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Syntheses of 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl purine nucleosides via selective glycosylation reactions of potassium salts of purine derivatives with the glycosyl bromide

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ABSTRACT

Syntheses of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-guanine (**1**) and -adenine (**2**) were accomplished from readily available 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose (**3**). A new and efficient approach for the synthesis of 1-α-bromide was developed using the mild bromination of α-1-*O*-benzoate (**3**). Selective coupling reactions of the bromosugar with purine potassium salts followed by derivatization/and or deprotection of the intermediate blocked 2′-fluoro β-arabinonucleosides resulted in formation of the target compounds with high overall yields.

Purine nucleosides with 2′-ara-fluorine substitution are of special interest because the location of the fluorine atom in the β-orientation of the carbohydrate moiety increases their metabolic stability to degradation by purine nucleoside phosphorylase, provides chemical stability of the glycosidic bond under acidic conditions and has an impact on the antiviral and anticancer activities of fluorinated nucleoside analogues. 9-(2′-Deoxy-2′-fluoro-β-D-arabinofuranosyl)guanine (**1**, 2′-F-araG) was found to display anticancer activity against human leukaemic T-cell lines,[1,2] and its adenine analogue (**2**, 2′-F-araA) (Fig. 1) has recently been shown to be more active against the protozoan parasite *Trichomonas vaginalis*[3] than metronidazole, the current drug for treatment of trichomoniasis.

It is also worth noting that 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides (2′-F-araNs) are key materials for the preparation of building blocks for the synthesis of arabinose-derived oligonucleotides which exhibit very promising gene silencing properties. 2′-Deoxy-2′-fluoro-D-arabinonucleic acids (2F′-ANA) can improve the activity of small interfering RNA and possess the enhanced stability of duplexes (2F′-ANA/RNA).[4–7]

The synthetic or chemo-enzymatic routes towards purine 2′-β-fluoro nucleosides have been developed using three main approaches: (a) convergent syntheses utilising 1-α-bromo, -*O*-acyl derivatives of 2-deoxy-2-fluoro-D-arabinofuranose as glycosylating agents in coupling reactions with various purine bases;[1,2,8–12] (b) chemo-enzymatic approaches including the synthesis of 2-deoxy-2-fluoro-D-arabinofuranosyl-α-1-phosphate and enzymatic glycosylation reactions by nucleoside phorsphorylases;[13,14] (c) direct introduction of the C2′-β-fluorine atom via nucleophilic displacement of an activated C2′-α-hydroxyl in selectively protected ribonucleoside using DAST.[15–17]

Various routes to synthesise 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides of the natural purines have been explored. Most of the known methods comprise of the coupling of glycosyl bromides with purine derivatives and further transformations of the intermediate protected N9-β-glycosides to guanine or adenine 2′-F-araN.[1,2,11,18] The major synthetic challenge to be solved for the development of efficient chemical approaches to purine 2′-F-araNs, in contrast to pyrimidine analogues,[19] is the stereo- and regioselective formation of a *N*-β-glycosidic bond in 2′-β-fluoro nucleosides during the glycosylation reaction.

In a continuation of our interest in the synthetic methodology of 2′-fluorosubstituted nucleosides, this letter deals with new syntheses of the purine 2′-F-araNs **1**–**2** via selective anion glycosylation of the purine potassium salt with a bromosugar as the key step starting from available 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose (**3**).

Firstly, an alternative method to the known procedure to prepare bromide **4** from **3** using HBr/AcOH[19] was studied. Bromination of the α-1-*O*-benzoate (**3**) with TMSBr[20] in anhydrous CH_2Cl_2 in the

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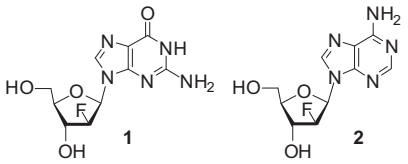


Figure 1. Structures of biologically active purine 2′-β-fluoro nucleosides.

Table 1
Reactions of K-salt of 6-chloro-2-aminopurine with the bromosugar **4**

Entry	Solvent	Ratio (β:α)[a] **5**/**6**	Yield[b] (%)
1	MeCN	9.2:1	61
2	MeCN/DCE	14:1	71

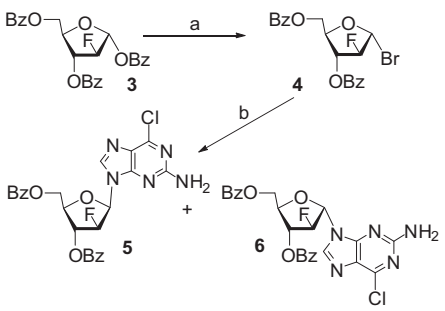
[a] Determined by ^{1}H NMR spectroscopy of the crude product.
[b] Isolated yield of protected purine N9-β-nucleoside.

presence of catalytic $ZnBr_2$, generated the desired 1-α-bromide **4** which was isolated as a colourless oil in 96% yield (Scheme 1). An efficient method developed for synthesis of the glycosyl bromide **4** from perbenzoylated 2-deoxy-2-fluoro-D-arabinofuranose **3** under mild conditions resulted in a single bromo anomer in high yield as in the case of the bromination method[19] with 30% HBr in acetic acid. The explored method will be of use in preparing 1-bromo derivatives from different acylated fluorodeoxy sugars.

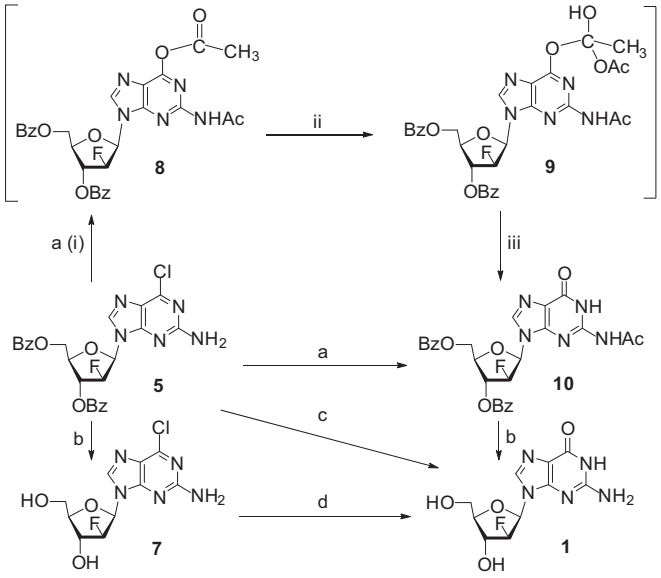
To improve the known synthetic methods for the preparation of **1**[1,2,10,11], the synthesis of the purine analogue **5**[9,18] from 1-α-bromide **4** (Scheme 1) and different conditions for the efficient conversion of the key intermediate to the guanine derivative were examined. Previous investigations on the glycosylations of modified purine bases with halogenoses have provided evidence for nucleobase-anion glycosylation[12,21] preceding with high anomeric selectivity and good yields for the β-glycosylation products. The coupling of potassium salts of halogenated 7-deazapurines or 8-aza-7-deazapurines, generated using powdered KOH[12,22–24] in the presence of tris[2-(2-methoxy)ethyl]amine as catalyst in MeCN, with bromide **4** gave rise to protected β-D-nucleosides in 50–85% isolated yields. It should be also noted that application of the solid–liquid phase-transfer glycosylation reactions of different 7-deazapurine bases resulted in the stereoselective or stereospecific formation of the β-D-anomers; however the main drawback of this approach is the formation of 3′-debenzoylated derivatives of N1-β-nucleosides (9–21%)[12,23] owing to a large excess of potassium hydroxide used in the glycosylation step. In the development of practical synthetic methods to purine 2′-F-araNs, Bauta and co-workers have thoroughly studied the condensation reactions of halogenated purines with bromide **4** in the presence of potassium *t*-butoxide[18,25] in various solvents to afford 2′-β-fluoro N9-β-nucleosides as main products, with the highest anomeric ratios (β/α) achieved in mixtures of solvents. The above results prompted us to employ the potassium salt glycosylation procedure for the synthesis of **5**. The coupling of **4** with the potassium salt of 2-amino-6-chloropurine, produced by the reaction with potassium *t*-butoxide in 1,2-dimethoxyethane (DME) at rt, was performed in anhydrous acetonitrile for 18 h at rt (Table 1). This reaction resulted in the formation of N9-β-anomer **5** and its α-anomer **6** in 61% and 6% yield, respectively, after column chromatography on silica gel (β/α ratio – 9.2/1). The heterogeneous nucleobase anion glycosylation reaction in a mixture of acetonitrile/1,2-dichloroethane (DCE) (entry 2, Table 1) at rt provided a higher yield of **5** (71%) after purification with better β/α selectivity (14/1) compared to previous conditions (entry 1) in acetonitrile and the known synthetic routes described for **5**.[9,11,18] The high anomeric ratio (**5**/**6**) achieved in a mixture of the polar acetonitrile and DCE of the lower polarity (via a preferred S_N2 pathway) allowed us to separate well the desired β-anomer from α-anomer by flash chromatography on silica gel.

Furthermore, three methods for the displacement of the 6-chloro atom of **5** and **7** to give the 6-oxo-functional group were investigated for the preparation of 2′-F-araG (**1**) (Scheme 2). The treatment of nucleoside **5** with 5 equiv of anhydrous NaOAc in a mixture of Ac_2O-AcOH gave rise to the acylated derivative of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine **10**.

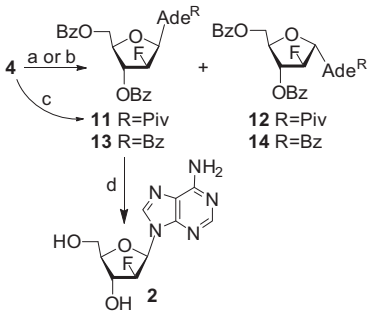
The chemical stability of the glycosidic bond in **5** made it possible to carry out the conversions of the nucleobase under heating (120–125 °C, 150 min) resulting in the formation of guanine nucleoside **10** in high yield (90–95%). The proposed mechanism for this transformation involves the generation of intermediate acetylated product **8** in the first step followed by selective 6-*O*-deacylation via formation of adduct **9** which gives the blocked nucleoside **10** after releasing a molecule of acetic anhydride (Scheme 2, i–iii). The structure of the latter was confirmed by ^{1}H, ^{13}C, ^{19}F NMR data and HRMS. It is noteworthy that Mansuri et al.[26] have briefly reported the preparation of **10** through the condensation of bromide **4** with the silylated N2-acetylguanine albeit without detailed experimental procedures and comprehensive NMR spectral data.



Scheme 1. Reagents and conditions: (a) TMSBr, $ZnBr_2$, CH_2Cl_2, 0 °C → rt, 96%; (b) **4**, K-salt of 2-amino-6-chloropurine, generated using *t*-BuOK in DME, solvent, 18 h, rt (Table 1, entries 1 and 2).



Scheme 2. Reagents and conditions: (a) 5.2 equiv AcONa in Ac_2O/AcOH (1:1, v/v), 120–125 °C; (b) saturated NH_3/MeOH, rt (**1**, 70% overall yield from **5**); (c) 4.3 equiv $HSCH_2CH_2OH$, 4.2 equiv MeONa in MeOH, reflux, (**1**, 72%); (b) saturated NH_3/MeOH, rt (**7**, 75%); (d) ADA, potassium phosphate buffer pH 7.4, rt (**1**, 87%).



Scheme 3. Reagents and conditions: (a) **4**, K-salt of N6-pivaloyladenine, generated using *t*-BuOK in DME, MeCN, CaH_2, rt; (b) **4**, K-salt of N6-pivaloyladenine or N6-benzoyladenine, generated using *t*-BuOK in DME, MeCN/DCE, CaH_2, rt; (c) **4**, K-salt of N6-pivaloyladenine or N6-benzoyladenine/THF, reflux (Table 2, entries 1–5); (d) saturated NH_3/MeOH, rt or MeONa/MeOH, heating (**2** from **11**, 82%; **2** from **13**, 80%).

Compound **10** could be used for the deprotection step without purification.

Removal of the acyl protective groups in **10** with ammonia in methanol for 48 h yielded arabinoside **1** in 70% yield over two steps. N9-β-D-arabinonucleoside **5** was also converted into 2′-F-araG (**1**) by treating with 2-mercaptoethanol and sodium methoxide in refluxing methanol to the target nucleoside **1** in 72% yield after column chromatography on a column of Silica Gel Woelm (20% water). The chlorine atom at the 6th position of purine **7** was converted to a hydroxyl group by utilising the hydrolase activity of adenosine deaminase. Treatment of intermediate **7** with adenosine deaminase from calf intestinal mucosa (ADA) in a phosphate buffer at rt afforded nucleoside **1** in 87% yield after purification on silica gel (Scheme 2). Thus, guanine nucleoside **1** was obtained from halogenose **4** and 2-amino-6-chloropurine in two or three steps with 46–51% overall yields. The nucleobase anion glycosylation[27] with potassium *t*-butoxide has also been utilized in the simultaneous study on the synthesis of purine 2′,3′-difluoro-D-arabinofuranosyl nucleosides, including 2-amino-6-chloropurine and guanine derivatives.

Next, 2′-F-araA (**2**) was prepared by analogy to the study using 2-amino-6-chloropurine (Scheme 3). Heterogeneous reactions of commercially available N6-benzoyladenine or N6-pivaloyladenine[28] and 1-α-bromide **4** were studied. The potassium salt of N6-pivaloyladenine was produced by the treatment of the corresponding purine with potassium *t*-butoxide in DME at 0 °C followed by removal of the solvent under reduced pressure. The glycosylation reaction of the prepared salt of N6-pivaloyladenine with **4** in anhydrous acetonitrile in the presence of calcium hydride for 18 h at rt resulted in the formation of β-anomer **11** and its α-anomer **12**, which were isolated by column chromatography in 54% overall yield (β/α ratio 7.4:1, entry 1, Table 2). Calcium hydride was added to reaction mixture as a drying agent to remove trace amounts of water from the solvents and increase the anomeric ratio during the glycosylation step.[25] The heterogeneous anion glycosylation reaction of N6-pivaloyladenine in a mixture of acetonitrile/DCE at rt provided good β/α selectivity (14:1) (entry 2, Table 2) and high yield of N9-β-nucleoside **11** (61%) along with its α-anomer **12** (5%) after separation by silica gel column chromatography.

The nucleobase anion glycosylation of N6-benzoyladenine with **4** under the similar reaction conditions afforded a more complex reaction mixture from which the N9-β-anomer **13** (48–50%) and its α-anomer **14** (5%) were isolated by column chromatography on silica gel (β/α ratio – 10.3/1, entry 4). The limitations of studied glycosylation reactions are the formation of N9-β- and -α-anomers, and a low solubility of the purine salts in the tested solvents that involve the need for a difficult separation of the isomeric protected nucleosides and require longer reaction times compared to the glycosylation methods explored for 7-deazapurines[22,23] in the presence of KOH, respectively. The pure adenine β-nucleosides **11** and **13** were isolated by column chromatography on silica gel. After crystallisation, the key intermediates were obtained as crystalline products.

From the above glycosylation reactions of purine derivatives it was found that the coupling of **4** with the potassium salts of N6-pivaloyladenine or 2-amino-6-chloropurine proceeded in a stereoselective manner, primarily via an S_N2 pathway, using a mixture of solvents of different types and polarities (acetonitrile/DCE) at ambient temperature to afford good isolated yields of protected β-nucleosides. In addition, the best stereochemical outcomes (β/α ratio – 86/1 and 32/1) were achieved by carrying out the condensation reactions of potassium salts of N6-pivaloyl- or -benzoyladenine with **4** at reflux in the lower polarity solvent THF (entries 3 and 5, Table 2), which is not favourable for S_N1 mechanism leading to β- and α-anomeric nucleosides with a low selectivity via oxonium ion intermediates. β-Anomers **11** and **13** were isolated in moderate yields of 49% and 46%, respectively, after flash chromatographic purification. The deprotection of **11** or **13** gave arabinoside **2** in 80–82% yields. The guanine and adenine nucleosides **1**–**2** were prepared in 36–48% overall yield from **3** using commercially available nucleobases and reagents.

In summary, simple and effective routes for the synthesis of purine 2′-F-araNs from benzoate **3** were described. A new method for preparation of the 1-α-bromosugar **4** was developed. It has been shown that the α-1-*O*-benzoate **3** can be converted to **4** in high yield by treating with TMSBr in the presence of $ZnBr_2$ as catalyst. The potassium salt glycosylation reactions of 2-amino-6-chloropurine and N6-acyladenines with the glycosyl bromide proceeded under mild conditions with high anomeric β-selectivity providing access to N9-β-glycosylated products as key intermediates in the synthesis of the target nucleosides. Convenient methods for the conversions of glycosides of 2-amino-6-chloropurine to the guanine nucleoside were explored. This synthetic methodology of purine nucleosides with the 2′-fluoro-β-D-arabinofuranosyl moiety will be useful for their practical synthesis and suitable for the preparation of novel purine 2′-fluorinated nucleoside analogues of biological interest.

Table 2
Reactions of potassium salts of adenine derivatives with the bromosugar **4** under various conditions according to Scheme 3

Entry	K-salt of purine	Conditions	Time (h)	Anomer ratios (β:α)[a] of prepared nucleosides	Yield[b] (%)
1	N6-pivaloyladenine	MeCN, CaH_2, rt	19	7.4:1 (**11**/**12**)	48
2	N6-pivaloyladenine	MeCN/DCE, CaH_2, rt	19	14:1 (**11**/**12**)	61
3	N6-pivaloyladenine	THF, 74–75 °C	4	86:1[c] (**11**/**12**)	49
4	N6-benzoyladenine	MeCN/DCE, CaH_2, rt	24	10.3:1 (**13**/**14**)	50
5	N6-benzoyladenine	THF, 74–75 °C	4	32:1[c] (**13**/**14**)	46

[a] Determined by ^1H NMR spectroscopy of the crude product in $CDCl_3$.
[b] Isolated yield of protected purine N9-β-nucleoside.
[c] Reaction mixture also contained unreacted bromide **4** (45–50% by ^1H NMR) after work-up.

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Supplementary data

Supplementary data (experimental procedures, characterization data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.11.091. These data include MOL files and InChiKeys of the most important compounds described in this article.

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