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Synthesis of N^4-aryl-β-D-glucopyranosylcytosines: a methodology study

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A number of leaving groups, including arylsulfonates, triazoles, 3-nitrotriazoles, and tetrazoles, have been studied for the substitution reaction by aryl and alkyl amines at the 4-position of β-D-glucopyranosyluracils. Examination of the stability, ease of purification and reactivity in the substitution reaction led to a number of optimized conditions with the most convenient involving substitution of triazole derivatives under microwave conditions in the presence of silica gel. Under these conditions, a number of N^4-aryl-substituted β-D-glucopyranosylcytosines were prepared as potential inhibitors of glycogen phosphorylase, a molecular target for type-2 diabetes mellitus.

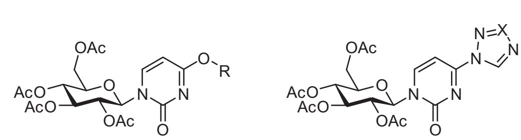
Introduction

Arylsulfonyltriazoles and tetrazoles were utilized during the development of the phosphotriester method for oligonucleotide synthesis, in order to improve the mildness and efficiency of the coupling step. However, it soon became apparent that these reagents also reacted with the unprotected carbonyl groups at O^4 of uridine and O^6 of guanosine, furnishing 4-arylsulfonate, 4-triazole and 4-tetrazole derivatives as side products, thus necessitating the protection of these functional groups.[1] The reactivity of 4-(1,2,4-triazolyl)- and 4-(3-nitro-1,2,4-triazolyl)-pyrimidin-2-one derivatives and their substitution by oxygen and nitrogen nucleophiles was first studied in detail by Reese,[2] who showed that these intermediates could be viable precursors for alkylamine and simple arylamine derivatives. In recent years, this methodology has been extensively used for converting uridine to cytidine[3–5] as well as other N^4-alkyl substituted nucleosides.[6–9]

Glycogen phosphorylase (GP) is a validated molecular target for the development of antidiabetic drugs.[10] In the course of our studies toward the design and synthesis of potent inhibitors of GP,[11,12] we required an efficient and general methodology for substitution of the O^4 position of β-D-glucopyranosyluracil by various arylamines. This interest required us to test the reactivity of a wide range of electron rich and poor arylamines for the synthesis of N^4-aryl-β-D-glucopyranosylcytosines. We report herein the results of a methodology study that was performed in order to determine the best activating group, additive and reaction conditions for the above reaction, which was amenable to a large variety of aryl and alkyl amines.

Results and discussion

Initially, we attempted to apply the conditions developed by Vorbrüggen and coworkers,[13] utilizing O^4-TMS as the leaving group (**1**, Fig. 1). However, reaction of trimethylsilylated 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose with 2-aminofluorene (2AF) did not lead to the formation of any product. It was noted that only primary and secondary alkylamines were utilized by Vorbrüggen whereas



1, R = TMS
2, R = Mst
3, R = TPS

4, X = CNO_2
5, X = CH
6, X = N

Figure 1. O^4-activated β-D-glycopyranosylpyrimid-2-ones studied in this work. (TMS: trimethylsilyl, Mst: 2,4,6-trimethylphenylsulfonyl, TPS: 2,4,6-triisopropylsulfonyl.)

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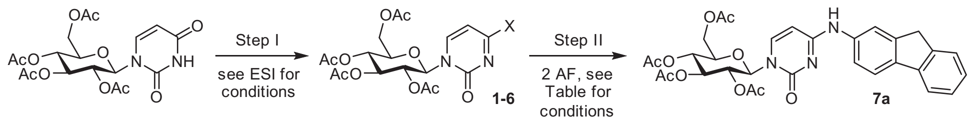
Table 1
Exploration of various activating groups for the reaction of 4-substituted pyrimidin-2-ones with 2AF



Entry	X	Conditions for step II[a]	Temp	Time	Yield % (over two steps)
1	$OSiMe_3$ (**1**)	HMDS	80 °C	10 h	–
2	OMst (**2**) in situ	DCE	rt	Overnight	(47)
3	OTPS (**3**) purified	Dioxane	70 °C	15 min	85 (20)
4	OTPS (**3**) in situ	DCE	70 °C	2 h	(53)
5	Triazole (**5**) purified	Pyridine	115 °C	10 h	55 (53)
6	Tetrazole (**6**) in situ	Pyridine	50 °C	5 d	(47)

[a] 1.5 equiv of 2-aminofluorene (2AF) was used in all reactions.

arylamines were expected to be less reactive (entry 1, Table 1). We then shifted our attention to the examination of various O^4-arylsulfonates.[14] Synthesis of O^4-mesitylenesulfonyl derivative **2** proceeded smoothly in the presence of mesitylenesulfonyl chloride and catalytic DMAP in dichloroethane, however the sulfonated intermediate was not stable enough to be isolated and characterized. Nevertheless, it could be reacted in situ with 2AF giving the desired product in 47% yield over 2 steps (entry 2, Table 1). Due to the instability of the mesitylenesulfonyl derivative we shifted our attention to the more hindered 2,4,6-triisopropylphenylsulfonyl (TPS) derivative. This was prepared using TPS chloride, triethylamine and catalytic DMAP in dichloroethane (rt, 36 h) and was isolated in a modest 23% yield after chromatography. Quantitative ^{1}H NMR of the crude product, after aqueous workup, indicated the presence of the TPS derivative in 57% yield. This suggested that the TPS derivative was also relatively unstable to chromatography.

When the isolated TPS derivative **3** (entry 3, Table 1) was reacted with 2AF in dioxane (70 °C, 15 min) the desired product was isolated in high yield (85%). However, the overall yield for the 2-step procedure was a modest 20%. In an attempt to further improve the yield, we attempted to react the in situ generated TPS derivative with 2AF. Using this one-pot, two-step procedure, we were able to raise the total yield to 53% over 2 steps and at the same time simplify the whole process (entry 4, Table 1). However, in this case, a small amount of a side product was formed (<5%), which was characterized as an N^4,N^4-diethyl cytosine derivative, that had most likely formed from the de-ethylation of a triethylammonium intermediate, since triethylamine remained in the reaction mixture from the initial TPS-formation step.

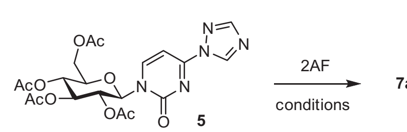
Following Reese's work[2] we then attempted to prepare the 4-(3-nitro-1,2,4-triazolyl)pyrimidin-2-one derivative **4**, which was expected to be a more stable crystalline intermediate. In the original work, an arabinosyl analogue had been prepared using diphenyl phosphorochloridate and 3-nitro-1,2,4-triazole in pyridine[2] and more recently with *p*-tosyl chloride, diphenyl phosphate and 3-nitro-1,2,4-triazole.[15] Under both reaction conditions, we were able to isolate, after chromatographic separation, the previously unreported 3-nitro-1,2,4-triazolyl derivative **4**, in microcrystalline form, albeit in low yield (15%). The low yield and long reaction time (72 h) required for the formation of **4** meant that this compound was not appropriate as a versatile intermediate for the substitution reaction with arylamines. On the other hand, the 4-(1,2,4-triazolyl)pyrimidin-2-one derivative **5** was synthesized using a slight modification of Reese's procedure and was obtained in high yield (95%) and virtually pure after aqueous workup. It was found that changing the sequence of reagent addition by adding Et_3N last, resulted in a faster and cleaner reaction. Compound **5** could easily be obtained as a white microcrystalline product after trituration of the off-white crude product with diethyl ether. When triazole derivative **5** was reacted with 2AF in pyridine at reflux, we observed slow conversion to the desired product leading to a 55% isolated yield after 10 h, without complete consumption of the starting material (entry 5, Table 1).

The last target-intermediate examined in the initial screening was the corresponding tetrazole **6**, which was prepared utilizing a solution of tetrazole in acetonitrile in the presence of diphenyl phosphate and *p*-tosyl chloride in pyridine.[16] Under these conditions we observed slow conversion to **6** (5 d) however the product proved to be unstable upon workup and therefore was reacted in situ with 2AF at 50 °C. Again we observed slow conversion (5 d) giving the desired product in 47% yield after chromatographic separation. It was concluded that the best conditions included either the in situ formation and reaction of TPS derivative **3** or the use of triazole intermediate **5**. (Table 1, entries 4 and 5, respectively). Nevertheless, since the reaction conditions involving **3** had already been optimized, we decided to proceed with optimization of the conditions involving triazolyl-intermediate **5**, using 2AF as a model arylamine.

An initial attempt to methylate derivative **5** using methyl iodide or dimethyl sulfate[17] in order to quaternize the triazole and render it a better leaving group was unsuccessful, leading to low yields and long reaction times. Therefore we studied the effect of a number of additives and solvents (Table 2).

The utilization of Hunig's base (entry 1) or DBU (entry 2) in dichloromethane did not improve the yield of the reaction, however the use of DABCO led to a significant increase in yield when

Table 2
Reaction condition optimization for the substitution of 4-(1,2,4-triazolyl)pyrimidin-2-one by 2AF



Entry	Conditions	Temp	Time	Yield %
1	DCM, DIPEA[a]	40 °C	72 h	55
2	DCM, DBU[a]	40 °C	2 h	40
3	Pyridine, DABCO[b]	115 °C	2.5 h	74
4	DCE, DABCO[b]	84 °C	2.5 h	70
5	Pyridine, MW[b,c]	80–115 °C	0.5 h	72
6	SiO_2, MW[b,d]	70 °C	10 min	90

[a] 2.5 equiv of 2AF was used.
[b] 1.5 equiv of 2AF was used.
[c] The reagents were suspended in pyridine (0.15 mL), MW power output 150 W.
[d] 2 g SiO_2 per mmol of **5** was added, MW power output 150 W.

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