitrile in 10 mM TEAA buffer pH 6.0 over 20 min at a flow rate of 1 mL min^{-1} . Detection at 254 nm.

Structures of **5a** (glycosylation at imidazole N1, noted N1'-Im in Scheme 3), **5b** (glycosylation at both imidazole N1 and pyrimidine N1, noted N3' Im and N1'), and **5c** (glycosylation at imidazole N3, noted N3' Im) were assigned based on NMR spectra (Figure 4). By reducing incubation time and enzyme concentration, it was possible to control further conversion of **5a** into **5b**, and thus isolate **5a** as the main product (Table 1, entry 24). Interestingly,

this enzymatic procedure is an attractive route to **5a** over the previously published ones,^[29,37] based on the coupling of 4,5-diiodoimidazole to protected 2-deoxysugar chloride, followed by the formation of the tricyclic inosine derivative and sugar deprotection.

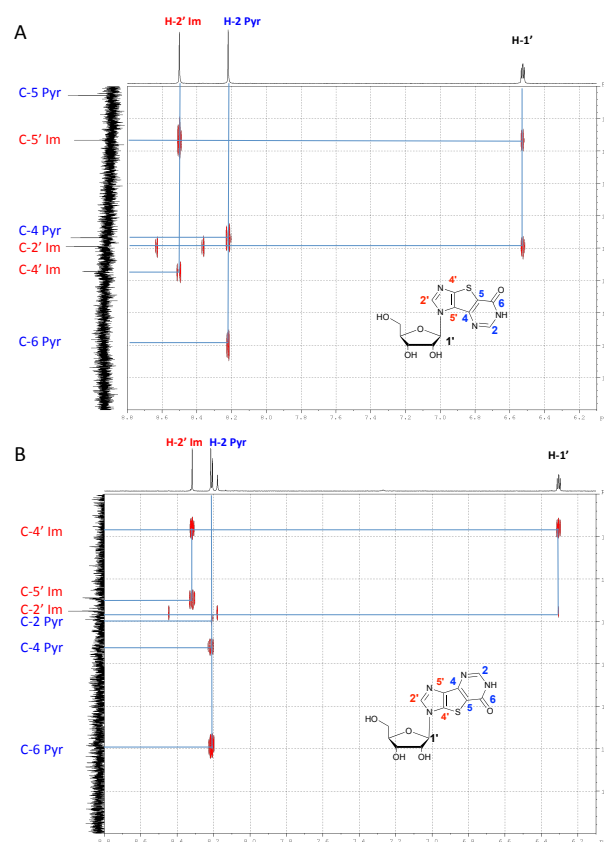
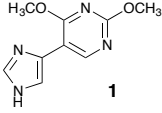
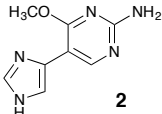
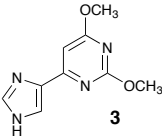
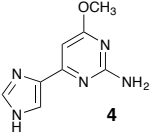
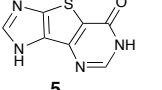


Figure 4. Expansion of the ^1H - ^{13}C HMBC NMR spectra of compounds **5a** (panel A) and **5c** (panel B).

COMMUNICATION

Table 1. Transglycosylation reactions of flex-bases (1–5) catalyzed by *L*/NDT^[a].

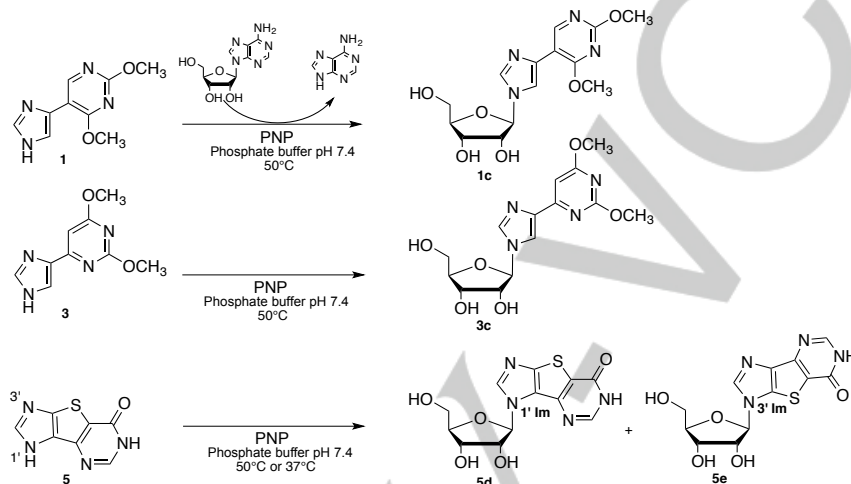
Entry	Acceptor	<i>L</i> /NDT (μL)	Incubation time (h)	Product (%) ^b		
				Starting material	N1 glycosylated product	N3 glycosylated product
1		1.25	3	61	17	22
2		1.25	10	53	28	20
3		2.5	3	40	74	13
4		2.5	10	24	65	6
5		5.0	3	15	80	5
6		5.0	10	10	88	2
7		7.5	3	12	86	2
8		7.5	10	10	89	1
9		10.0	3	11	87	2
10		10.0	10	10	89	1
11		1.25	3	24	30	46
12		1.25	10	17	45	38
13		2.5	3	18	42	40
14		2.5	10	10	78	12
15		1.25	3	70	30	-
16		1.25	10	20	80	-
17		2.5	3	7	93	-
18		2.5	10	4	96	-
19		1.25	0.5	0	100	-
20		0.63	0.5	18	82	-
21		0.15	3	40	60	-
22		1.25	3	2	62 and 32 ^c	4
23		1.25	10	2	46 and 44 ^c	6
24		0.63	3	3	71 and 23 ^c	3
25		0.63	10	2	58 and 34 ^c	6

[a] Reaction conditions: 1 μmol acceptor in 5% v/v DMSO, 4 μmol thymidine in 10 mM citrate buffer pH 6.5 (0.1 mL) in the presence of *L*/NDT (μL as indicated) at 37 °C. [b] Percent Conversion was determined by reverse-phase HPLC analysis of an aliquot of the incubation mixture monitored at 254 nm. [c] Product from glycosylation at both imidazole and pyrimidine nitrogens of **5**.

COMMUNICATION

Enzymatic glycosylation mediated by PNP.

We also tested the ability of purine nucleoside phosphorylase (PNP) to use flex-bases **1**, **3** and the thieno-expanded tricyclic base **5** for ribonucleoside synthesis (Scheme 4). Reaction mixtures (0.1 mL) contained the flex-base (10 mM), adenosine (20 mM) as a donor and bacterial PNP (0.2 U/ μ mol) in 10 mM phosphate buffer (pH 7.4). Incubation was initially performed at 50°C. Conversion of **3** into the corresponding ribonucleoside **3c** is slow and reached a plateau at 75% after 16 h at 50°C, as previously observed for PNP-catalyzed ribosylation of **1**.^[42]



Scheme 4. PNP mediated transglycosylation of flex bases **1**, **3** and **5**

In conclusion, the four flex-bases **1–4** and the tricyclic nucleobase **5** were found to be substrates for the nucleoside 2'-deoxyribosyltransferase from *L. leichmannii* (LNDT) and bacterial purine nucleoside phosphorylase (PNP). Experimental conditions were designed to manage the regioselectivity in the NDT-catalyzed transfer reaction (i.e. at N1 versus N3 position of the imidazole ring) and also the bis-glycosylation side reaction (at the pyrimidine nitrogen). Thus, *proximal* fleximers in both deoxy and ribose series are easily obtained from flex-bases **1** and **2** by transglycosylation reaction catalyzed by NDT and PNase, respectively. On the other hand, *distal* fleximers are not accessible from the corresponding flex-bases **3** and **4**, since glycosylation catalyzed by LNDT or PNP occurs at the N1 position of the imidazole ring. Importantly, enzymatic glycosylation of the tricyclic base **5** into the deoxy- and ribonucleoside **5a** and **5d** should provide a simple and efficient chemoenzymatic access to the target *distal* deoxy- and ribonucleoside fleximers, thereby overcoming the tedious and low-yielding approach that has been used to date. The chemoenzymatic approach compares very well with the classical chemical routes to *distal* and *proximal* fleximer nucleosides, as only one anomer and one regioisomer were obtained. This strategy will now be useful for the preparation of other *distal* fleximers, i.e. A and G analogues.

Acknowledgements

The authors from IP would like to thank Frédéric Bonhomme (UMR3523) for assisting with HRMS analysis, Dr Bruno Vitorge

In the case of flex-base **5**, conversion is much more effective (80% after 2 h at 50°C) and two mono-glycosylated products (**5d** and **5e**) were formed, the target compound **5d** being the major one (Scheme 4). When incubation was performed at 37°C, the formation of **5e** increased as compared to 50°C, allowing further characterization (Figure S3). The glycosylation sites of **5d** and **5e** at the N1' or N3' position of imidazole ring, respectively, were assigned based on NMR analysis (see Supporting Information). It should be noted that chemical glycosylation of **5** using classical procedures yielded nucleoside **5e** (glycosylation at N3' position of imidazole ring) as the sole product.^[29]

and Dr Inaki Guijarro (BioNMR platform) for providing access to Avance 600 and Avance Neo 800 spectrometers. This work was funded by the Institut Pasteur and the Centre National de la Recherche Scientifique (CNRS) (S.V.-G. and S.P.) and the National Institutes of Health/NIAID R21 AI118470-01 (KSR) and T32 GM066706 (KSR and TK).

Keywords: biocatalysis • fleximer • nucleoside • nucleoside 2'-deoxyribosyltransferase • purine nucleoside phosphorylase

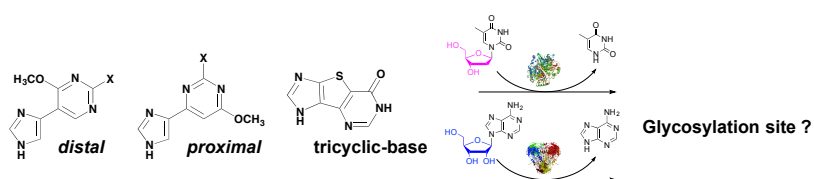
- [1] Townsend, L. B.; Tipson, R. S. *Nucleic acid chemistry: improved and new synthetic procedures, methods, and techniques*; Wiley, 1978.
- [2] Merino, P. *Chemical synthesis of nucleoside analogues*; Wiley: Hoboken, N.J., 2013.
- [3] Vorbrüggen, H.; Ruh-Pohlenz, C. *Handbook of Nucleoside Synthesis*; John Wiley & Sons, Inc: New York, NY, 2001.
- [4] Downey, A. M.; Pohl, R.; Roithova, J.; Hocek, M. *Chemistry* **2017**, *23*, 3910-3917.
- [5] Fernández-Lucas, J.; Camarasa, M.-J. *Enzymatic and Chemical Synthesis of Nucleic Acid Derivatives*; WILEY-VCH, 2018.
- [6] Mikhailopulo, I. A. *Curr. Org. Chem.* **2007**, *11*, 317-335.
- [7] Mikhailopulo, I. A.; Miroshnikov, A. I. *Acta Naturae* **2010**, *2*, 36-58.
- [8] Condezo, L. A.; Fernández-Lucas, J.; García-Burgos, C. A.; Alcántara, A. R.; Sinisterra, J. V. *Biocatalysis in the Pharmaceutical and Biotechnology Industries*; Patel, R. N., Ed.; CRC Press: 2006.
- [9] Pérez, E.; Sánchez-Murcia, P. A.; Jordaán, J.; Blanco, M. D.; Mancheño, J. M.; Gago, F.; Fernández-Lucas, J. *ChemCatChem*, **2018**, *10*(19), 4406-4416.
- [10] Fresco-Taboada, A.; de la Mata, I.; Arroyo, M.; Fernández-Lucas, J. *Applied microbiology and biotechnology*, **2013**, *97*(9), 3773-3785.
- [11] Krenitsky, T. A.; Koszalka, G. W.; Tuttle, J. V. *Biochemistry* **1981**, *20*, 3615-3621.
- [12] Hennen, W. J.; Wong, C. H. *J Org Chem* **1989**, *54*, 4692-4695.
- [13] Slater, M. J.; Gowrie, C.; Freeman, G. A.; Short, S. A. *Bioorg Med Chem Lett* **1996**, *6*, 2787-2790.
- [14] Fateev, I. V.; Antonov, K. V.; Konstantinova, I. D.; Muravyova, T. I.; Seela, F.; Esipov, R. S.; Miroshnikov, A. I.; Mikhailopulo, I. A. *Beilstein J. Org. Chem.* **2014**, *10*, 1657-1669.

COMMUNICATION

- [15] Fateev, I. V.; Kharitonova, M. I.; Antonov, K. V.; Konstantinova, I. D.; Stepanenko, V. N.; Esipov, R. S.; Seela, F.; Temburnikar, K. W.; Seley-Radtke, K. L.; Stepchenko, V. A.; Sokolov, Y. A.; Miroshnikov, A. I.; Mikhailopulo, I. A. *Chem-Eur J* **2015**, *21*, 13401-13419.
- [16] Danzin, C.; Cardinaud, R. *Eur J Biochem* **1976**, *62*, 365-372.
- [17] Kaminski, P. A. *J Biol Chem* **2002**, *277*, 14400-14407.
- [18] Cardinaud, R.; Holguin, J. *Biochim Biophys Acta* **1979**, *568*, 339-347.
- [19] Van Draanen, N. A.; Freeman, G. A.; Short, S. A.; Harvey, R.; Jansen, R.; Szczech, G.; Koszalka, G. W. *J. Med. Chem.* **1996**, *39*, 538-542.
- [20] Carson, D. A.; Wasson, D. B. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 829-834.
- [21] Betbeder, D.; Hutchinson, D. W. *Nucleosides Nucleotides* **1990**, *9*, 569-577.
- [22] Pochet, S.; Dugué, L.; Meier, A.; Marlière, P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1679-1684.
- [23] Ye, W. J.; Paul, D.; Gao, L. N.; Seckute, J.; Sangaiah, R.; Jayaraj, K.; Zhang, Z. F.; Kaminski, P. A.; Ealick, S. E.; Gold, A.; Ball, L. M. *Plos One* **2014**, *9*(12), e115082.
- [24] Muller, M.; Hutchinson, L. K.; Guengerich, F. P. *Chem. Res. Toxicol.* **1996**, *9*, 1140-1144.
- [25] Stachelska-Wierzchowska, A.; Wierzchowski, J.; Gorka, M.; Bzowska, A.; Wielgus-Kutrowska, B. *Molecules* **2019**, *24*.
- [26] Seley, K. L.; Quirk, S.; Salim, S.; Zhang, L.; Hagos, A. *Bioorg Med Chem Lett* **2003**, *13*, 1985-1988.
- [27] Seley, K. L.; Salim, S.; Zhang, L. *Org Lett* **2005**, *7*, 63-66.
- [28] Seley, K. L.; Zhang, L.; Hagos, A. *Org Lett* **2001**, *3*, 3209-3210.
- [29] Seley, K. L.; Zhang, L.; Hagos, A.; Quirk, S. *J. Org. Chem.* **2002**, *67*, 3365-3373.
- [30] Peters, H. L.; Ku, T. C.; Seley-Radtke, K. L. *Curr. Med. Chem.* **2015**, *22*, 3910-3921.
- [31] Zucman-Rossi, J.; Clement, B.; Buendia, M. A.; Lerat, H.; Beers, B. V.; Bedossa, P.; Taieb, J.; Rosenbaum, J. *Bull. Cancer* **2009**, *96*, 45-50.
- [32] Polak, M.; Seley, K. L.; Plavec, J. *J. Am. Chem. Soc.* **2004**, *126*, 8159-8166.
- [33] Quirk, S.; Seley, K. L. *Biochemistry* **2005**, *44*, 10854-10863.
- [34] Quirk, S.; Seley, K. L. *Biochemistry* **2005**, *44*, 13172-13178.
- [35] C. Ku, T.; Seley-Radtke, K. *Enzymatic and Chemical Synthesis of Nucleic Acid Derivatives*; WILEY-VCH, 2018.195-235.
- [36] Chen, Z.; Jochmans, D.; Ku, T.; Paeshuyse, J.; Neyts, J.; Seley-Radtke, K. L. *Acs Infect. Dis.* **2015**, *1*, 357-366.
- [37] Wauchope, O. R.; Velasquez, M.; Seley-Radtke, K. *Synthesis-Stuttgart* **2012**, *44*, 3496-3504.
- [38] Wauchope, O. R.; Tomney, M. J.; Pepper, J. L.; Korba, B. E.; Seley-Radtke, K. L. *Org. Lett.* **2010**, *12*, 4466-4469.
- [39] Tomney, M.; Korba, B.; Zimmermann, S.; Seley-Radtke, K. *Antivir. Res.* **2010**, *86*, A68-A68.
- [40] Zhang, Z. B.; Wauchope, O. R.; Seley-Radtke, K. L. *Tetrahedron* **2008**, *64*, 10791-10797.
- [41] Seley, K. L.; Januszczyk, P.; Hagos, A.; Zhang, L.; Dransfield, D. T. *J Med Chem* **2000**, *43*, 4877-4883.
- [42] Vichier-Guerre, S.; Dugué, L.; Bonhomme, F.; Pochet, S. *Org. Biomol. Chem.* **2016**, *14*, 3638-3653.
- [43] Vichier-Guerre, S.; Dugué, L.; Bonhomme, F.; Pochet, S. *Org. Biomol. Chem.* **2017**, *15*, 8193-8203.
- [44] Vichier-Guerre, S.; Dugué, L.; Pochet, S. *Tetrahedron Lett.* **2014**, *55*, 6347-6350.
- [45] Ku, T.; Lopresti, N.; Shirley, M.; Mori, M.; Marchant, J.; Heng, X.; Botta, M.; Summers, M. F.; Seley-Radtke, K. L. *Bioorg. Med. Chem.* **2019**, *27*, 2883-2892.
- [46] del Arco, J.; Perona, A.; Gonzalez, L.; Fernandez-Lucas, J.; Gago, F.; Sanchez-Murcia, P. A. *Org. Biomol. Chem.* **2019**, *17*, 7891-7899.

COMMUNICATION

Entry for the Table of Contents



Thieno-expanded tricyclic base and several *distal* and *proximal* flex-bases were converted into their corresponding fleximer nucleosides by enzymatic transglycosylation reactions catalyzed by *N*-deoxyribosyltransferase from *L. leichmannii* (LINDT) or *E. coli* purine nucleoside phosphorylase (PNP).