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Synthesis of a Nucleobase-Modified ProTide Library

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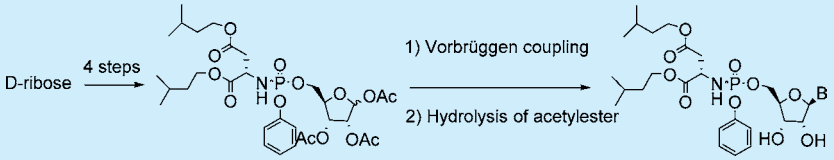
Supporting Information



ABSTRACT: A new method for the construction of (aryloxy)phosphoramidate nucleoside prodrugs is presented. An (aryloxy)phosphoramidate ribose derivative as key building block was used for coupling with a number of nucleobases under Vorbrüggen reaction conditions yielding the protected ProTides in excellent yields. Selective hydrolysis of the acetoxy groups on the sugar moiety afforded a series of the desired ProTides. The advantage of this approach, when compared to classical procedures, is the greater flexibility for achieving structural variety of the nucleobase moiety.

Nucleoside analogues have widespread utility as antiviral and antitumor agents.[1] Their antiviral or antitumor effects depend on the sequential intracellular conversion of the parent nucleoside into the monophosphate, the diphosphate, and then the triphosphate metabolite. In general, for antiviral nucleosides the nucleoside−triphosphate acts as a chain terminator for the viral DNA or RNA polymerase. For anticancer nucleosides, different molecular mechanisms are known, such as inhibition of the human DNA polymerase by the nucleoside triphosphate, inhibition of the ribonucleotide reductase by the nucleoside diphosphate, or inhibition of thymidylate synthase by nucleoside monophosphate.[2] Notably, all these mechanisms require phosphorylation of the nucleoside in the target cells to at least the monophosphate level.[3] One of the most successful approaches to overcome the dependence of bioactive nucleoside analogues on kinase-mediated activation is the ProTide methodology, based on aryloxyphosphoramidate chemistry.[4] It allows one to bypass the first (and often rate limiting) step of kinase-mediated activation of nucleosides. This method has been extensively applied to anti-HIV,[5] anti-HCV,[6] and antitumoral nucleosides.[7] (Aryloxy)phosphoramidate nucleoside prodrugs are generally prepared by coupling of the desired nucleosides with phosphorochloridate by either activation as an imidazolium intermediate by treatment with *N*-Me-imidazole (NMI) or by deprotonation of the primary hydroxyl group of the nucleoside with *t*-BuMgCl and subsequent substitution with the chlorophosphoramidate (Figure 1).[8] Numerous nucleoside prodrugs have been synthesized using one of these procedures. These methodologies present some limitations, mainly in the formation of byproducts, which arise from phosphorylation of unprotected hydroxyl groups on the sugar backbone. In addition, the nature of the nucleobase plays an important role in the reaction behavior. Whereas uracil and adenine as bases are not usually phosphorylated, the preparation of cytosine, guanine, diaminopurine, and hypoxanthine analogues is more problematic due to competitive phosphorylation of the nucleobase and/or poor solubility of the substrate in the reaction solvents. The formation of byproducts has been reduced by appropriate protection of the sugar hydroxyl groups and the competitive sites on the nucleobase moiety.

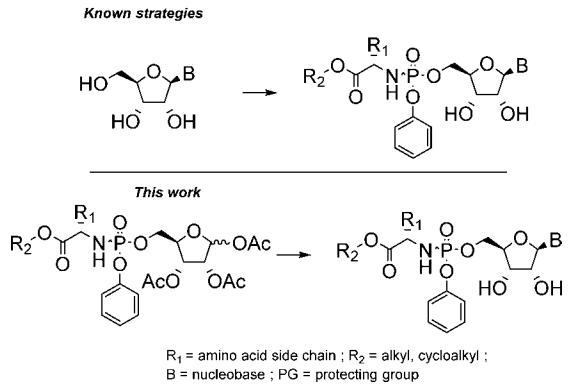


Figure 1. Synthesis of ProTides.

An additional drawback of the current approaches, which has not received much attention in literature, is the fact that these methods require the preparation of a (protected) nucleoside derivative, from which the corresponding phosphoramidate prodrug is synthesized. If necessary, the last step involves removal of the protecting groups on the sugar and/or nucleobase moiety. This is not optimal from a medicinal chemistry standpoint. It is not amenable to high-throughput chemistry. For each new ProTide, a novel nucleoside derivative has to be synthesized, and in the last step, this is converted to its (aryloxy)phosphoramidate prodrug. In the early phases of drug discovery, medicinal chemists prefer synthetic sequences in which structural variety is introduced at a late stage of the synthesis. This allows for large-scale synthesis of a key

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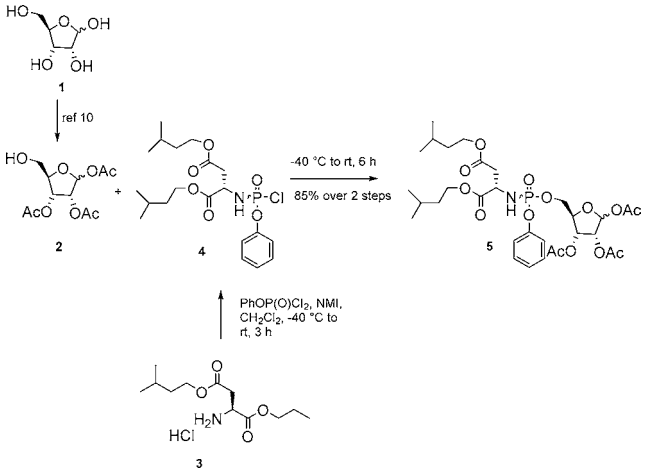
intermediate from which, by a number of simple transformations, structural variety can be introduced. With these limitations in mind, and as part of our ongoing interest in the synthesis of novel ProTides,[9] we sought to develop a strategy that utilizes a key intermediate from which in a single step a variety of nucleobases can be introduced and hence can be used for the assembly of a nucleobase-modified ProTide library. This requires the availability of a protected ribose derivative in which the primary hydroxyl group is derivatized as an (aryloxy)phosphoramidate moiety. The protected D-ribose derivative **2** was prepared from D-ribose **1**, with a slightly modified literature procedure.[10] Protection of the primary hydroxyl group as a monomethoxytrityl group was followed by acetylation of the remaining hydroxyl groups. Acidic deprotection affords the triacetylated D-ribose derivative **2** in 80% yield over three steps. It was feasible to separate the mixture of α- and β-anomers of compound **2** by flash chromatography on silica gel. However, since the configuration of the anomeric hydroxyl group has no influence on the diastereoselective introduction of the base moiety, the mixture **2** was used as such in the next step. Commercially available phenyl phosphorodichloridate was reacted with the diisoamyl esterified L-aspartic acid hydrochloride salt **3**[9a] to afford phosphorochloridate **4**, which was allowed to react in situ with compound **2** in the presence of NMI in anhydrous dichloromethane. The key intermediate **5** was isolated in 85% yield after flash chromatography on silica gel as a mixture of four isomers due to the chirality of the phosphorus atom and the anomeric center of the sugar moiety (Scheme 1). This mixture moves as a single, inseparable

Scheme 1. Synthesis of ProTide Sugar 5

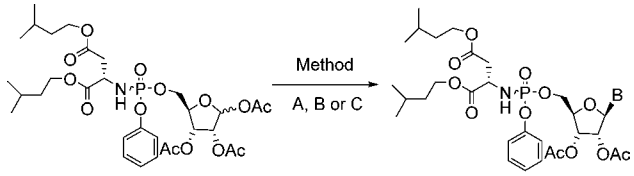


spot on TLC. We gave this sugar derivative **5** the name ProTide sugar. The choice of L-aspartic acid diisoamyl ester as the amino acid motif was dictated by the fact that this often imparts higher antiviral activity to nucleoside analogues, when compared to the classical L-alanine.[9a]

Having key intermediate **5** in hand, we started to explore the reaction conditions for its coupling with a number of nucleobases. In nucleoside chemistry, the Vorbrüggen type of coupling is the method of choice for construction of the glycosidic bond.[11] It is based on the coupling of trimethylsilylated heterocyclic bases with protected sugar derivatives having an acetoxy group at the anomeric center, and it is catalyzed by a Lewis acid. Due to anchimeric assistance from a 2′-acyloxy group, this reaction proceeds diastereoselectively to afford exclusively nucleosides with a β-configuration at the anomeric center. However, depending on the nucleobase and sugar, regioselectivity in the glycosylation can be problematic, leading to regioisomeric mixtures.

Uracil was selected as a prototype nucleobase. Two sets of reactions conditions were explored (Table 1). *N*,*O*-bis-(trimethylsilyl)acetamide (BSA) was selected as the silylating agent, trimethylsilyltriflate (TMSOTf) as the Lewis acid, and

Table 1. Coupling of ProTide Sugar 5 with Nucleobases



5 6a-m

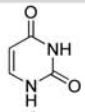
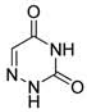
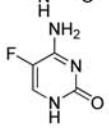
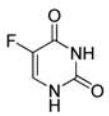
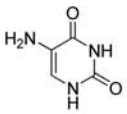
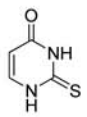
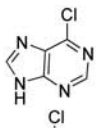
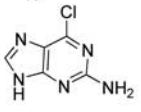
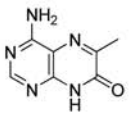
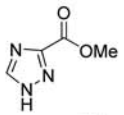
entry	base	product	method/yield (%)
a		**6a**: B= uracil (N-1 isomer)	Method A: 87 Method B : 89
b		**6b**: B= 6-aza-uridine (N-1 isomer)	Method A: 85 Method B: 94
c		**6c**: B= 5-F-cytosine (N-1 isomer)	Method A: 79 Method B: 88
d		**6d**: B= 5-F-uracil (N-1 isomer) **6e**: B= 5-F-uracil (N-3 isomer)	Method B: 89 **6d**/**6e** : 70/18
e		**6f**: B= 5-NH_2-uridine (N-3 isomer)	Method B: 75
f		**6g**: B= 2-thio-uridine (N-1 isomer) **6h**: B= 2-thio-uridine (N-3 isomer)	Method B: 82 **6g**/**6h** : 27/55
g		**6i**: B= 6-Cl-purine (N-9 isomer)	Method A: 92 Method C: 85
h		**6j**: B= 6-Cl-2-NH_2-purine (N-9 isomer)	Method A: 84 Method C: 84
i		**6k**: B= 4-amino-6-methyl-7-oxo-pteridine	Method B: 76
j		**6l**: B= methyl 1H-1,2,4-triazole-3-carboxylate	Method B: 92
k		**6m**: B= 3-cyano-2-oxo-pyridine	Method B: 58

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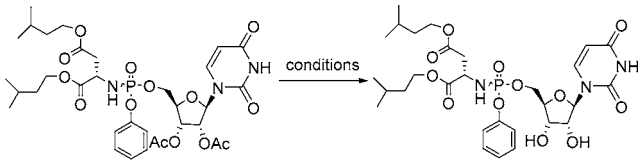
Table 2. Selective *O*-Deacetylation Conditions



entry	conditions	result
a	1 N HCl or 1 N K_2CO_3	rt: recovered starting material; reflux: complex reaction mixture
b	1 N HCl or 1 N K_2CO_3 in CH_3OH	hydrolysis of acetyl and isoamyl esters
c	7 N NH_3 in MeOH/CH_2Cl_2, rt	hydrolysis of acetyl esters and formation of L-aspartic acid dimethyl ester
d	2 N NH_3 in iPrOH, 48 h, rt	hydrolysis of acetyl esters
e	25% aq NH_3, iPrOH, 2 h, rt	hydrolysis of acetyl esters

acetonitrile as the reaction solvent (method A). Because of the presumed lability of the phosphoramidate part, the Vorbrüggen glycosylation reaction was initially run at room temperature. The desired ProTide **6a** was isolated in good yield after a reaction time of 24−48 h. The reaction time could be dramatically reduced, without a significant drop in reaction yield, by running the same reaction at reflux temperature. Alternatively, hexamethyldisilazane (HMDS) could be used as the silylating agent with tin(IV) chloride as a Lewis acid (method B). According to this procedure, the nucleobase was first treated with HMDS in the presence of a catalytic amount of ammonium sulfate, after which the silylated heterocycle was coupled with ProTide sugar **5** in 1,2-dichloroethane or acetonitrile in the presence of $SnCl_4$ to yield ProTide **6a** in excellent yield. In the literature, most of the $SnCl_4$-mediated glycosylation reactions are quenched with a saturated $NaHCO_3$/$KHCO_3$ solution. This very often results in emulsions that are problematic to the workup procedure. However, quenching the reaction mixture with a saturated NH_4Cl solution led to a good separation between the organic and aqueous phases, making extractions much easier. Because of the convenience of this procedure, method B was applied for most of the subsequent compounds. Given the good results obtained with uracil, a set of other pyrimidines was selected (Table 1). The reactions with 6-azauridine (entry b) and 5-F-cytosine (entry c) proceeded regioselectively, and only the N-1 regioisomers were isolated in excellent yields (using both methods A and B). In contrast, when 5-F-uracil was used for the Vorbrüggen condensation, a significant amount of the N-3 glycosylated product was isolated (entry d), which could be easily separated from the N-1 isomer by flash chromatography on silica gel. When 5-aminouracil (entry e) was selected as the nucleobase, only the N-3 glycosylated isomer **6f** was isolated. When the experimental reaction conditions of method A were applied for the coupling of 2-thiouracil with ProTide sugar **5** (entry g), the desired ProTide was not obtained. At room temperature, no reaction took place and only starting material was recovered, whereas at reflux temperature, a complex reaction mixture was formed. When the experimental conditions of procedure B were applied using 1,2-dichloroethane as solvent, this resulted in a 1:2 mixture of the N-1/N-3 regioisomers (82% combined yield).

Besides pyrimidine-like bases, a number of purine nucleobases were also introduced. In the Vorbrüggen coupling of ProTide sugar **5** with 6-chloropurine (entry g) and 2-amino-6-chloropurine (entry h), a mixture of two regioisomers, due to N-7 and N-9 glycosylation, was isolated if the reaction was run at room temperature. When the same reaction was performed at reflux temperature, only the N-9 glycosylated purine derivatives **6i** and **6j** were isolated. An independent proof of regioselectivity was obtained by resynthesis of **6i** and **6j** by a reported procedure (method C).[12] The chlorine-substituted purines are ideal substrates for further transformation into adenine and 2,6-diaminopurine by substitution of the chloride with sodium azide, followed by catalytic hydrogenation yielding an amino group, as reported previously.[13]

In order to demonstrate the general applicability of this approach, a number of modified nucleobases were selected. The synthesis of the pteridine-based nucleoside **6k** proceeded smoothly and in excellent yield (entry i). Glycosylation of methyl 1*H*-1,2,4-triazole-3-carboxylate (entry j) gave the desired ProTide **6l** in excellent yield, whereas glycosylation of pyridin-2(1*H*)-one (entry k) gave a lower yield. On the other hand, 4-chloropyrrolo[2,3-*d*]pyrimidine (also called 6-chloro-7-deaza-purine) did not undergo coupling with ProTide sugar **5** under different Vorbrüggen reaction conditions (data not shown). As it is well known that pyrrolo[2,3-*d*]pyrimidine nucleosides are harder to prepare than the corresponding purine nucleosides, due to reactivity differences of the pyrrole versus the imidazole system, this is not surprising.[14] Having the desired protected ProTides **6a−m** in hand, we turned our attention toward the selective *O*-deacetylation without affecting the diisoamyl ester and phenoxy moieties of the prodrug part. The uridine-containing (aryloxy)phosphoramidate prodrug **6a** was studied as a prototype example (Table 2). Initial attempts focused on mild, water-based, hydrolysis conditions (such as 1 N HCl or 1 N K_2CO_3) at room temperature (entry a). The results were disappointing due to the poor solubility of the substrates in water. Heating the same reaction resulted in complex reaction mixtures. Switching from water to methanol as the reaction solvent (1 N HCl or 1 N K_2CO_3 in methanol) led to concomitant hydrolysis of the acetyl groups on the ribose part and the isoamylester groups in the prodrug moiety (entry b). Encouraging results were obtained when the reactants were subjected to a 7 N ammonia solution in methanol at room temperature using dichloromethane as reaction solvent (entry c). Careful monitoring of the reaction time and concentration of ammonia used was critical for selective deacetylation. Ultimately, employing a 7 N ammonia in methanol solution and dichloromethane in a 1:1 ratio for 12 h at room temperature was found to be optimal for selective hydrolysis of the acetyl ester groups. However, the desired product was always accompanied by the corresponding L-aspartic acid dimethyl ester derivative, formed by transesterification reaction with methanol. Due to their very

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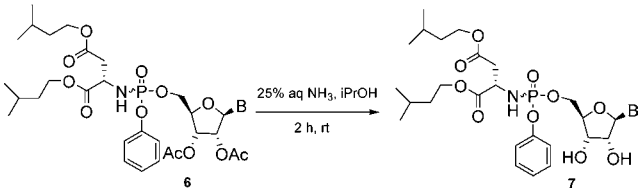
similar polarity, separation of both compounds by silica gel flash chromatography was tedious and difficult.

In an attempt to avoid the formation of this side product, we ran the same reaction in a solvent mixture of 2 N ammonia in 2-propanol and dichloromethane and found that the reaction proceeded sluggishly. However, running the reaction in 2 N ammonia in 2-propanol (without dichloromethane) for 48 h at room temperature (entry d) led to full deprotection of the acetyl groups without formation of transesterification-based products. In an effort to shorten the reaction time, a mixture of 25% ammonia in water and 2-propanol (1:2 volume ratio) was evaluated (entry e). The reaction was run at room temperature, and the acetyl groups were completely deprotected within 2 h to yield the desired product in good yield. These optimized reaction conditions were applied to the protected ProTides **6b−m** (Table 3). The procedure works very well for most of the substrates with isolated yields of 80% or more.

Table 3. Selective *O*-Deacetylation of ProTides 6b−m



entry	substrate	B	product	yield (%)
a	**6b**	6-azauridine (N-1 isomer)	**7b**	83
b	**6c**	5-fluorocytosine (N-1 isomer)	**7c**	85
c	**6d**	5-fluorouracil (N-1 isomer)	**7d**	80
d	**6e**	5-fluorouracil (N-3 isomer)	**7e**	86
e	**6f**	5-aminouridine (N-3 isomer)	**7f**	90
f	**6g**	2-thiouridine (N-1 isomer)	**7g**	82
g	**6h**	2-thiouridine (N-3 isomer)	**7h**	86
h	**6i**	6-chloropurine (N-9 isomer)	**7i**	81
i	**6j**	6-chloro-2-aminopurine (N-9 isomer)	**7j**	80
j	**6k**	4-amino-6-methyl-7-oxopteridine	**7k**	87
k	**6l**	methyl 1*H*-1,2,4-triazole-3-carboxylate	**7l**	85[a]
l	**6m**	3-cyano-2-oxopyridine	complex reaction mixture	

[a]Yield of product **7l** where B = 1,2,4-triazole-3-carboxamide.

In some cases, the formation of overreacted products (due to hydrolysis of the isoamyl esters) was observed on TLC. This was, however, not a major issue since they can be often easily separated from the desired compound by flash chromatography on silica gel. It is noteworthy that the chlorine at position 6 of the purine moiety of compounds **6i** and **6j** is stable under these reaction conditions, affording ProTides **7i** and **7j**, whereas the methyl ester on the imidazole ring of **6j** was transformed into the corresponding carboxamide. On the other hand, for the pyridine analogue **6m**, no desired product was isolated due to the decomposition of the substrate under the reaction conditions.

In summary, a conceptually new method for the construction of (aryloxy)phosphoramidate nucleoside prodrugs is presented. It involves the synthesis of ProTide sugar **5** as a key building block that can be used for coupling with a number of nucleobases, affording exclusively the β-nucleosides, although mixtures of regioisomers were formed in some cases. Finally, a method has been elaborated for the selective hydrolysis of the acetoxy groups on the sugar moiety, leaving the prodrug moiety intact. This methodology is useful for the construction of a ProTide library with structural variation of the nucleobase moiety.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02764.

Experimental procedures and spectral data of compounds **2**, **5**, **6a−m**, and **7a−l** (PDF)

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Notes

The authors declare no competing financial interest.

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