Organic & Biomolecular Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: S. Vichier-Guerre, L. Dugué, F. Bonhomme and S. Pochet, *Org. Biomol. Chem.*, 2017, DOI: 10.1039/C7OB01850A.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

View Article Online
DOI: 10.1039/C7OB01850A

An expedient synthesis of flexible nucleosides via a regiocontrolled enzymatic glycosylation of functionalized imidazoles

S. Vichier-Guerre, L. Dugué, F. Bonhomme and S. Pochet*

Unité de Chimie et Biocatalyse, Institut Pasteur, CNRS, UMR3523, 28 rue du Dr Roux, 75724 Paris Cedex 15

*Corresponding author: sylvie.pochet@pasteur.fr

Abstract

A versatile two-step synthesis of C4 and C5-arylated 2'-deoxyribosylimidazoles was elaborated using enzymatic *N*-transglycosylation followed by microwave-assisted Pd-catalysed arylation reactions. We report herein the reaction conditions that permit to manage the regioselectivity (N3 versus N1-isomers) in the enzymatic glycosylation of 4-iodoimidazole using the nucleoside *N*-deoxyribosyltransferase from *L. leichmannii*. Regiocontrolled glycosylation was also observed among several other imidazole derivatives studied, providing simple access to isomers not readily accessible by chemical routes. Finally, a series of flexible nucleosides was obtained in one-step from 4 or 5-iodo-imidazole nucleoside by Suzuki-Miyaura cross-coupling reaction with (hetero)aryl-boronic acids in aqueous media. Moreover, this chemoenzymatic approach is compatible with a one-pot two-step process affording a straightforward access to a broad array of potential anticancer and antiviral drugs as well as new DNA building blocks.

Keywords: Chemoenzymatic synthesis, Flexible Nucleoside, Microwave irradiation, Nucleoside 2'-deoxyribosyltransferase, Regioselective glycosylation, Suzuki-Miyaura cross-coupling

View Article Online
DOI: 10.1039/C7OB01850A

Introduction

Nucleoside analogues constitute a major class of biologically active compounds that are widely used as anticancer and antiviral drugs.[1, 2] Most of these nucleoside analogues are routinely produced by multi-step and time-consuming chemical procedures, including the stereocontrolled formation of the glycosidic linkage and several protection/deprotection steps of the sugar and nucleobase moieties. Over the past few decades, biocatalysts have emerged as a powerful and attractive alternative to conventional chemical synthesis. In particular, enzymes that catalyze the formation of the glycosidic bond have been successfully exploited in the synthesis of natural and unnatural nucleosides.[3-6] Two main classes of enzymes are currently used for transferring pentosyl residue from a donor nucleoside to an acceptor base: nucleoside phosphorylases (NP, EC 2.4.2.1) and nucleoside *N*-deoxyribosyltransferases (NDT, EC 2.4.2.6). Interestingly, NDTs have been shown to tolerate a wide range of modified nucleobases from azole derivatives[7-9] to expanded-size purines.[10-12]

In the last decade, a class of shape-modified nucleosides called fleximers, where the purine base has been split into its imidazole and pyrimidine subunits, has been introduced by Seley-Radke *et al*.[13]The imidazole ring remains attached to the sugar moiety, while the pyrimidine ring is linked via a single C–C bond to the C4 (proximal fleximer) or C5 (distal fleximer) of the imidazole (Fig. 1).[14, 15] This connectivity confers additional degrees of conformational freedom and torsional flexibility to the molecule, while still retaining the requisite hydrogen bonding and aromatic features necessary for base pairing and molecular recognition. These modified nucleobases have attracted significant attention as biochemical tools or potential therapeutic agents.[16-18]

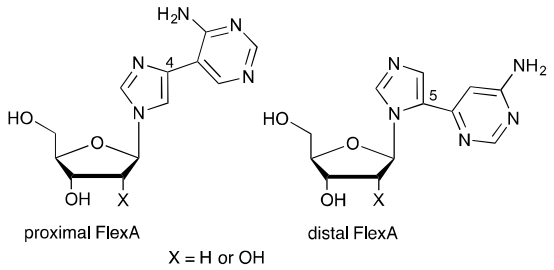
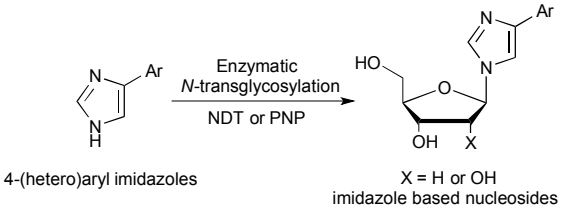


Fig. 1 Chemical structures of distal and proximal FlexA

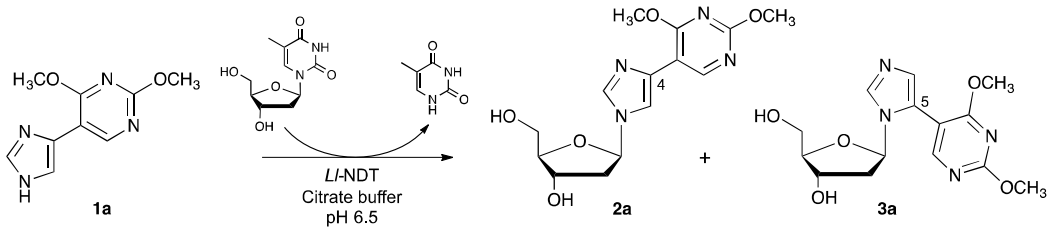
The chemical synthesis of these nucleoside analogues remains challenging.[15] The distal fleximers were initially prepared from their tricyclic “expanded purine” counterparts through desulfurization with Raney nickel.[15, 19] The proximal fleximers were synthesized via cross-coupling reactions to connect the imidazole and pyrimidine rings.[15] We recently described the use of nucleoside transferases (NDT-II from *L. leichmannii* and PNP from *E. coli*) to easily convert a chemically diverse set of 4-aryl and 4-heteroaryl imidazole derivatives[20] into the corresponding nucleoside derivatives (Scheme 1).[12]

View Article Online
DOI: 10.1039/C7OB01850A



Scheme 1 Synthetic route to imidazole-based nucleosides

During the course of our study, we noticed that efficient 2'-deoxyribosylation of the pyrimidinone derivative **1a** at both N1 and N3 sites using NDT-II from *L. leichmannii* (Scheme 2). More precisely, the transferase reaction between **1a** and thymidine led to the initial formation of the N1 and N3 glycosylated products (**2a** and **3a**, respectively). When the conversion was allowed to proceed to completion, **2a** was isolated as the sole product suggesting that the N3 isomer **3a** (kinetic product) was progressively converted into the thermodynamically more stable N1 isomer **2a**. However, in the case of the other studied 4-arylated imidazole derivatives, the corresponding target N1 nucleosides were isolated as the sole products.[12]



Scheme 2 NDT-catalysed transglycosylation of **1a** into **2a** and **3a**.

While the nucleobase exchange reaction is highly regioselective for natural substrates, base analogues may be glycosylated at multiple sites.[11, 21, 22] There are only few examples of 2'-deoxyribosylimidazole derivatives being synthesized by biocatalysts. Betbeder *et al.*[7] have described the transglycosylation of ethyl 5-aminoimidazole 4-carboxamide using NDT from *L. leichmannii* to produce 5-amino-1-(2-deoxy-ß-D-ribosyl)imidazole 4-carboxamide as the sole product. Regioselective glycosylation at N1 position was also reported starting from imidazole 4-carboxamide.[8] However, using the whole cells of *E. coli* as a biocatalyst, Ewing *et al.*[23] have reported the 2-deoxy-D-ribosylation of ethyl 5-aminoimidazole 4-carboxylate affording a mixture of ß-N1 and N3 isomers.

We thus surmised that it might be possible to control the regioselectivity in the glycosylation of 4-iodoimidazole, thus providing a simple access to the N1 and N3 isomers in good yields. The resulting 4- and 5-iodo-imidazole derivatives could be further engaged in cross-coupling reactions with boronic acid derivatives, affording C-4 or C-5 aryl-imidazole nucleosides. We report here how this scheme was validated experimentally.

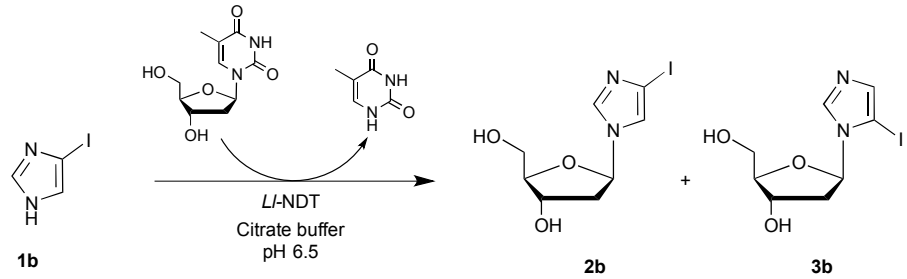
View Article Online
DOI: 10.1039/C7OB01850A

Results and Discussion

First, we studied the NDT-catalysed transfer reaction between 4-iodoimidazole (**1b**) and thymidine as a function of enzyme concentration, incubation time and temperature. Reaction mixtures (1 mL) typically contained 10 mM 4-iodoimidazole, 40 mM thymidine and variable amount of enzyme in 10 mM citrate buffer (pH 6.0) containing 5% v/v DMSO. Conversion rate was monitored by reverse phase HPLC.

As shown in Table 1, for a given enzyme concentration (0.5 mg/mL), conversion of 4-iodoimidazole (**1**) at 50°C, which is reported to be the optimal temperature for NDT,[24] led to the simultaneous formation of two glycosylated products, which were isolated by reverse-phase HPLC and identified by NMR spectroscopy as the N1 and N3 isomers (**2b** and **3b**) (Table 1, entry 1). The ^{1}H-^{13}C HMBC spectra (see Supporting Information) indicate a clear correlation between H-1' and C(H)-5 for compound **2b** and between H-1' and C(q)-5 for compound **3b**.

Table 1 NDT-catalysed transglycosylation reactions of 4-iodoimidazole **1b**[a]



Entry	Enzyme (µg)	Incubation temperature (°C)	Incubation time (h)	% Products[b]		
				1b	**2b**	**3b**
1	500	50	3	0	82	18
2	250	50	3	13	50	37
3	125	50	3	17	35	48
4	62.5	50	3	22	31	47
5	500	20	3	32	26	40
6	500	20	10	9	42	49
7	250	20	3	60	15	25
8	250	20	10	23	30	47
9	125	20	3	75	9	16
10	125	20	10	30	27	43
11	500	37	3	6	60	33
12	250	37	3	12	44	44
13	125	37	3	24	34	42
14	62.5	37	3	37	21	42

[a] Reaction conditions: 10 mM acceptor in 5% v/v DMSO, 40 mM thymidine in 10 mM citrate buffer pH 6.5 (1 mL), variable amounts of enzyme (from 12.5 to 100 µL at 5 mg/mL), incubation temperature (°C) and time (h) as indicated in Table 1.
[b] Conversions were determined by reverse-phase HPLC analysis of an aliquot of the incubation mixtures monitored at 230 nm.

As previously observed for the conversion of imidazole derivative **1a** into 2'-deoxynucleosides **2a/3a**, the

View Article Online
DOI: 10.1039/C7OB01850A

rate of the glycosylated products changed as a function of time (Fig. 2A). When complete conversion of **1b** was observed, the amount of the N1 product **2b** had reached 82%, while the early amount of N3 isomer **3b** had decreased (down to 18% after 3 h). This interconversion indicates that **3b** acts also as a donor for the transfer reaction, **3b** being a better substrate than **2b**. Indeed, when the nucleoside **3b** was incubated in the presence of thymine and NDT in the above conditions, we observed the initial conversion of thymine into thymidine, which in turn is slowly converted into **2b** (see Fig. 3A). Under the same conditions, the nucleoside **2b** appeared quite stable toward the deoxyribosyl transfer reaction, as only a trace of thymidine was detected after a long incubation time (see Fig. 3B).

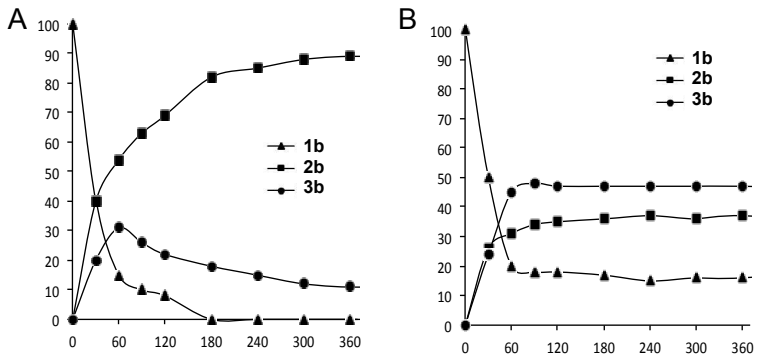


Fig. 2 HPLC monitoring at 230 nm of the crude reaction mixture of **1b** with thymidine in the presence of NDT (500 µg/mL panel A or 125 µg/mL panel B) at 50°C. Experiments were done in triplicate. See experimental section for elution conditions.

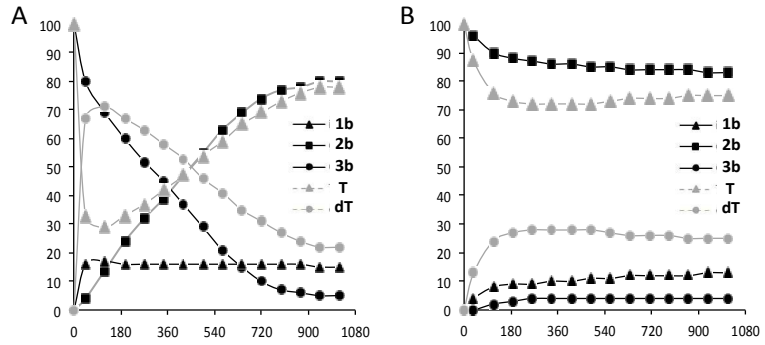


Fig. 3 HPLC monitoring at 230 nm of the crude reaction mixture of **3b** (panel A) or **2b** (panel B) with thymine in the presence of NDT (125 µg/mL) at 50°C. Experiments were done in duplicate. See experimental section for elution conditions.

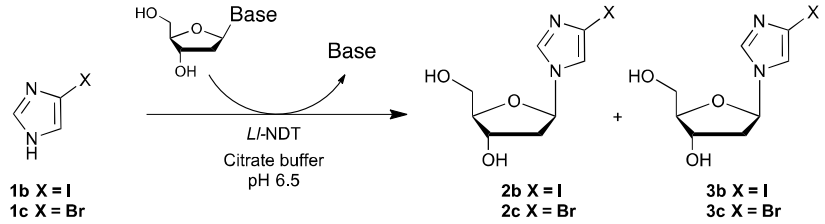
Reducing the enzyme concentration by a factor 4 (compared entries 1 and 3, Table 1) resulted in a nearly complete conversion of **1b** (17% of remaining **1b** after 2h at 50°C) with the formation of the N3 derivative **3b** as a major product (47%) compared to the N1 product **2b** (36%). No change in the products ratio was observed after 2 h, the reaction having reached equilibrium (Fig. 2B). When incubation was performed at 20°C, as anticipated the conversion rate was slow and long incubation time was required to achieve a nearly complete conversion (9% of remaining **1b** after 10h), **3b** was found to be the major glycosylated

View Article Online
DOI: 10.1039/C7OB01850A

product whatever the concentration enzyme is used (Table 1, entries 5-10). An incubation temperature of 37°C led to a similar products profile as compared to 20°C, conversion of **1b** was only reached faster (Table 1, entries 11-14). The highest enzyme concentration (entry 11) caused an increase in **2b**/**3b** ratio due to the reversibility of the reaction.

We also examined the influence of the 2'-deoxyribosyl donor, 2'-deoxycytidine (dC) or 2'-deoxyadenosine (dA) in place of thymidine (dT), on the kinetic of the reaction and the products distribution, while maintaining the incubation temperature at 37°C and the enzyme concentration at 125 µg/mL.

Table 2 Effect of donor-acceptor on the NDT products[a]



1b X = I
1c X = Br

2b X = I
2c X = Br

3b X = I
3c X = Br

Entry	Acceptor	Donor	% Conversion at 3 h[b]						% Conversion at 8 h[b]					
1	**1b**	dT	**1b**	24	**2b**	34	**3b**	42	**1b**	11	**2b**	47	**3b**	42
2	**1b**	dC	**1b**	43	**2b**	22	**3b**	35	**1b**	29	**2b**	29	**3b**	42
3	**1b**	dA	**1b**	63	**2b**	18	**3b**	19	**1b**	48	**2b**	28	**3b**	24
4	**1c**	dT	**1c**	36	**2c**	17	**3c**	47	**1c**	28	**2c**	37	**3c**	35
5	**1c**	dC	**1c**	48	**2c**	15	**3c**	37	**1c**	36	**2c**	28	**3c**	36

[a] Reaction conditions: 10 mM acceptor **1b** or **1c** in 5% v/v DMSO, 40 mM donor as indicated in the Table, 10 mM citrate buffer pH 6.5 in the presence of 125 µg/mL NDT, incubation temperature of 37°C.
[b] Conversions were determined by reverse-phase HPLC analysis of an aliquot of the incubation mixtures monitored at 230 nm.

As shown in Table 2, thymidine was found to be the best donor (i.e. conversion of **1b** was nearly complete after 3 h as compared to dC and dA, entries 1-3). The N3 isomer **3b** was early formed as the major compound whatever the donor used and was further converted into N1 isomer **2b** when the reaction has gone almost to completion (or reached equilibrium). When 4-bromoimidazole (**1c**) was used as the acceptor, comparable results were obtained with the concomitant formation of **2c** and **3c**, but the conversion was a little slower than using **1b** (Table 2, entries 4 & 5).

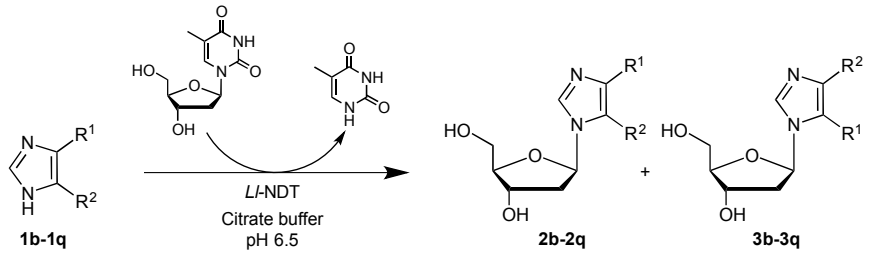
Based on these data, it appears possible to manage the regioselectivity of the NDT-catalysed reaction using 4-iodoimidazole as substrate by varying enzyme concentration and incubation time for altering the products distribution (i.e. the N3/N1 ratio).

We next studied the scope and limitations of the transfer reaction in terms of regioselectivity using a small set of 4-substituted and 4,5-disubstituted imidazole derivatives (**1d-1r**). The efficiency and selectivity profile were determined by HPLC analysis of the crude reaction mixtures. The glycosylated

View Article Online
DOI: 10.1039/C7OB01850A

products were isolated and further characterized by NMR and mass spectrometry. Conversions after 3 h of incubation are summarized in Table 3. Imidazole bearing a small functional group at position 4, such as hydroxymethyl (**1d**), carboxamide (**1e**), methyl (**1f**) and ethyl ester (**1g**) carboxylic acid, carboxyaldehyde (**1h**) and carbonitrile (**1i**), were found to be substrates with different reactivity patterns in terms of conversion rate and N1/N3 ratio (Table 3, entries 3-8). Thus, **1d** and **1e** were rapidly converted under the conditions used and the corresponding N1-glycosylated bases (**2d** and **2e**, respectively) were isolated as the sole products in nearly quantitative yield.[8]

Table 3 NDT-catalysed transglycosylation reactions of imidazole derivatives **1b-1q**[a]



Entry	R^1	R^2	Remaining acceptor[b] (%)		N1 isomer[b] (%)		N3 isomer[b] (%)	
1	I	H	**1b**	24	**2b**	34	**3b**	42
2	Br	H	**1c**	36	**2c**	17	**3c**	47
3	CH_2OH	H	**1d**	0	**2d**	100	**3d**	nd
4	$CONH_2$	H	**1e**	4	**2e**	96	**3e**	nd
5	$COOCH_3$	H	**1f**	22	**2f**	68	**3f**	10
6	$COOCH_2CH_3$	H	**1g**	10	**2g**	88	**3g**	2
7	CHO	H	**1h**[c]	31	**2h**	41	**3h**	28
8	CN	H	**1i**[c]	44	**2i**	39	**3i**	17
9	CH_2COOCH_3	H	**1j**	26	**2j**	70	**3j**	4
10	CH_2COOH	H	**1k**	100[d]	**2k**	nd	**3k**	nd
11	CH=CHCOOH	H	**1l**	78	**2l**	22	**3l**	nd
12	$CH_2CH_2NH_2$	H	**1m**	100[d]	**2m**	nd	**3m**	nd
13	$CH_2CH(NH_2)COOH$	H	**1n**	100	**2n**	nd	**3n**	nd
14	$CH_2CH(NH_2)CH_2OH$	H	**1o**	100	**2o**	nd	**3o**	nd
15	CN	NH_2	**1p**	13 (8[e])	**2p**	4 (39[e])	**3p**	83 (53[e])
16	$CONH_2$	CN	**1q**	60 (44[e])	**2q**	7 (33[e])	**3q**	33 (23[e])

[a] Reaction conditions: 10 mM acceptor in 5% v/v DMSO, 40 mM thymidine in 10 mM citrate buffer pH 6.5, in the presence of 125 µg NDT, incubation at 37°C for 3 h.
[b] % conversions after 3 h at 37°C determined by reverse-phase HPLC analysis of an aliquot of the incubation mixtures monitored at 230 nm.
[c] The corresponding N1/N3-glycosylated products were not separable by HPLC under the analytical conditions used. The N1/N3 ratio was determined by NMR analysis.
[d] 20% conversion in the presence of 500 µg NDT after 48 h at 37°C.
[e] % conversions after 16 h for compound **1p** or 24 h for compound **1q** at 50°C.
nd : not detected

Conversion of imidazole derivatives **1f-1j** proceeded more slowly. HPLC analyses showed the formation of both N1 and N3 products that further shifted toward glycosylation at N1 (Table 3, entries 5-8).

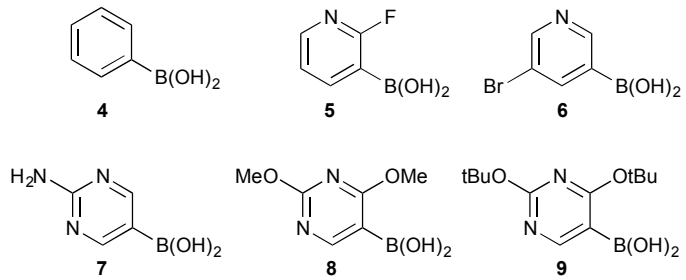
View Article Online
DOI: 10.1039/C7OB01850A

Surprisingly, 4-acetic acid imidazole (**1k**) was found to be a poor substrate compared to the methyl ester derivative (**1j**). A 4-fold higher enzyme concentration and long incubation time were required to observe a 20% conversion rate into N1/N3 products. When more sterically demanding substituents at position 4 of the imidazole ring (**1l-1o**, entries 11-14) were used as substrates, no or poor conversion were detected even in the presence of higher enzyme concentration, incubation time and temperature. We[12] and others[10, 11] previously shown that the catalytic active site of NDT tolerates a variety of large heterocycles as acceptors. The lack of transfer activity observed for some imidazole derivatives such as histidine, which occurs in the lactobacteria cells hosting the NDT enzyme, might be explained by counterselection of the active site during evolution.

Finally we examined the recognition of 4,5-disubstituted imidazoles **1p** and **1q**, precursors of purine analogues.[25, 26] Chemical glycosylation of **1p** have been previously reported via the sodium salt procedure affording both N1 and N3 glycosylated products in 66% yield with a 1:2 ratio, respectively.[25] An additional deprotection step is needed to isolate compounds **2p** and **3p** (62% and 70%, respectively). Both imidazole derivatives **1p** and **1q** were found to be substrates of NDT and converted into the N1 and N3 isomers in good overall yields with a ratio depending on the experimental conditions (Table 3, entries 15-16). The achievable NDT-catalysed interconversion of **3p** (or **3q**) into **2p** (or **2q**) is of significant interest to facilitate access to minor isomers compared to the classical synthetic approach.

The 4- and 5-halogeno-imidazole deoxynucleosides provide valuable starting points for further chemical diversification. Thus, the palladium-catalysed Suzuki-Miyaura reaction[27] represents one of the most powerful methods for the C-modification of nucleobases and nucleosides.[28] The C–C cross-coupling reactions are usually performed in organic solvents using protected nucleobases and nucleosides. More recently, alternative methodologies involving aqueous-phase coupling reactions from unprotected halonucleobases and halonucleosides were developed.[29-31]

Applying our conditions optimized above to the transglycosylation of **1b** on a 5.5-mmol scale, we successfully isolated the N1/N3 isomers **2b/3b** in large quantity and in good yields. We next studied conditions for cross-coupling Suzuki-Miyaura reaction starting from 4(or 5)-iodo-imidazole derivatives **2b** (or **3b**) with a set of aryl- and heteroarylboronic acids (Fig. 4).

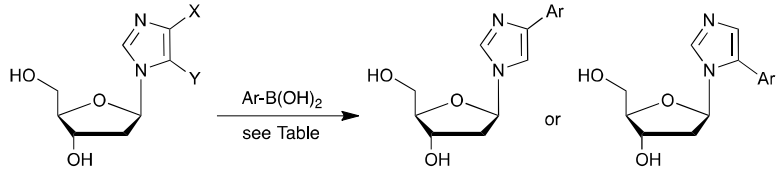


View Article Online
DOI: 10.1039/C7OB01850A

Fig. 4 Aryl and heteroarylboronic acids (**4-9**) used in this study

We recently reported the efficient arylation of 4-iodoimidazole under microwave (MW) irradiation.[20] First we applied these conditions (Table 4, conditions A) to 4-iodo derivative **2b**, which was reacted with 2 equiv. of phenylboronic acid (**4**) in the presence of Na_2CO_3 and $Pd(Ph_3)_4$ in a 1/1 mixture of DMF/water at 110°C for 1 h. While the starting material was nearly consumed, the targeted product **10.1** was isolated in moderate yield (Table 4, entry 1). We next considered the optimized coupling conditions involving 5-iodo-2'-deoxyuridine.[32] Thus, performing the reaction in water without co-solvents in the presence of $Pd(OAc)_2$ and PPh_3 under MW irradiation at 110°C, slightly improved the yield of **10.1** (Table 4, entry 2), affording **10 .1** in 54% yield (data not shown).

Table 4 Suzuki cross-coupling reaction of **2b** and **3b** with arylboronic acids **4-9**



2b X = I, Y = H
3b X = H, Y = I

10 **11**

Entry	Nucleoside	Ar-B(OH)$_2$	Conditions[a]	Temperature/ Time	Products	Yield (%)
1	**2b**	**4**	A	110°C/1 h	**10.1**	38
2	**2b**	**4**	B	120°C/1 h	**10.1**	45
3	**2b**	**4**	C	90°C[b]/2 h	**10.1**	35
4	**2b**	**4**	C	150°C/5 min	**10.1**	57
5	**2b**	**4**	C	120°C/5 min	**10.1**	78
6	**2b**	**5**	C	120°C/10 min	**10.2**	30
7	**2b**	**5**	A	110°C/1 h	**10.2**	60
8	**2b**	**6**	C	120°C/10 min	**10.3**	39
9	**2b**	**6**	A	80°C/1 h	**10.3**	48
10	**2b**	**7**	C	120°C/5 min	**10.4**	70
11	**2b**	**8**	C	120°C/10 min	**10.5**	58
12	**2b**	**8**	A	120°C/1 h	**10.5**	51
13	**2b**	**9**	C	120°C/20 min	**10.6/10.7**	20/12
14	**2b**	**9**	C	150°C/1 min	**10.6/10.7**	37/3
15	**2b**	**9**	A	110°C/1 h	**10.6**	76
16	**3b**	**4**	C	120°C/10 min	**11.1**	70
17	**3b**	**5**	A	110°C/1 h	**11.2**	60
18	**3b**	**6**	A	110°C/1 h	**11.3**	22
19	**3b**	**6**	A	80°C/1 h	**11.3**	40
20	**3b**	**7**	C	120°C/5 min	**11.4**	60

View Article Online
DOI: 10.1039/C7OB01850A

21	**3b**	**8**	C	120°C/10 min	**11.4**	25
22	**3b**	**8**	A	110°C/1 h	**11.4**	46
23	**3b**	**9**	A	110°C/1 h	**11.6**	80

[a] Reagents and conditions: **2b** or **3b** (0.1 mmol), arylboronic acid (1.25 mmol), 1M Na_2CO_3 (3 mmol)
Method A: $Pd(PPh_3)_4$ (0.08 mmol) in DMF/H_2O (1/1, 14 mL/mmol);
Method B: $Pd(OAc)_2$ (0.08 mmol), PPh_3 (0.08 mmol) in H_2O (14 mL/mmol);
Method C: $Pd(PPh_3)_4$ (0.08 mmol), TPPS (0.08 mmol), in CH_3CN/H_2O (1/1, 14 mL/mmol);
[b] Conventional heating

Finally, the use of $Pd(OAc)_2$/TPPS catalytic system[29] in a mixture of acetonitrile/water in the presence of Na_2CO_3 (conditions C) gave a complete conversion of **2b** in 5 min under MW irradiation at 120°C, affording **10.1** in 78% yield (Table 4, entry 3). Lower or higher temperature resulted in rather low product yields (entries 4 and 5). It is worth noting that bromide **2c** was found to react slowly under these optimal conditions.

Coupling reactions of **2b** with the pyridinyl- and pyrimidinyl-boronic acid derivatives **5-9** (Fig. 3) were next attempted under these conditions, resulting in the formation of compounds **10.2-10.6** with yield ranging from 48 to 76% (Table 4, entries 6-15). The use of Cs_2CO_3 in place of Na_2CO_3 did not improve the coupling yield of **5** (data not shown). In the case of the 3,5-pyrimidinone derivative **9**, the partial deprotection of *t*-butoxy group was observed using the $Pd(OAc)_2$/TPPS system (conditions C) and two products **10.6** and **10.7** were isolated, even if the reaction temperature was increased and reaction time was shortened. The use of $Pd(Ph_3)_4$ as catalyst (conditions A) gave the fully protected product **10.6** in high yield.
These optimal conditions were next applied to the coupling reaction of 5-iodo derivative **3b** with the set of boronic acid derivatives **5-9**. Similar trends were observed and the C-5 aryl-imidazole derivatives **11.1-11.6** were isolated in 40–80% yield (Table 4, entries 16-23).

Finally, we examined the feasibility of a one-pot two-step process, performing the Suzuki-Miyaura arylation without work-up of the intermediate iodoimidazole derivative. As a proof of concept, we carried out the enzymatic step under conditions that yielded a mixture of N1 and N3 isomers **2b**/**3b** (80% conversion). After enzyme denaturation, the crude reaction mixture was treated with phenyl boronic acid **4**. The coupling products **2b** and **3b** were isolated in 56% overall yield after HPLC purification.

Conclusion

We have developed a straightforward and highly efficient two-step chemoenzymatic procedure for the synthesis of C-4 and C-5 aryl-imidazole 2'-deoxynucleosides. We found experimental conditions that

View Article Online
DOI: 10.1039/C7OB01850A

affect the regioselectivity in the glycosylation of 4-iodoimidazole using NDT-II from *L. leichmannii*, making possible the preparation of the kinetic product of the reaction, i.e. the N3 glycosylated isomer, in good yield. Regiocontrolled enzymatic glycosylation was also observed among several other imidazole derivatives studied, providing simple access to regioisomers not readily accessible by chemical routes. These results illustrate that NDT can accommodate a variety of nucleobases, because of its flexibility in both accepting and donating hydrogen bonds.[11] To our knowledge, regioselectivity control in the transfer reactions catalysed by NDT has never been reported so far. Once in hand, the 4 and 5-iodo-imidazole nucleosides have been successfully engaged in Suzuki-Miyaura cross-coupling reactions with a set of (hetero)aryl-boronic acid derivatives. We also demonstrated that our synthetic approach is compatible with a one-pot two-step process, i.e. enzymatic glycosylation of 4-iodoimidazole followed by Suzuki-Miyaura arylation without work-up of the iodoimidazole nucleoside. Compared to the chemical routes described so far, the chemoenzymatic strategy reported here is easier and less labor consuming, providing a straightforward access to original proximal or distal fleximers. These results will be beneficial to the further development of these artificial nucleobases as drug candidates or DNA building blocks. Studies are currently underway to explore their potential as polymerase substrates.

Experimental Section

General information

All chemicals were obtained from commercial sources and used without further purification unless otherwise noted. All solvents were analytical grade purity. Microwave reactions were performed on a microwave Biotage initiator 2 oven under an inert atmosphere of argon. Reactions were monitored by thin layer chromatography (TLC) on pre-coated Merck silica gel plates (60 F_{254}/0.2 mm), by LC-MS (Agilent 1100 series) or by HPLC (Agilent 1100 series) using a C18 reverse phase column. Analytical HPLC was carried out on an Agilent system (1100 series) equipped with a diode-array detector using a C18 reverse phase column (Kromasil, 5 µm, 100 Å; column 1: 4.6 x 150 mm or column 2: 4.6 x 150 mm) at a flow rate of 1mL/min and a linear gradient of acetonitrile in 10 mM triethylammonium acetate buffer (TEAA) over 20 min (Gradients: G1, 5 to 40%; G2, 0 to 7%; G3, 0 to 20%; G4, 5 to 25%; G5, 0 to 10%; G6, 5 to 60%). Purification by HPLC was carried out on Agilent 1100 Series system on a C18 reverse phase column (Kromasil, 5 µm, 100 Å, 10 x 250 mm) using a flow rate of 4.0 mL/min and an isocratic or linear gradient of acetonitrile in 10 mM TEAA over 20 min. Yields refer to chromatographically and spectroscopically pure (> 95 %) compounds. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 400 operating at 400.13 MHz and 100.62 MHz, respectively. Chemical shifts are given in ppm (δ) relative to residual solvent peak, coupling constants (*J*) are reported in Hertz and standard abbreviations are used. Assignment of ^{1}H and ^{13}C signals was performed by analysis of the correlated homonuclear ^{1}H,^{1}H-COSY and heteronuclear

View Article Online
DOI: 10.1039/C7OB01850A

^{1}H,^{13}C-HMBC, ^{1}H,^{13}C-HSQC spectra. High-resolution mass spectra were recorded on a Waters Q-TOF micro MS instrument under electrospray ionization in positive ionization mode using a mobile phase of acetonitrile/water with 0.1 % formic acid.

General procedure for the enzymatic glycosylation of imidazole derivatives

Nucleoside 2-deoxyribosyltransferases class II from *L. leichmannii* (*Ll*NDT-II or NDT) was produced and purified as described.[33]

Analytical scale synthesis: Typically, enzymatic reaction mixtures (1 mL) contained imidazole derivative (0.01 mmol) as acceptor and thymidine (0.04 mmol) as donor. A solution of acceptor and donor in DMSO (5% v/v) was added slowly to 10 mM citrate buffer (pH 6.0) to reach a final volume of 1 mL. The reaction was started by adding NDT (variable amounts at 5 µg/µL) and run at 20°C, 37°C and 50°C (see Tables 1 -3). Conversion was monitored by analytical reverse phase HPLC.

Preparative scale synthesis: Experimental conditions described above were adapted by increasing the volume and mass of reactants. The amount of NDT at 5 µg/µL, the reaction time and temperature were adjusted in order to obtain in a single experiment both regioisomers in a significant amount, while recovering relatively little amounts of starting material. The products were isolated after purification by reverse phase HPLC.

1-(2-Deoxy-β-D-ribofuranosyl)-4-iodo-1*H*-imidazole (2b) and 1-(2-deoxy-β-D-ribofuranosyl)-5-iodo-1*H*-imidazole (3b): Starting from **1b** (1.0 g, 5.20 mmol) and thymidine (5.0 g, 20.0 mmol) in the presence of NDT (62.5 mg) at 20°C for 20 h (78% conversion). **2b:** 709 mg (45% yield); t_R = 11.35 min (column 1, G1); Analytical and spectral data are in accordance with previously published data;[34] HRMS (ESI-TOF) m/z calcd for [$C_8H_{11}IN_2O_3$+H]: 310.9893, found: 310.9885. **3b:** 445 mg (28% yield); t_R = 11.68 min (column 1, G1); ^{1}H NMR (DMSO-d_6): δ2.27 (ddd, *J* = 3.3, 6.0, 13.3 Hz, 1H, H-2'), 2.41 (ddd, *J* = 6.0, 7.3, 13.3 Hz, 1H, H-2"), 3.51 (m, 2H, H-5', H-5"), 3.83 (m, 1H, H-4'), 4.31 (m, 1H, H-3'), 4.90 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.30 (d, *J* = 4.0 Hz, 1H, OH-3'), 5.91 (dd, *J* = 6.1, 7.5 Hz, 1H, H-1'), 7.02 (d, *J* = 1.0 Hz, 1H, H-4), 8.11 (d, *J* = 1.0 Hz, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 40.9, 62.0, 71.0, 87.2, 88.3, 136.2, 138.4, 137.2; HRMS (ESI-TOF) m/z calcd for [$C_8H_{11}IN_2O_3$+H]: 310.9893, found: 310.9892.

4-Bromo-1-(2-Deoxy-β-D-ribofuranosyl)-1*H*-imidazole (2c) and 5-bromo-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole (3c): Starting from **1c** (340 mg, 2.35 mmol) and thymidine (2.3 g, 9.50 mmol) in the presence of NDT (30 mg) at 37°C for 2.5 h (86% conversion). **2c:** 133 mg (21% yield); t_R = 10.36 min (column 1, G1); ^{1}H NMR (DMSO-d_6): δ2.22 (ddd, *J* = 3.3, 6.0, 13.3 Hz, 1H, H-2'), 2.33 (ddd, *J* = 6.0, 7.3, 13.3 Hz, 1H, H-2"), 3.50 (m, 2H, H-5', H-5"), 3.81 (m, 1H, H-4'), 4.28 (m, 1H, H-3'), 4.89 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.24 (d, *J* = 4.0 Hz 1H, OH-3'), 6.01 (t, *J* = 6.5 Hz, 1H, H-1'), 7.51 (d, *J* = 1.6 Hz, 1H, H-5), 7.86 (d, *J* = 1.6 Hz, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ41.2, 62.1, 71.1, 86.4, 88.3, 114.8, 117.0, 137.2; HRMS (ESI-TOF) m/z calcd for [$C_8H_{11}{}^{79}BrN_2O_3$+H]: 263.0031, found: 263.0031. [$C_8H_{11}{}^{81}BrN_2O_3$+H]: 265.0012, found: 265.0025. **3c:** 200

View Article Online
DOI: 10.1039/C7OB01850A

mg (32% yield); t_R = 11.43 min (column 1, G1); ^{1}H NMR (DMSO-d_6): δ 2.28 (ddd, *J* = 3.4, 6.3, 13.4 Hz, 1H, H-2'), 2.44 (ddd, *J* = 5.9, 7.3, 13.4 Hz, 1H, H-2"), 3.50 (m, 2H, H-5', H-5"), 3.83 (m, 1H, H-4'), 4.32 (m, 1H, H-3'), 4.95 (br s, 1H, OH-5'), 5.32 (br s, 1H, OH-3'), 5.96 (dd, *J* = 6.3, 7.1 Hz, 1H, H-1'), 7.01 (d, *J* = 1.1 Hz, 1H, H-4), 8.01 (d, *J* = 1.1 Hz, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 40.6, 61.9, 70.9, 85.3, 88.3, 102.3, 129.5, 137.2; HRMS (ESI-TOF) m/z calcd for [$C_8H_{11}{}^{79}BrN_2O_3$+H]: 263.0031, found: 263.0031, for [$C_8H_{11}{}^{81}BrN_2O_3$+H]: 265.0012, found: 265.0021.

4-Hydroxymethyl-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole (2d) and 5-hydroxymethyl-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole (3d): Starting from **1d** as chlorhydrate salt (14 mg, 0.10 mmol) and thymidine (100 mg, 0.4 mmol) in the presence of NDT (1 mg) at 50°C for 2 h (80% conversion). A mixture of **2d** and **3d** (10/1): 10.4 mg (47% yield); t_R = 6.93 min (column 2, G2); ^{1}H NMR (DMSO-d_6): δ 2.18 (ddd, *J* = 3.1, 5.9, 13.2 Hz, 1H, H-2' of **2d**), 2.25 (ddd, *J* = 2.9, 6.1, 13.2 Hz, 0.1H, H-2' of **3d**), 2.35 (ddd, *J* = 5.9, 7.3, 13.2 Hz, 1H, H-2" of **2d**), 2.35 (ddd, *J* = 5.9, 7.3, 13.2 Hz, 0.1H, H-2" of **3d**), 3.48 (m, 2.2H, H-5' and H-5" of **2d**, H-5' and H-5" of **3d**), 3.78 (m, 1.1H, H-4' of **2d** and **3d**), 4.28 (m, 1.1H, H-3' of **2d** and **3d**), 4.21 (d, *J* = 5.5 Hz, 2H, CH_2 of **2d**), 4.45 (d, *J* = 4.5 Hz, 0.2H, CH_2 of **3d**), 4.81 (t, *J* = 5.6 Hz, 1H, $CH_2\underline{OH}$ of **2d**), 4.86 (m, 1.1H, OH-5' of **2d** and **3d**), 5.08 (t, *J* = 5.0 Hz, 0.1H, $CH_2\underline{OH}$ of **3d**), 5.22 (m, 1.1H, OH-3' of **2d** and **3d**), 5.98 (dd, *J* = 6.0, 6.7 Hz, 1H, H-1' of **2d**), 6.02 (dd, *J* = 6.0, 7.3 Hz, 0.1H, H-1' of **3d**), 6.78 (s, 0.1H, H-4 of **3d**), 7.16 (s, 1H, H-5 of **2d**), 7.74 (d, *J* = 1.3 Hz, 1H, H-2 of **2d**), 7.88 (br s, 0.1H, H-2 of **3d**); ^{13}C NMR (DMSO-d_6): δ 41.2(**2d**), 41.3(**3d**), 51.2 (C5), 53.1 (**3d**), 58.2 (**2d**), 62.1 (**3d**), 62.4 (**2d**), 71.0 (**3d**), 71.3 (**2d**), 84.6 (**3d**), 85.8 (**2d**), 87.9 (**3d**), 88.0 (**2d**), 114.4 (**2d**), 127.8 (**3d**), 131.8 (**3d**), 136.3 (**2d**), 143.6 (**2d**); HRMS (ESI-TOF) m/z calcd for [$C_9H_{14}N_2O_4$+H]: 215.1032, found: 215.1031.

1-(2-Deoxy-β-D-ribofuranosyl)-1*H*-imidazole-4-carboxamide (2e): Starting from **1e** (7.9 mg, 0.07 mmol) and thymidine (70 mg, 0.29 mmol) in the presence of NDT (350 µg) at 50°C for 2 h (97% conversion). **2e**: 14.2 mg (85% yield); t_R = 8.04 min (column 2, G3); Spectral data in accordance with previously published data[8]; HRMS (ESI-TOF) m/z calcd for [$C_9H_{13}N_3O_4$+Na]: 250.0804, found: 250.0814.

Methyl-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-4-carboxylate (2f) and methyl-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-5-carboxylate (3f): Starting from **1f** (20 mg, 0.16 mmol) and thymidine (150 mg, 0.62 mmol) in the presence of NDT (250 µg) at 37°C for 2 h (51% conversion). **2f**: 12.3 mg (29% yield); t_R = 12.28 min (column 2, G3); ^{1}H NMR (DMSO-d_6): δ 2.26 (ddd, *J* = 3.3, 6.1, 13.4 Hz, 1H, H-2'), 2.37 (ddd, *J* = 5.8, 7.1, 13.4 Hz, 1H, H-2"), 3.53 (m, 2H, H-5', H-5"), 3.74 (s, 3H, CH_3), 3.84 (m, 1H, H-4'), 4.31 (m, 1H, H-3'), 4.94 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.27 (d, *J* = 3.8 Hz, 1H, OH-3'), 6.08 (t, *J* = 6.7 Hz, 1H, H-1'), 7.99 (d, *J* = 1.4 Hz, 1H, H-5), 8.08 (d, *J* = 1.4 Hz, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 41.6, 51.5, 62.0, 71.1, 86.5, 88.4, 124.5, 133.0, 138.0, 163.1; HRMS (ESI-TOF) m/z calcd for [$C_{10}H_{14}N_2O_5$+Na]: 265.0800, found: 265.0802. **3f**: 8.1 mg (19% yield); t_R = 16.65 min (column 2, G3); ^{1}H NMR (DMSO-d_6): δ 2.23 (dt, *J* = 6.1, 13.2 Hz, 1H, H-2'), 2.34 (ddd, *J* = 4.5, 6.2, 13.2 Hz, 1H, H-2"), 3.58 (m, 2H, H-5', H-5"), 3.78 (s, 3H, CH_3), 3.84 (m, 1H, H-4'),

View Article Online
DOI: 10.1039/C7OB01850A

4.27 (m, 1H, H-3'), 4.98 (br s, 1H, OH-5'), 5.27 (br s, 1H, OH-3'), 6.56 (t, *J* = 6.0 Hz, 1H, H-1'), 7.66 (d, *J* = 1.0 Hz, 1H, H-4), 8.34 (d, *J* = 1.0 Hz, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 42.4, 52.0, 61.4, 70.0, 86.4, 88.2, 121.8, 137.9, 140.5, 160.4; HRMS (ESI-TOF) m/z calcd for [$C_{10}H_{14}N_2O_5$+Na]: 265.0800, found: 265.0806.

Ethyl-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-4-carboxylate (2g) and ethyl-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-5-carboxylate (3g): Starting from **1g** (100 mg, 0.8 mmol) and thymidine (650 mg, 2.6 mmol) in the presence of NDT (750 µg) at 37°C for 2 h (38% conversion). **2g**: 33.8 mg (18% yield); t_R = 9.25 min (column 1, G4); Spectral data in accordance with previously published data[8]; HRMS (ESI-TOF) m/z calcd for [$C_{11}H_{16}N_2O_5$+Na]: 279.0957, found: 279.0949. **3g**: 3.6 mg (4% yield); t_R = 14.27 min (column 1, G4); ^{1}H NMR (DMSO-d_6): δ 1.29 (t, *J* = 7.4, 3H, CH_3), 2.21 (dt, *J* = 6.0, 13.2 Hz, 1H, H-2'), 2.36 (ddd, *J* = 4,5, 6.0, 13.2 Hz, 1H, H-2"), 3.59 (m, 2H, H-5', H-5"), 3.87 (m, 1H, H-4'), 4.26 (m, 3H, CH_2, H-3'), 4.99 (m, 1H, OH-5'), 5.25 (br s, 1H, OH-3'), 6.56 (t, *J* = 6.1 Hz, 1H, H-1'), 7.65 (d, *J* = 0.9 Hz, 1H, H-4), 8.33 (br s, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 14.6, 42.6, 60.8, 61.4 70.0, 86.4, 88.1, 122.1, 137.8, 140.4, 160.0; HRMS (ESI-TOF) m/z calcd for [$C_{11}H_{16}N_2O_5$+Na]: 279.0957, found: 279.0955.

1-(2-Deoxy-β-D-ribofuranosyl)-1*H*-imidazole-4-carbaldehyde (2h) and 1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-5-carbaldehyde (3h): Starting from **1h** (9.6 mg, 0.1 mmol) and thymidine (116 mg, 0.48 mmol) in the presence of NDT (350 µg) at 50°C for 2 h (65% conversion). **2h**: 7.2 mg (31% yield); t_R = 8.85 min (column 2, G2); ^{1}H NMR (DMSO-d_6): δ 2.30 (ddd, *J* = 3.5, 6.0, 13.2 Hz, 1H, H-2'), 2.38 (ddd, *J* = 5.8, 7.2, 13.2 Hz, 1H, H-2"), 3.53 (m, 2H, H-5', H-5"), 3.85 (m, 1H, H-4'), 4.32 (m, 1H, H-3'), 4.95 (br s, 1H, OH-5'), 5.30 (br s, 1H, OH-3'), 6.12 (t, *J* = 6.8 Hz, 1H, H-1'), 8.09 (d, 1H, H-5), 8.24 (s, 1H, H-2), 9.73 (s, 1H, CHO); ^{13}C NMR (DMSO-d_6): δ 41.6, 62.0, 71.1, 86.7, 88.5, 126.0, 139.0, 142.2, 185.8; HRMS (ESI-TOF) m/z calcd for [$C_9H_{12}N_2O_4$+Na]: 235.0695, found: 235.0693. **3h**: 6.0 mg (26% yield); t_R = 13.37 min (column 2, G2); ^{1}H NMR (DMSO-d_6): δ 2.22 (m, 1H, H-2'), 2.37 (m, 1H, H-2"), 3.57 (m, 2H, H-5', H-5"), 3.86 (m, 1H, H-4'), 4.28 (m, 1H, H-3'), 5.00 (br s, 1H, OH-5'), 5.27 (br s, 1H, OH-3'), 6.53 (t, *J* = 6.2 Hz, 1H, H-1'), 7.93 (s, 1H, H-4), 8.44 (s, 1H, H-2), 9.73 (s, 1H, CHO); ^{13}C NMR (DMSO-d_6): δ 42.3, 61.4, 70.1, 86.6, 88.4, 130.9, 141.8, 144.4, 180.5; HRMS (ESI-TOF) m/z calcd for [$C_9H_{12}N_2O_4$+Na]: 235.0695, found: 235.0693.

1-(2-Deoxy-β-D-ribofuranosyl)-1*H*-imidazole-4-carbonitrile (2i) and 1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-5-carbonitrile (3i): Starting from **1i** (11 mg, 0.12 mmol) and thymidine (115 mg, 0.47 mmol) in the presence of NDT (350 µg) at 50°C for 2 h (67% conversion). **2i**: 13.7 mg (56% yield); t_R = 14.95 min (column 2, G5); ^{1}H NMR (DMSO-d_6): δ 2.26-2.41 (m, 2H, H-2', H-2"), 3.52 (m, 2H, H-5', H-5"), 3.84 (m, 1H, H-4'), 4.31 (m, 1H, H-3'), 6.10 (t, *J* = 6.3 Hz, 1H, H-1'), 8.12 (d, *J* = 1.1 Hz, 1H, H-5), 8.31 (d, *J* = 1.1 Hz, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 41.5, 61.9, 70.9, 86.8, 88.6, 112.9, 115.9, 127.9, 139.0; HRMS (ESI-TOF) m/z calcd for [C_9H_{11} N_3O_3+H]: 210.0879, found: 210.0885. **3i**: 0.9 mg (4% yield); t_R = 16.48 min (column 2, G5); ^{1}H NMR (DMSO-d_6): δ 2.36 (ddd, *J* = 3.8, 6.3, 13.5 Hz, 1H, H-2'), 2.51 (m, 1H, H-2"), 3.52 (m, 2H, H-5', H-5"), 3.84 (m, 1H, H-4'), 4.31 (m, 1H, H-3'), 4.89 (br s, 1H, OH-5'), 5.37 (br s, 1H, OH-3'), 6.12 (t, *J* = 6.3 Hz,

View Article Online
DOI: 10.1039/C7OB01850A

1H, H-1'), 7.89 (d, *J* = 1.1 Hz, 1H, H-4), 8.31 (d, *J* = 1.1 Hz, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 40.5, 61.7, 70.8, 86.2, 88.7, 103.3, 111.9, 140.6, 141.2; HRMS (ESI-TOF) m/z calcd for [C_9H_{11} N_3O_3+H]: 210.0879, found: 210.0885.

Methyl 2-[1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazol-4-yl]acetate (2j) and methyl 2-[1-(2-deoxy-D-ribofuranosyl)-1*H*-imidazol-5-yl]acetate (3j): Starting from **1j** (7 mg, 0.05 mmol) and thymidine (48 mg, 0.22 mmol) in the presence of NDT (315 µg) at 50°C for 4 h (84% conversion). **2j**: 5.9 mg (42% yield); t_R = 12.95 min (column 2, G3); ^{1}H NMR (DMSO-d_6): δ 2.20 (ddd, *J* = 3.2, 6.3, 13.4 Hz, 1H, H-2'), 2.31 (ddd, *J* = 5.7, 7.5, 13.4 Hz, 1H, H-2"), 3.49 (m, 2H, H-5', H-5"), 3.53 (s, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.79 (m, 1H, H-4'), 4.28 (m, 1H, H-3'), 4.88 (br s, 1H, OH-5'), 5.23 (br s, 1H, OH-3'), 5.98 (dd, *J* = 6.1, 7.4 Hz, 1H, H-1'), 7.21 (s, 1H, H-5), 7.80 (d, *J* = 1.3 Hz, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 34.3, 41.2, 52.0, 62.3, 71.2, 86.0, 88.0, 115.5, 134.9, 136.4, 171.6; HRMS (ESI-TOF) m/z calcd for [$C_{11}H_{16}N_2O_5$+Na]: 279.0957, found: 279.0955. **3j**: 5.9 mg (42% yield); t_R = 13.82 min (column 2, G3); ^{1}H NMR (DMSO-d_6): δ 2.21 (ddd, *J* = 3.3, 6.0, 13.4 Hz, 1H, H-2'-C4), 2.31 (ddd, *J* = 6.0, 7.4, 13.4 Hz, 1H, H-2"), 3.47 (m, 2H, H-5', H-5"), 3.63 (s, 3H, CH_3), 3.78 (m, 3H, CH_2, H-4'), 4.28 (m, 1H, H-3'), 4.84 (t, *J* = 5.5 Hz, 1H, OH-5'), 5.23 (d, *J* = 4.1 Hz, 1H, OH-3'), 5.87 (dd, *J* = 6.2, 7.4 Hz, 1H, H-1'), 6.77 (br s, 1H, H-4), 7.87 (br s, 1H, H-2); ^{13}C NMR (DMSO-d_6): ^{13}C NMR (DMSO-d_6): δ 29.8, 41.1, 52.4, 62.1, 71.0, 84.5, 88.0, 124.6, 128.6, 136.0, 170.7; HRMS (ESI-TOF) m/z calcd for [$C_{11}H_{16}N_2O_5$+Na]: 279.0957, found: 279.0951.

1-(2-Deoxy-β-D-ribofuranosyl)-1*H*-imidazole-4-acrylic acid (2l) and 1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-5-acrylic acid (3l): Starting from **1l** (16 mg, 0.11 mmol) and thymidine (112 mg, 0.46 mmol) in the presence of NDT (100 µg) at 50°C overnight (89% conversion). A mixture of **2l** and **3l** (10/1.5): 25.1 mg (85% yield); t_R = 8.21 min (column 2, G5); ^{1}H NMR (DMSO-d_6): δ 2.25 (ddd, *J* = 3.4, 6.2, 13.1 Hz, 1.15H, H-2' **2l** and **3l**), 2.34 (ddd, *J* = 5.9, 7.1, 13.1 Hz, 1.15H, H-2" **2l** and **3l**), 3.50 (m, 2.3H, H-5' and H-5" **2l** and **3l**), 3.81 (m, 1H, H-4' **2l**), 3.85 (m, 0.15H, H-4' **3l**), 4.30 (m, 1.15H, H-3' **2l** and **3l**), 6.03 (t, *J* = 6.0 Hz, 1H, H-1' **2l**), 6.11 (t, *J* = 6.2 Hz, 0.15H, H-1' **3l**), 6.30 (d, *J* = 15.7 Hz, 1H, =CH **2l**), 6.31 (d, *J* = 16 Hz, 0.15H, =CH **3l**), 7.40 (d, *J* = 15.7 Hz, 1H, =CH **2l**), 7.50 (d, *J* = 16 Hz, 0.15H, =CH **3l**), 7.57 (s, 0.15H, H4 **3l**), 7.74 (d, *J* = 0.6 Hz, 1H, H5 **2l**), 7.94 (br s, 1H, H2 **2l**), 8.08 (s, 0.15H, H2 **3l**); ^{13}C NMR (DMSO-d_6): δ 40.3 (**3l**), 41.2 (**2l**), 62.0 (**3l**), 62.2(**2l**), 71.0 (**3l**), 71.1(**2l**), 84.4 (**3l**), 86.1 (**2l**), 88.2 (C4), 88.3 (**3l**), 116.2 (**2l**), 118.2 (**3l**), 121.0 (**2l**), 128.4 (**3l**), 130.0 (**3l**), 132.2 (**3l**), 137.0 (**2l**), 138.0 (**2l**), 138.6 (**2l** and **3l**), 168.1 (**3l**), 168.4 (**2l**); HRMS (ESI-TOF) m/z calcd for [$C_{11}H_{14}N_2O_5$+H]: 255.0981, found: 255.0985.

5-Amino-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-4-carbonitrile (2p) and 4-amino-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-5-carbonitrile (3p): Starting from **1p** (13 mg, 0.12 mmol) and thymidine (116 mg, 0.48 mmol) in the presence of NDT (1.25 mg) at 50°C for 20 h (100% conversion). **2p**: 5.4 mg (20% yield); t_R = 13.31 min (column 1, G1); Spectral data in accordance with previously published data;[25] HRMS (ESI-TOF) m/z calcd for [$C_9H_{11}N_4O_3$+H]: 225.0988, found: 225.0978. **3p:** 16.5 mg (61% yield); t_R = 11.24 min

View Article Online
DOI: 10.1039/C7OB01850A

(column 1, G5); Spectral data in accordance with previously published data;[25] HRMS (ESI-TOF) m/z calcd for $[C_9H_{11}N_4O_3+H]$: 225.0988, found: 225.0985.

5-Cyano-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-4-carboxamide (2q) and 4-cyano-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazole-5-carboxamide (3q): Starting from **1q** (17 mg, 0.12 mmol) and thymidine (118 mg, 0.48 mmol) in the presence of NDT (625 µg) at 50°C for 2 h (51% conversion). **2q**: 3.22 mg (9.7% yield); t_R = 8.56 min (column 1, G5); ^{1}H NMR (DMSO-d_6): δ 2.39 (ddd, *J* = 4.0, 6.3, 10.2 Hz, 1H, H-2'), 2.56 (m, 1H, H-2"), 3.52 (m, 2H, H-5', H-5"), 3.88 (m, 1H, H-4'), 4.34 (m, 1H, H-3'), 4.92 (br s, 1H, OH-5'), 5.38 (br s, 1H, OH-3'), 6.14 (t, *J* = 6.3 Hz, 1H, H-1'), 7.59 (br s, 1H, $CONH_2$), 7.71 (br s, 1H, $CONH_2$), 8.34 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 40.5, 61.5, 70.6, 86.7, 88.9, 104.7, 111.1, 139.4, 145.8, 161.9. HRMS (ESI-TOF) m/z calcd for $[C_{10}H_{12}N_4O_4+Na]$: 275.0756, found: 275.0753. **3q:** 4.25 mg (13% yield); t_R = 9.56 min (column 1, G1); ^{1}H NMR (DMSO-d_6): 2.35 (m, 2H, H-2' and H-2"), 3.56 (m, 2H, H-5', H-5"), 3.85 (m, 1H, H-4'), 4.29 (m, 1H, H-3'), 5.00 (br s, 1H, OH-5'), 5.30 (br s, 1H, OH-3'), 6.29 (t, *J* = 6.1 Hz, 1H, H-1'), 8.1 (br s, 1H, $CONH_2$), 8.2 (br s, 1H, $CONH_2$), 8.34 (s, 1H, H-2); ^{13}C NMR (DMSO-d_6): δ 42.1, 61.4, 70.4, 86.7, 88.6, 114.0, 114.9, 134.6, 139.0, 159.7; HRMS (ESI-TOF) m/z calcd for $[C_{10}H_{12}N_4O_4+Na]$: 275.0756, found: 275.0750.

General procedure for the Suzuki-Miyaura cross coupling reactions

Procedure A: A 1M Na_2CO_3 aqueous solution (0.5 mL) purged with argon were introduced into a mixture purged with argon of **2b** or **3b** (0.1 mmol), boronic acid (0.16 mmol) and $Pd(PPh_3)_4$ (80 mg, 0.005 mmol) in DMF (1.5 mL). The mixture was heated under microwave irradiation (for temperature and reaction time see Table 4). When the reaction was complete, the reaction mixture was filtered and products were isolated from the crude reaction mixture by HPLC on a C18 column followed by lyophilisation.

Procedure B: Under argon, **2b** (0.1 mmol), PPh_3 (1.46 mg, 0.006 mmol), Na_2CO_3 (15.9 mg, 0.15 mmol), and boronic acid **4** (0.15 mmol) were dissolved in water (1 mL). Then $Pd(OAc)_2$ (0.9 mg, 0.004 mmol) was added to the mixture. The mixture was heated under microwave irradiation for 1 h at 120°C. The product **10.1** was isolated from the crude reaction mixture by HPLC on a C18 column followed by lyophilisation.

Procedure C: A mixture of water–acetonitrile (2:1, 1.2 ml) was added through a septum to an argon-purged vial containing **2b** or **3b** (0.1 mmol), boronic acid (0.125 mmol), $Pd(OAc)_2$ (1.12 mg, 0.005 mmol), TPPS (14.2 mg, 0.025 mmol), Na_2CO_3 (32 mg, 0.3 mmol). The mixture was stirred under heating or under microwave irradiation (for temperature and reaction time see Table 4). Products were isolated from the crude reaction mixture by HPLC on a C18 column followed by lyophilisation.

Analytical and spectral data for compounds **10.1** to **10.5**, **10.7** and **11.5** were in accordance with previously published data.[12]

1-(2-Deoxy-β-D-ribofuranosyl)-4-phenyl-1*H*-imidazole (10.1): Following procedure C, 20.4 mg (78% yield).

View Article Online
DOI: 10.1039/C7OB01850A

2-Fluoro-3-[1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazol-4-yl]-pyridine (10.2): Following procedure A, 14.8 mg (60% yield).

5-Bromo-3-[1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazol-4-yl]-pyridine (10.3): Following procedure A, 16.3 mg (48% yield).

2-Amino-4-[1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazol-5-yl]-pyrimidine (10.4): Following procedure C, 19.4 mg (70% yield).

2,4-Di-methyl-4-[1-(2-deoxy-D-ribofuranosyl)-1*H*-imidazol-4-yl]-pyrimidine (10.5): Following procedure C, 18.4 mg (58% yield).

2,4-Di-*tert*-butoxy-5-[1-(2-deoxy-D-ribofuranosyl)-1*H*-imidazol-4-yl]-pyrimidine (10.6): Following procedure A, 31.0 mg (76% yield). Purification by silica gel column, gradient 0% to 12% MeOH in CH_2Cl_2; t_R = 18.79 min (column 1, G6); ^{1}H NMR (DMSO-d_6): δ 1.57 (s, 9H, *t*Bu), 1.67 (s, 9H, *t*Bu), 2.26 (m, 1H, H-2'), 2.35 (m, 1H, H-2"), 3.51 (m, 2H, H-5', H-5"), 3.83 (m, 1H, H-4'), 4.33 (m, 1H, H-3'), 4.89 (t, *J* = 5.1 Hz, 1H, OH-5'), 5.28 (d, *J* = 3.6 Hz, 1H, OH-3'), 6.10 (t, *J* = 6.8 Hz, 1H, H-1'), 7.54 (s, 1H, H-5 Im), 7.93 (s, 1H, H-2 Im), 8.81 (s, 1H, H Pyr); ^{13}C NMR: 28.6, 41,3, 62.3, 71.3, 80.0, 82.6, 86.1, 88.1, 110.6, 116.1, 133.9, 137.0, 155.0, 162.0, 165.5; HRMS (ESI-TOF) m/z calcd for [$C_{20}H_{30}N_4O_5$+H]: 407.2294, found: 407.2276.

5-[1-(2-Deoxy-β-D-ribofuranosyl)-*1H*-imidazol-4-yl]-pyrimidine-2,4-(1*H*,3*H*)-dione (10.7): Following procedure C, 3.5 mg (12% yield).

1-(2-Deoxy-β-D-ribofuranosyl)-5-phenyl-1*H*-imidazole (11.1): Following procedure C, 18.3 mg (70% yield); t_R = 14.07 min (column 1, G6); ^{1}H NMR (DMSO-d_6): δ 2.22 (ddd, *J* = 2.8, 5.8, 13.2 Hz, 1H, H-2'), 2.55 (ddd, *J* = 6.0, 8.1, 13.2 Hz, 1H,H-2"), 3.53 (m, 2H, H-5', H-5"), 3.81 (m, 1H, H-4'), 4.31 (m, 1H, H-3'), 4.92 (br s, 1H, OH-5'), 5.23 (br s, 1H, OH-3'), 5.09 (dd, *J* = 6.0, 7.8 Hz, 1H, H-1'), 7.01 (s, 1H, H-4 Im), 7.41 (m, 1H, H-4 Ph), 7.48 (m, 4H, H-2, H-3, H-5, H-6 Ph), 8.11 (s, 1H, H-2 Im); ^{13}C NMR (DMSO-d_6): δ 40.2, 54.9, 61.7, 70.8, 83.7, 87.7, 127.3, 127.9, 128.5, 128.8, 129.4, 132.7, 136.3; HRMS (ESI-TOF) m/z calcd for [$C_{14}H_{16}N_2O_3$+H]: 261.1239, found: 261.1247.

2-Fluoro-3-[1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazol-5-yl]-pyridine (11.2): Following procedure A, 16.3 mg (60% yield); t_R = 11.56 min (column 1, G6); ^{1}H NMR (DMSO-d_6): δ 2.22 (ddd, *J* = 3.0, 6.0, 13.3 Hz, 1H, H-2'), 2.45 (m, 1H, H-2"), 3.48 (m, 2H, H-5', H-5"), 3.76 (m, 1H, H-4'), 4.28 (m, 1H, H-3'), 4.90 (br s, 1H, OH-5'), 5.23 (br s, 1H, H-3'), 5.73 (dd, *J* = 6.1, 7.7 Hz, 1H, H-1'), 7.11 (br s, 1H, H-4 Im), 7.51 (ddd, *J* = 2.0, 4.8, 7.1 Hz, 1H, H-5 Pyr), 8.06 (ddd, *J* = 2.0, 7.4, 9.7 Hz, 1H, H-4 Pyr) 8.18 (d, *J* = 0.9 Hz, 1H, H-2 Im), 8.32 (ddd, *J* = 1.1, 1.9, 4.8 Hz, 1H, H-6 Pyr); ^{13}C NMR (DMSO-d_6): δ 41.1, 62.1, 71.2, 84.7, 88.2, 112.7 (d, *J* = 31 Hz), 122.9 (d, *J* = 4 Hz), 124.5 (d, *J* = 4 Hz), 130.3, 137.6, 143.1 (d, *J* = 3 Hz), 148.0 (d, *J* = 14 Hz), 160.2 (d, *J* = 236 Hz); HRMS (ESI-TOF) m/z calcd for [$C_{13}H_{14}FN_3O_3$+H]: 280.1097, found: 280.1094.

5-Bromo-3-[1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazol-5-yl]-pyridine (11.3): Following procedure A, 14.1 mg (40% yield); t_R = 11.72 min (column 1, G6); ^{1}H NMR (DMSO-d_6): δ 2.26 (ddd, *J* = 3.4, 6.2, 13.4 Hz,

View Article Online
DOI: 10.1039/C7OB01850A

1H, H-2'), 2.61 (ddd, *J* = 6.2, 7.6, 13.4 Hz, 1H, H-2"), 3.49 (m, 2H, H-5', H-5"), 3.82 (m, 1H, H-4'), 4.30 (m, 1H, H-3'), 4.89 (t, *J* = 5.5 Hz, 1H, OH-5'), 5.27 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.89 (dd, *J* = 6.2, 7.4 Hz, 1H, H-1'), 7.23 (d, *J* = 0.9 Hz, 1H, H-4 Im), 8.18 (br s, 1H, H-2 Im), 8.19 (t, *J* = 2.0 Hz, 1H, H-6 Pyr), 8.70 (d, *J* = 1.9 Hz, 1H, H-4 Pyr), 8.73 (d, *J* = 1.9 Hz, 1H, H-2 Pyr); ^{13}C NMR (DMSO-d_6): δ 40.2, 62.1, 71.2, 84.3, 88.4, 120.7, 128.0, 128.5, 130.2, 138.0, 147.7, 149.8; HRMS (ESI-TOF) m/z calcd for [$C_{13}H_{14}{}^{79}BrN_3O_3$+H]: 340.0297, found: 340.0298, for [$C_{13}H_{14}{}^{81}BrN_3O_3$+H]: 342.0278, found: 342.0284.

2-Amino-5-[1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazol-5-yl]-pyrimidine (11.4): Following procedure C, 16.8 mg (60% yield); t_R = 7.20 min (column 1, G1); ^{1}H NMR (DMSO-d_6): δ 2.22 (ddd, *J* = 3.1, 6.1, 13.3 Hz, 1H, H-2'), 2.54 (ddd, *J* = 6.1, 8.0, 13.4 Hz, 1H, H-2"), 3.49 (m, 2H, H-5', H-5"), 3.79 (m, 1H, H-4'), 4.28 (m, 1H, H-3'), 4.89 (br s, 1H, OH-5'), 5.25 (d, *J* = 3.7 Hz, 1H, OH-3'), 5.77 (dd, *J* = 6.1, 8.0 Hz, 1H, H-1'), 6.89 (s, 2H, NH_2), 6.99 (d, *J* = 0.9 Hz, 1H, H-4 Im), 8.09 (d, *J* = 0.9 Hz, 1H, H-2 Im), 8.29 (s, 2H, H-4, H-6 Pyr); ^{13}C NMR (DMSO-d_6): δ 40.3, 62.2, 71.2, 84.1, 88.2, 112.6, 127.8, 128.2, 136.6, 158.2, 163.5; HRMS (ESI-TOF) m/z calcd for [$C_{12}H_{15}N_5O_3$+H]: 278.1253, found: 278.1257.

2,4-Di-methyl-5-[1-(2-deoxy-D-ribofuranosyl)-1*H*-imidazol-4-yl]-pyrimidine (11.5): Following procedure A, 14.8 mg (46% yield).

2,4-Di-*tert*-butoxy-5-[1-(2-deoxy-D-ribofuranosyl)-1*H*-imidazol-5-yl]-pyrimidine (11.6): Following procedure A from 0.05 mmol of **3b**, 16.2 mg (80% yield). Purification by silica gel column, gradient 0% to 12% MeOH in CH_2Cl_2; t_R = 17.28 min (column 1, G6); ^{1}H NMR (DMSO-d_6): δ 1.55 (s, 9H, *t*Bu), 1.60 (s, 9H, *t*Bu), 2.18 (ddd, *J* = 2.6, 5.7, 13.1 Hz, 1H, H-2'), 2.43 (ddd, *J* = 5.8, 8.1, 13.4 Hz, 1H, H-2"), 3.47 (m, 2H, H-5', H-5"), 3.74 (m, 1H, H-4'), 4.28 (m, 1H, H-3'), 4.89 (t, *J* = 5.0 Hz, 1H, OH-5'), 5.22 (d, *J* = 4 Hz, 1H, OH-3'), 5.66 (dd, *J* = 5.8, 8.1 Hz, 1H, H-1'), 6.91 (s, 1H, H-4 Im), 8.08 (s, 1H, H-2 Im), 8.16 (s, 1H, H Pyr); ^{13}C NMR (DMSO-d_6): δ 28.4, 28.5, 41.1, 62.2, 71.3, 80.8, 83.0, 84.4, 87.9, 105.9, 125.3, 128.9, 136.8, 159.5, 164.1, 167.7; HRMS (ESI-TOF) m/z calcd for [$C_{20}H_{30}N_4O_5$+H]: 407.2294, found: 407.2309.

On-pot two-step procedure

A mixture of 4-iodoimidazole (20 mg, 0. 1 mmol), thymidine (100 mg, 0.04 mmol) and NDT (2 mg) in 10 mM citrate buffer (pH 6.0, 8 mL) was heated at 50°C over 2 h 30 (80% conversion by LC-MS monitoring). Enzyme denaturation was performed by heating for 1 min at 100 °C under MW irradiation. The crude reaction mixture was then added to through a septum to an argon-purged vial containing boronic acid (0.125 mmol), $Pd(OAc)_2$ (1.12 mg, 0.005 mmol), TPPS (14.2 mg, 0.025 mmol) and Na_2CO_3 (32 mg, 0.3 mmol) in acetonitrile (4 mL). The mixture was heated for 5 min at 120°C under MW irradiation. Products were isolated from the crude reaction mixture by reverse phase HPLC followed by lyophilisation, affording **10.1** (8.4 mg, 32%) and **11.1** (6.2 mg, 24%).

View Article Online
DOI: 10.1039/C7OB01850A

Acknowledgements

The authors gratefully acknowledge financial support from the Institut Pasteur and CNRS.

Notes and references

NMR spectra of new compounds are given as supplementary information.

1. L. P. Jordheim, D. Durantel, F. Zoulim and C. Dumontet, *Nat. Rev. Drug Discov.*, 2013, **12**, 447-464.
2. E. De Clercq, *Cur. Med. Chem.*, 2015, **22**, 3866-3880.
3. A. K. Prasad, S. Trikha and V. S. Parmar, *Bioorg. Chem.*, 1999, **27**, 135-154.
4. I. A. Mikhailopulo and A. I. Miroshnikov, *Acta Naturae*, 2010, **2**, 36-59.
5. I. A. Mikhailopulo and A. I. Miroshnikov, *Mendeleev Commun.*, 2011, **21**, 57-68.
6. A. Fresco-Taboada, I. Mata, M. Arroyo and J. Fernández-Lucas, *Appl Microbiol Biotechnol*, 2013, **97**, 3773-3785.
7. D. Betbeder and D. W. Hutchinson, *Nucleosides Nucleotides*, 1990, **9**, 569-577.
8. S. Pochet, L. Dugué, A. Meier and P. Marlière, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 1679-1684.
9. S. Pochet and L. Dugué, *Nucleosides Nucleotides*, 1998, **17**, 2003-2009.
10. M. Müller, L. K. Hutchinson and F. P. Guengerich, *Chem. Res. Toxicol.*, 1996, **9**, 1140-1144.
11. W. Ye, D. Paul, L. Gao, J. Seckute, R. Sangaiah, K. Jayaraj, Z. Zhang, P. A. Kaminski, S. E. Ealick, A. Gold and L. M. Ball, *PLoS ONE*, 2014, **9**, e115082.
12. S. Vichier-Guerre, L. Dugué, F. Bonhomme and S. Pochet, *Org. Biomol. Chem.*, 2016, **14**, 3638-3653.
13. K. L. Seley, L. Zhang and A. Hagos, *Org. Lett.*, 2001, **3**, 3209-3210.
14. K. L. Seley, S. Salim, L. Zhang and P. I. O'Daniel, *J. Org. Chem.*, 2005, **70**, 1612-1619.
15. O. R. Wauchope, M. Velasquez and K. Seley-Radtke, *Synthesis*, 2012, **44**, 3496-3504.
16. S. Zimmermann, E. Neill, G. Ebiloma, L. Wallace, H. De Koning and K. Seley-Radtke, *Molecules*, 2014, **19**, 21200-21214.
17. H. L. Peters, D. Jochmans, A. H. de Wilde, C. C. Posthuma, E. J. Snijder, J. Neyts and K. L. Seley-Radtke, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 2923-2926.
18. M. K. Yates, M. R. Raje, P. Chatterjee, C. F. Spiropoulou, S. Bavari, M. Flint, V. Soloveva and K. L. Seley-Radtke, *Bioorg. Med. Chem. Let.*, 2017, **27**, 2800-2802.
19. K. L. Seley, L. Zhang, A. Hagos and S. Quirk, *J. Org. Chem.*, 2002, **67**, 3365-3373.
20. S. Vichier-Guerre, L. Dugué and S. Pochet, *Tetrahedron Lett.*, 2014, **55**, 6347-6350.
21. J. Holguin-Hueso and R. Cardinaud, *FEBS Letters*, 1972, **20**, 171-173.
22. M. C. Huang, J. A. Montgomery, M. C. Thorpe, E. L. Stewart, J. A. Secrist and R. L. Blakley, *Arch. Biochem. Biophys.*, 1983, **222**, 133-144.
23. D. F. Ewing, A. Holý, I. Votruba, R. W. Humble, G. Mackenzie, F. Hewedi and G. Shaw, *Carbohydr. Res.*, 1992, **216**, 109-118.
24. A. H. Roush and R. F. Betz, *J. Biol. Chem.*, 1958, **233**, 261-266.
25. Z. Kazimierczuk and F. Seela, *Liebigs Ann. Chem.*, 1991, **1991**, 695-697.
26. R. K. Ujjinamatada, R. L. Paulman, R. G. Ptak and R. S. Hosmane, *Bioorg. Med. Chem.*, 2006, **14**, 6359-6367.
27. A. Suzuki, *Ang. Chem. Int. Ed.*, 2011, **50**, 6722-6737.
28. L. A. Agrofoglio, I. Gillaizeau and Y. Saito, *Chem. Rev.*, 2003, **103**, 1875-1916.
29. E. C. Western, J. R. Daft, E. M. Johnson, P. M. Gannett and K. H. Shaughnessy, *J. Org. Chem.*, 2003, **68**, 6767-6774.
30. P. Capek, R. Pohl and M. Hocek, *Org. Biomol. Chem.*, 2006, **4**, 2278-2284.
31. K. Shaughnessy, *Molecules*, 2015, **20**, 9419-9454.

View Article Online
DOI: 10.1039/C7OB01850A

32. N. Fresneau, M.-A. Hiebel, L. Agrofoglio and S. Berteina-Raboin, *Molecules*, 2012, **17**, 14409-14417.
33. P. A. Kaminski, P. Dacher, L. Dugué and S. Pochet, *J. Biol. Chem.*, 2008, **283**, 20053-20059.
34. R. Tawarada, K. Seio and M. Sekine, *J. Org. Chem.*, 2008, **73**, 383-390.