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An Alternative Pathway to Ribonucleoside β-Hydroxyphosphonate Analogues and Related Prodrugs

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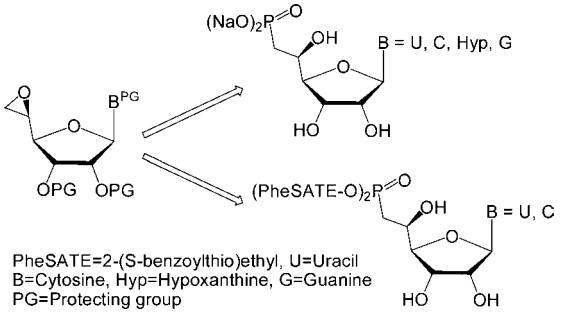
ABSTRACT



Nucleoside β-(*S*)-hydroxyphosphonate analogues have recently proven to be interesting bioactive compounds as 5′-nucleotidase inhibitors. These derivatives were obtained in a pyrimidine series through an ex-chiral pool pathway or the stereoselective reduction of a β-ketophosphonate intermediate. Herein, an original synthesis of these compounds using nucleoside epoxide intermediates, containing either a pyrimidine or a purine as nucleobase, was explored and allowed the direct synthesis of the corresponding bis *S*-acyl-2-thioethyl (SATE) prodrugs.

Nucleos(t)ide analogues are an important family of bioactive compounds widely used as anticancer and antiviral drugs.[1a−c] Nevertheless, their clinical use is currently limited by the appearance of resistance mechanisms mainly associated with nucleos(t)ide metabolism.[1a,d] As an example, the involvement of 5′-nucleotidase II (cN-II) is proposed as a new resistance mechanism to cytotoxic nucleosides.[1d] To date, our research interests have focused on the development of 5′-monophosphate nucleoside mimics containing a nonhydrolyzable P−C bond rather than one with a P−O bond, as potential therapeutic agents.[2] Thus, we previously reported the synthesis of nucleoside β-hydroxyphosphonate analogues from the ex-chiral pool[3] or by reduction of a β-ketophosphonate intermediate[4] (Figure 1). These pathways allowed synthesis of the desired mononucleotide analogues in the pyrimidine series whereas the synthesis of purine analogues remained unachieved, especially for the guanine derivative. Furthermore, extensive assays were performed to prepare the corresponding prodrugs, incorporating biolabile protecting groups such as pivaloyloxymethyl (POM) or *S*-acyl-2-thioethyl (SATE) moieties, using previously described methodologies such as the coupling reaction of a β-hydroxyphosphonic acid (or its salts) with 2-(*S*-benzoylthio)ethanol in the presence of TPSCl or MSNT,[5] an alkylation reaction using pivaloyloxymethyliodide,[6] or the transesterification of

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dimethylphosphonate protecting groups.[7] However, none of these were satisfactory.

Thus, we sought to investigate an original synthetic strategy aiming to obtain the β-hydroxyphosphonate nucleoside from a nucleoside epoxide intermediate, which could be opened by an appropriate phosphite entity giving rise to the expected products.

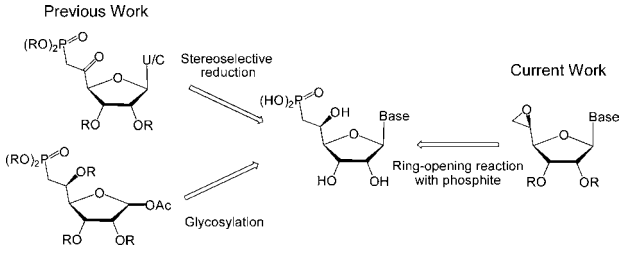

Figure 1. Key steps toward the synthesis of nucleoside β-hydroxyphosphonates.

Although there are abundant literature reports of the ring-opening reactions of epoxides by various nucleophiles,[8] only a few examples describe the use of trialkylphosphites and/or dialkyl phosphite salts.[9] Among the last, most of the reactions involved strong bases[9a,10] and harsh reaction conditions incompatible with the chemical stability of biolabile groups. Thus, the methodology developed herein allows the regioselective formation of β-hydroxyphosphonate nucleosides under mild conditions, compatible with the stability of the future prodrug moiety. It starts from a nucleoside epoxide intermediate and a silylated bis(alkyl)phosphite[9a] in the presence of a Lewis acid such as $BF_3\cdot$etherate.

At first, we examined the key ring-opening reaction using a model substrate (Scheme 1). For this purpose, the epoxide **2** was synthesized from commercially available 1,2:5,6-di-*O*-isopropylidene-D-allofuranose in three steps, including an intramolecular Mitsunobu reaction with retention of configuration.[11]

Optimization of the experimental conditions (selected data are reported in Table 1) showed that either *N,O*-bis(trimethyl)silyl acetamide (*N,O*-BSA, entries 6 and 7) or trimethylsilyl chloride (TMSCl, entries 8 and 9) are efficient reagents for the silylation step, promoting the use of toluene and/or dichloromethane as solvent. This study also revealed that, for an improved reaction yield, the use of all reagents in excess was required.

Scheme 1. Synthesis of the Osidic β-(*S*)-Hydroxyphosphonate Analogue **3**

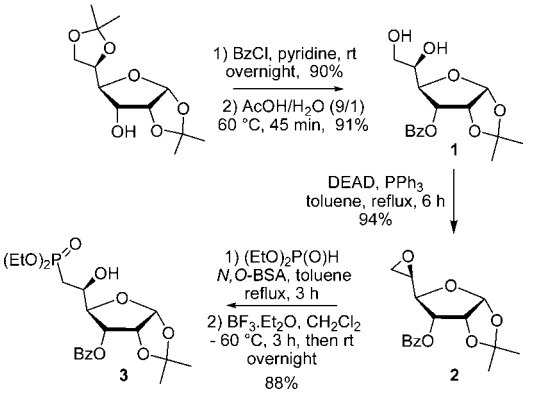

Table 1. Ring-Opening Reaction of Epoxide **2** with Diethyl Phosphite

	reagents					
	silylation step: solvent and *t*	$(EtO)_2P(O)H$ (equiv)	*N,O*-BSA (equiv)	$BF_3\cdot Et_2O$ (equiv)	solvent for **2**	yield[a] (%)
1	toluene, Δ	3	3.2	3	toluene	44
2	toluene, rt	3	3.2	3	toluene	14
3	CH_3CN, Δ	3	3.2	3	CH_3CN	0
4	toluene, Δ	3	3.2	3	CH_2Cl_2	37
5	toluene, Δ	3	3.2	3	CH_3CN	46
6	toluene, Δ	6	6.4	6	CH_2Cl_2	88
7	CH_2Cl_2, Δ	6	6.4	6	CH_2Cl_2	79
8	toluene, rt	6	(TMSCl) 12 (NEt_3) 12	6	CH_2Cl_2	40
9	toluene, rt	6	(TMSCl) 12 (NEt_3) 12	6	CH_2Cl_2	98[b]

[a] Isolated yield of derivative **3** after silica gel column chromatography. [b] Tedious filtration of the reaction mixture containing the silylated phosphite was performed before addition to the epoxide, to eliminate the triethylammonium salts. Δ means that the reaction was carried out under reflux conditions.

Following this, the applicability of the ring-opening reaction to an epoxy-nucleoside intermediate was investigated in the uracil series (Scheme 2). Peracetylation of the 1,2:5,6-di-*O*-isopropylidene-D-allofuranose was performed as previously reported[12] giving rise to compound **4**, as a mixture of α/β anomers, in 88% yield. Uracil was condensed with **4** under Vorbrüggen conditions[13] leading to

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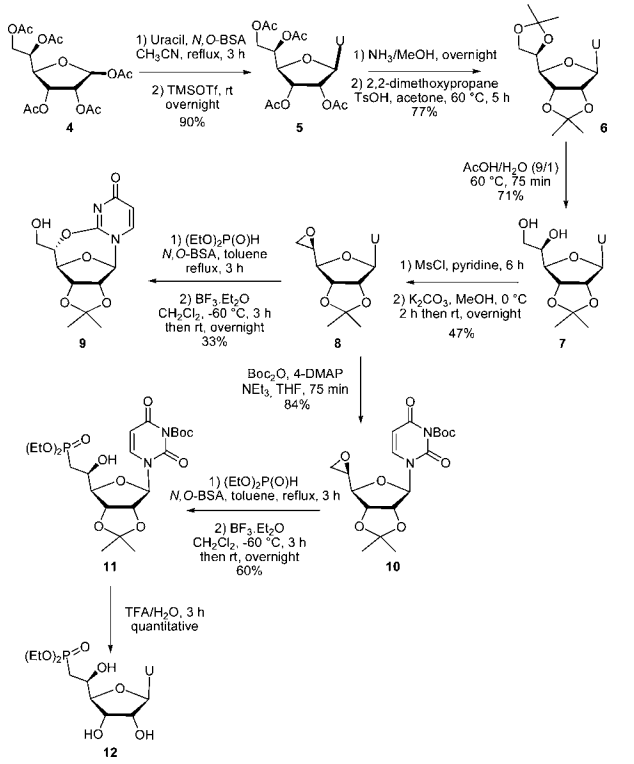
intermediate **5**, as a mixture of α/β products (ratio of 1/3). At this stage, the two anomers could not be separated, and the acetyl protecting groups were replaced by isopropylidene groups using a two-step procedure, thus allowing an efficient separation of the α and β anomers of nucleoside **6** by silica gel chromatography. A selective removal of the hydroxyl protecting groups from the 5′- and 6′-positions led to diol **7** as a precursor of the required epoxide. Treatment of compound **7** under Mitsunobu conditions was performed, but due to purification issues it was only isolated in poor yield. Therefore, the hydroxyl in position 6′ of the diol was selectively mesylated.[14] Intramolecular nucleophilic substitution then gave rise to **8** in 47% yield (Scheme 2). A first attempt of the ring-opening reaction was carried out using previously optimized conditions (Table 1, entry 6). Unfortunately, we only observed formation of the 2,5′-anhydro derivative **9** through an S_N2 process (Scheme 2). To avoid this side reaction, the N-3 position of uracil was protected by a *tert*-butoxycarbonyl (Boc) group,[15] which was cleavable under similar conditions to those of the 2′,3′-isopropylidene protecting group. The fully protected compound **10** was opened with silylated diethyl phosphite under conditions analogous to some of those reported for the opening of **2** in Table 1. When the conditions of Table 1, entries 6 and 9 were applied, the expected β-(*S*)-hydroxyphosphonate **11** was obtained in 60% yield and 65% yield, respectively. Thus, both procedures behaved similarly with nucleoside epoxides as substrates. Finally, the protecting groups were removed with aqueous trifluoroacetic acid, giving rise to derivative **12** quantitatively.

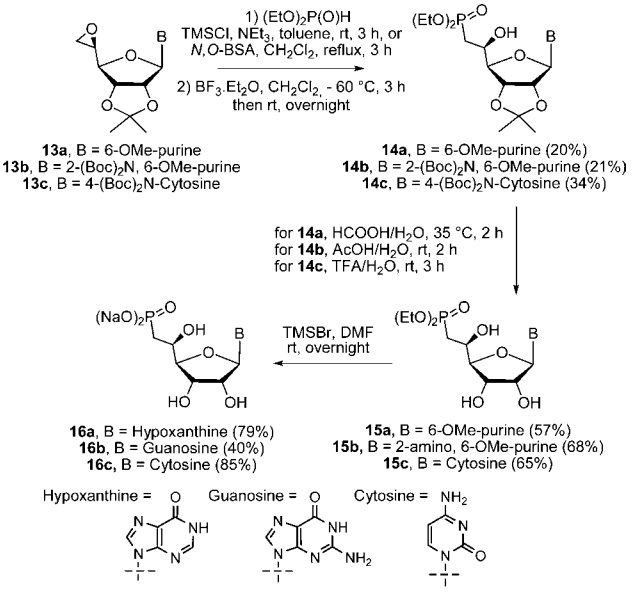
With this synthetic route for the uracil containing derivative established, we extended it to the preparation of the corresponding analogues in the cytosine, hypoxanthine, and guanine series. The first goal was to synthesize the epoxide intermediates (Scheme 3, compounds **13a**–**c**). As for compound **10**, similar synthetic pathways were developed (see SI for details), starting with the condensation of 6-chloro-purine or 2-amino-6-chloro-purine with compound **4** under Saneyoshi[16] or Vorbrügen[13] conditions, respectively. The corresponding cytosine starting material was obtained from the uracil-peracetylated analogue **5**, via a two-step procedure involving the use of Lawesson's reagent.[17]

Thus, the ring-opening reactions of epoxides **13a**–**c** in the presence of silylated diethyl phosphite and of BF_3·etherate were performed under the previously defined conditions, affording the desired β-(*S*)-hydroxyphosphonate **14a**–**c** in lower yields than those for the uracil analogue. We hypothesized the lower stability of the starting material (formation of side-products was observed during the reaction) as well as the influence of steric hindrance on the β-face of the nucleoside, especially for the purine-containing derivatives. Removal of the sugar and nucleobase protecting groups was carried out under acidic conditions. Finally, hydrolysis of the di(ethyl)phosphonoester was accomplished using trimethylsilyl bromide in DMF, with the phosphonic acids **16a**–**c** being isolated as sodium salts.

Scheme 2. Synthesis of the β-(*S*)-Hydroxy(diethyl)phosphonate Analogue of Uridine **12** through a Ring Opening Reaction



Scheme 3. Synthesis of β-(*S*)-Hydroxyphosphonate Derivatives **16a**–**c**



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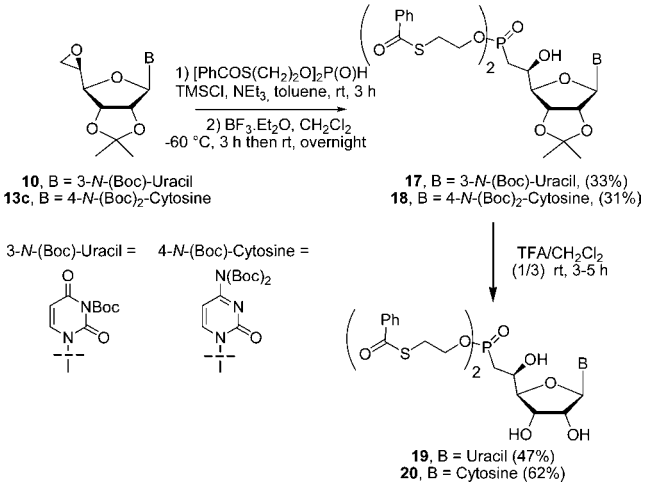
Scheme 4. Synthesis of the Bis(phenyl)SATE Prodrugs of β-(*S*)-Hydroxyphosphonate Derivative in the Uracil and Cytosine Series



As we previously reported that, within the β-hydroxyphosphonate ribonucleoside series, the cytosine-containing analogue was found to be a more potent inhibitor of 5′-nucleotidase II (cN-II),[2b] we applied our strategy to the synthesis of the corresponding bis(phenylSATE) prodrug in a pyrimidine series (compounds **19** and **20**, Scheme 4). The ring-opening reaction was performed using bis(phenylSATE) phosphite (synthesized beforehand from 2-(*S*-benzoylthio)ethanol and PCl_3 in the presence of triethylamine and then *in situ* hydrolysis of the trialkylphosphite by silica, in 87% yield over the two steps).[18] Thus, the fully protected prodrugs **17** and **18** were isolated in 33 and 31% yield, respectively. The 2′,3′-isopropylidene and Boc protecting groups were removed under acid conditions as previously described.

In conclusion, the objective of this work, namely the description of a new strategy for the synthesis of ribonucleoside β-hydroxyphosphonate analogues and of a bis(phenylSATE) prodrug of a cN-II inhibitor of interest, has been met. It is based on the regioselective ring-opening of a nucleoside epoxide by a required phosphite under mild conditions, owing to the potential instability of the biolabile prodrug under nucleophilic and/or basic conditions. This synthetic pathway was first validated and optimized for monosaccharide epoxide before being applied to epoxy-nucleosides. It provided access to a novel β-(*S*)-hydroxyphosphonate analogue of guanosine, which was not available from a previously reported synthetic approach, as well as bis(phenylSATE) prodrugs of the uracil and cytosine series.

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Supporting Information Available. Experimental procedure, Scheme S1, and spectral data for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.