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Stereoselective synthesis of nucleoside monophosphate HepDirect™ prodrugs

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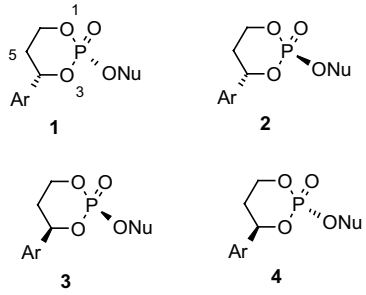
Abstract—Synthesis of HepDirect™ prodrugs of nucleoside monophosphates via phosphorylation with a chiral reagent forms a new asymmetric center at phosphorus and produces two diastereomers. Coupling of chiral phosphoramidite **6** derived from (*S*)-diol **5** with ara-A followed by oxidation of the intermediate phosphite gave ara-AMP prodrugs **8** (4*S*,2*S* isomer) and **9** (4*S*,2*R* isomer). Several methods were explored to identify routes for the selective synthesis of each diastereomer. Two successful stereoselective approaches to ara-AMP prodrugs **8** and **9** are described.

Nucleosides are building blocks of life and are useful in treating infectious diseases and cancers.[1] Nucleoside based drugs are converted in the host to the active form, the nucleoside triphosphate (NTP), by enzymatic reactions that transform the nucleoside first to the nucleoside monophosphate (NMP) and in turn to the diphosphate (NDP) and then to the triphosphate (NTP). Efficacy of most of these drugs is dependent on the efficiency of the phosphorylation or kinase activity to convert the nucleoside to the corresponding NMP. Often, conversion to the monophosphate is the rate-limiting step that dictates the pharmacological activity of these molecules.[2] However, direct introduction of nucleoside monophosphates into the body faces absorption and cell penetration issues due to their ionic nature. Hence, biologically activated NMP esters known as prodrugs could deliver nucleotides to cells by masking the ionic nature of NMPs and bypassing the kinase.[3] While several classes of such NMP prodrug approaches are known, most are inefficient due to their instability and rapid activation outside of the target tissue. To overcome this, a new class of substituted cyclic propyl biocleavable phosphodiesters was designed, wherein the esters are activated by a two-step process.[4] It was shown that these prodrugs are selectively activated by CYP3A4, a cytochrome P-450 isoenzyme, which is abundant in human liver.[5] These substituted cyclic (HepDirect™) prodrugs bypass the nucleoside kinase and are capable of producing higher NTP levels specifically in the liver than the corresponding nucleoside.

Non-selective HepDirect prodrug formation results in four diastereomers when made via coupling of a nucleoside with a phosphorylating agent derived from a racemic 1-arylpropane-1,3-diol. Starting with an enantiomerically enriched diol, phosphorylation results in two diastereomers, which are isomers at the newly formed phosphorus asymmetric center.[6] These two HepDirect nucleoside prodrug isomers are identified as *cis*-[4*S*,2*R* (**1**) or 4*R*,2*S* (**3**)] and *trans*-[4*R*,2*R* (**2**) or 4*S*,2*S* (**4**)] isomers based on the relative stereochemistry of the activating aryl group and the nucleoside (Fig. 1).



Ar = Aryl, Nu = Nucleoside attached through 5'-alcohol

Figure 1.

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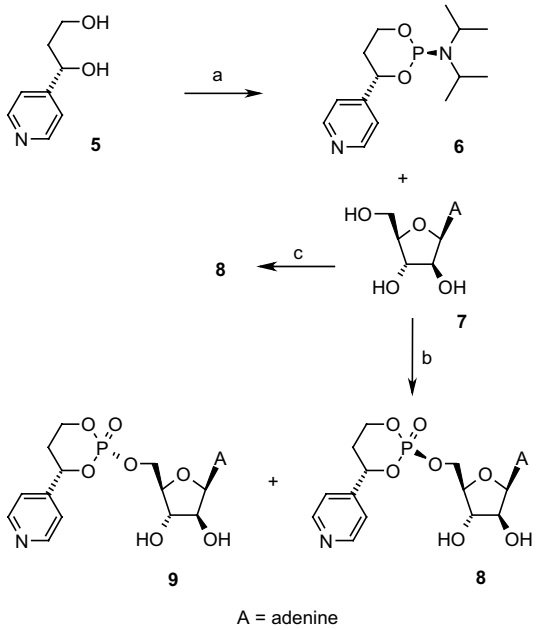
With the advance of our research programs, individual prodrug isomers were required for in vitro and in vivo characterization, as well as more facile drug development. Hence, a new method was needed for the stereoselective synthesis of *cis*- and *trans*-isomers of HepDirect NMP prodrugs.

While there are several methods available to prepare enantiomerically pure substituted 1-aryl-propane-1,3-diols,[7] there are no reported methods to selectively generate the stereogenic phosphorus center in 4-substituted-2-oxo-1,3,2-dioxa-phosphorane systems. We initiated a program to explore optimal methods to selectively prepare both diastereomers resulting from phosphorylation of a chiral diol. Phosphorylation of nucleosides via P(III) chemistry is a well known and facile process to form a P–O bond.[8] The initial objective was to apply this method to examine any preference in the formation of ara-AMP HepDirect prodrug isomers (**8** or **9**). Enantiomerically enriched (*S*)-1-(4-pyridyl)-propane-1,3-diol (**5**) was made by a lipase-mediated resolution in the presence of porcine pancreatic lipase (PPL) and vinyl acetate.[7b,c] The resolution was carried out at the diacetate stage, resulting in 35–40% conversion and >95% ee. Conversion and ee were monitored by HPLC and NMR in the presence of $Eu(hfc)_3$. Hydrolysis of the acetate groups resulted in enantiomerically enriched diol **5**.[7e]

Phosphoramidite **6** was synthesized from diol **5** by reaction with commercially available diisopropylphosphoramidous dichloride (Scheme 1). Compound **6** was found to be a stable intermediate, which was isolated following a silica gel column chromatography in 48% yield as a low melting solid. Phosphorylation of commercially available ara-A (**7**) with phosphoramidite **6** was carried out in the presence of the highly reactive coupling agent benzimidazolium triflate.[9] The reaction rates were found to be comparable with commercially available 5-(methylthio)-1*H*-tetrazole, whereas the phosphorylation was much slower with the conventional agent, tetrazole. The phosphorylation was instantaneous with benzimidazolium triflate resulting in the coupled phosphite, which was oxidized in situ by slow addition of ^{t}BuOOH at −40 °C. Purification by column chromatography provided the ara-AMP prodrug in 78% yield, which was shown to be a mixture of two diastereomers by NMR (^{1}H and ^{31}P).

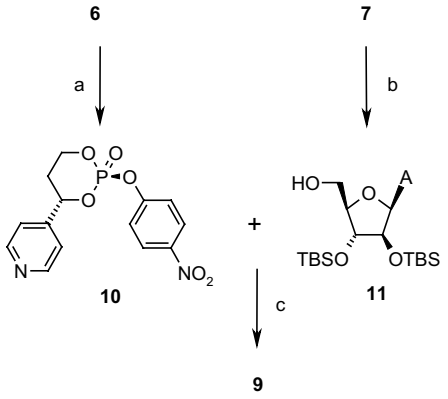


Scheme 1. Reagents and conditions: (a) diisopropyl phosphoramidous dichloride, THF, Et_3N, −78 to 25 °C, 48%; (b) (i) benzimidazolium triflate, DMF, 0 °C, 10 min; (ii) 5–6 M TBHP in decane, −40 to 25 °C, 78%; (c) benzimidazolium triflate, DMF, 60 °C, 3 h; (ii) 5–6 M TBHP in decane, 65%.

HPLC and NMR analysis of the reaction mixture indicated that the two diastereomers were formed in nearly equal amounts. Differentiation of *cis*-(**9**) and *trans*-(**8**) isomers by relative orientation of the benzylic methine proton was established by NOE studies on similar prodrugs and literature data for 2-oxo-1,3,2-dioxaphosphorane (6-membered phosphoranes).[10] In general, ^{1}H NMR shift of the benzylic methine proton for the *cis*-isomer (**9**) is downfield by 0.2 ppm compared to the *trans*-isomer (**8**). Phosphorus (^{31}P) NMR also indicated the same trend, resulting in a 1.4 ppm downfield shift in the case of the *cis*-isomer (**9**) compared to the *trans*-isomer (**8**). These effects were most characteristic when DMSO-d_6 was used as the NMR solvent. Attempts to improve the selective formation of one isomer over the other by changing the reaction conditions (e.g., solvent, leaving group, or coupling agents) were ineffective.

It is well known that the P(III) to P(V) oxidation is a stereospecific reaction.[11] Hence, the thermodynamic equilibration of the phosphite mixture obtained from the phosphorylation of ara-A with enantiomerically enriched phosphoramidite (**6**) prior to oxidation was predicted to enable formation of a single stereoisomer at the phosphorus center. Phosphorylation of ara-A by phosphoramidite **6** gave the intermediate mixture of phosphites as described earlier. Thermal epimerization was optimized to give the thermodynamically more stable single phosphite isomer (60 °C, 3 h) without any decomposition of the intermediate phosphite mixture. The conversion was monitored by ^{1}H and ^{31}P NMR. The reaction was then subjected to the stereospecific oxidation resulting in one phosphate isomer in 65% yield. HPLC and NMR analysis indicated the exclusive formation of the *trans*-isomer **8**. This sequence revealed a new method for the stereoselective synthesis of six-membered 2,4-substituted 2-oxo-1,3,2-phosphoranes via thermal equilibration. The procedure provides a method for preparation of diastereomers of structures **2** and **4** exclusively depending on the starting enantiomer of the 1-aryl-propane-1,3-diol.

Alternatively, to gain access to isomers with the *cis*-stereochemistry (as in **1** or **3**), we planned to take advantage of the selective *trans*-phosphate formation method and the known propensity of phosphates to undergo S_N2 type reactions resulting in an inversion of configuration.[6,12] Consequently, a sequence was devised to obtain



Scheme 2. Reagents and conditions: (a) (i) 4-nitrophenol, 5-(methylthio)-1*H*-tetrazole, DMF, 8 h; (ii) 5–6 M TBHP, −40 to 25 °C, 78%; (b) (i) TBSCl, imidazole, DMF, 74%; (ii) TFA–water–THF (1:1:4), 58%; (c) (i) ^{t}BuMgCl, DMF, −10 to 25 °C, 52%; (ii) tetraethylammonium fluoride, THF, 65%.

9 by phosphorylation of the 5′-alkoxide of a nucleoside with an activated *trans*-phosphate intermediate **10**. The *trans*-phosphorylating agent **10** was made as shown in Scheme 2 starting from **6** by phosphorylation of 4-nitrophenol. Equilibration of the phosphite intermediate was optimally achieved by stirring at room temperature for longer reaction times (8 h). Oxidation resulted in formation of the single desired *trans*-isomer of phosphorylating agent **10** in 78% yield. Coupling of the resulting phosphorylating agent **10** with **7** was attempted with several bases (e.g., NaH, LiH, KO^{t}Bu, KNH_2, etc.) under a variety of reaction conditions to effect the desired S_N2 substitution. However, none of these were found to give the *cis*-diastereomer exclusively. Most reactions resulted in mixtures of isomers and/or extensive hydrolysis of phosphate esters. Reactions with NaH formed exclusively the *trans*-isomer in low yield by an unknown reaction mechanism. However, it was found that the 5′-magnesium alkoxide made by treatment of the 2′,3′-^{t}butyldimethylsilyloxy protected nucleoside with ^{t}BuMgCl[13] resulted in the exclusive formation of the desired *cis*-isomer **9** in 52% yield with no scrambling of the phosphorus stereocenter. It was found that 2′,3′-protection was necessary to avoid precipitation of the intermediate magnesium salt, which occurred even in polar solvents such as DMF or *N*-methylpyrrolidine. Deprotection of the silyl groups as shown in Scheme 2 provided *cis*-ara-AMP HepDirect prodrug **9**.

Both DMF and THF were found to be suitable solvents for the coupling reaction. Optimization identified the nitrophenoxy leaving group as ideal (just enough reactivity for complete inversion and yet avoiding epimerization) in comparison to chloro, 4-chlorophenyl, and 2,4-dichlorophenyl groups. Protection by 2′,3′-isopropylidene in the case of ribonucleosides and 2′,3′-silyl for arabino-, xylo-, or deoxy-type nucleosides (obtained via persilylation and selective 5′-desilylation sequence[14]) was also found to be optimal. The method was applicable to a wide variety of both natural and non-natural nucleoside analogs for synthesis of *cis*-prodrug isomers (**1** or **3**).

In summary, we have identified and optimized two methods for preparing single diastereomers of HepDirect prodrugs of ara-AMP. A P(III) strategy using phosphoramidite gave a single phosphite stereoisomer, which upon stereospecific oxidation resulted in the *trans*-phosphate isomer (**8**). A second strategy utilizing the *trans*-phosphorylating agent **10** was delineated and optimized to give the *cis*-diastereomer (**9**) via stereoselective phosphorylation of the 5′-magnesium alkoxide of a 2′,3′-protected nucleoside.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.04.103.

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