Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 7675–7681 **7675**
DOI: 10.1021/jm100817f

Diastereoselective Synthesis of Aryloxy Phosphoramidate Prodrugs of 3′-Deoxy-2′,3′-didehydrothymidine Monophosphate§

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Received July 2, 2010

The first diastereoselective synthesis of aryloxy phosphoramidate prodrugs of 3′-deoxy-2′,3′-didehydrothymidine monophosphate (d4TMP) is reported. In our approach, (*S*)-4-isopropylthiazolidine-2-thione **1** was used as a chiral auxiliary to introduce the stereochemistry at the phosphorus atom. In the last step of the developed reaction sequence, the nucleoside analogue d4T was introduced to a stereochemically pure phosphordiamidate which led to the formation of the almost diastereomerically pure phosphoramidate prodrugs **8a**−**d** (≥95% de). As expected, the individually prepared diastereomers of the phosphoramidate prodrugs showed significant differences in the antiviral activity. Moreover, the difference was strongly dependent on the aryl substituent attached to the phosphoramidate moiety.

Introduction

Chiral phosphorus compounds are important target structures. It has been proven that the configuration at the phosphorus atom has an influence on the biological activity while interacting with biomolecules. Therefore, the stereoselective synthesis of such compounds is an important aim to achieve. Stec et al. reported the oxathiaphospholane method, a nonenzymatic approach that allows the diastereoselective synthesis of phosphorothioate oligonucleotides.[1] Wada et al. developed a new approach to synthesize diastereomerically pure backbone-modified DNA analogues, which is based on the oxazaphospholidine method.[2] Another important application of chiral phosphorus compounds is as pronucleotides that act as lipophilic nucleotide precursors in antiviral chemotherapy. Pronucleotides are membrane-permeable nucleotide precursors that allow the intracellular delivery of nucleoside monophosphates. The 5′-monophosphates are converted into the 5′-di- and 5′-triphosphates, which are the ultimate biological active compounds. Several pronucleotide strategies have been reported in order to improve the biological activity of nucleoside analogues,[3] e.g., the phosphoramidates,[4,5] the *cyclo*Sal-phosphate triesters,[6] the mixed-*S*-acyl-2-thioethyl compounds,[7] the HepDirect technique,[8] and recently the bis(acyloxybenzyl) approach for the intracellular delivery of nucleoside diphosphates.[9] All these pronucleotide approaches use different means of activation, e.g., by enzymatic reactions or chemical cleavage. However, the first four compounds are derivatives of phosphoric acid that contain four different residues attached to the phosphorus center. As a consequence, the phosphorus atom is a chiral center that leads to diastereomers of the pronucleotides due to the additional chiral centers in the nucleoside analogues. So far, all these pronucleotides cannot be prepared in the form of single diastereomers because of lack of control of the stereochemistry at the phosphorus atom during synthesis; e.g., the reported synthesis of the phosphoramidates afforded the prodrugs as a mixture of two diastereomers in an almost 1:1 ratio.[4] Moreover, the chromatographic separation of the diastereomers was often impossible or a very difficult task to achieve. Liquid chromatography on chiral stationary phases provided successful results in the separation of the diastereomers in only a few cases.[10,11] However, in those cases in which it was possible to separate the diastereomers of the phosphoramidates or the *cyclo*Sal compounds, the biological activity was found to be strongly dependent on the configuration at the phosphorus atom.

For example the S_P-diastereomer of the 9-[2-(*R*)-phosphonomethoxypropyl]adenine prodrug (GS-7340) is 10-fold more potent against HIV than the R_P-diastereomer.[12] The biological activity against other viruses like hepatitis C was also dependent on the phosphorus configuration. As mentioned above, phosphoramidates of 2′-*C*-methylcytidine were synthesized as mixtures first and then separated into individual diastereomers using reversed phase HPLC for antiviral evaluation. The antiviral evaluation of these separated diastereomers proved that the more lipophilic diastereomer was also about 8-fold more antivirally active than the other diastereomer.[13] Additionally, not only the antiviral activity but also the cytostatic activity depended on the phosphorus configuration. 1-Naphthyl phosphoramidates of [5-(*E*-bromovinyl)]-2′deoxyuridine were synthesized as a mixture, and after separation, the diastereomers were evaluated in a breast cancer cell line. Again, the S_P-diastereomer was about 10 times more active than the diastereomeric mixture, whereas the R_P-diastereomer was slightly less active than the mixture.[11] All these examples show the importance of the phosphorus configuration on the biological activity. For this reason the development of a diastereoselective synthesis of phosphoramidate prodrugs would be of great interest. Here, we report on a successful chemical approach.

§Dedicated to Professor Jürgen Heck on the occasion of his 60th birthday.

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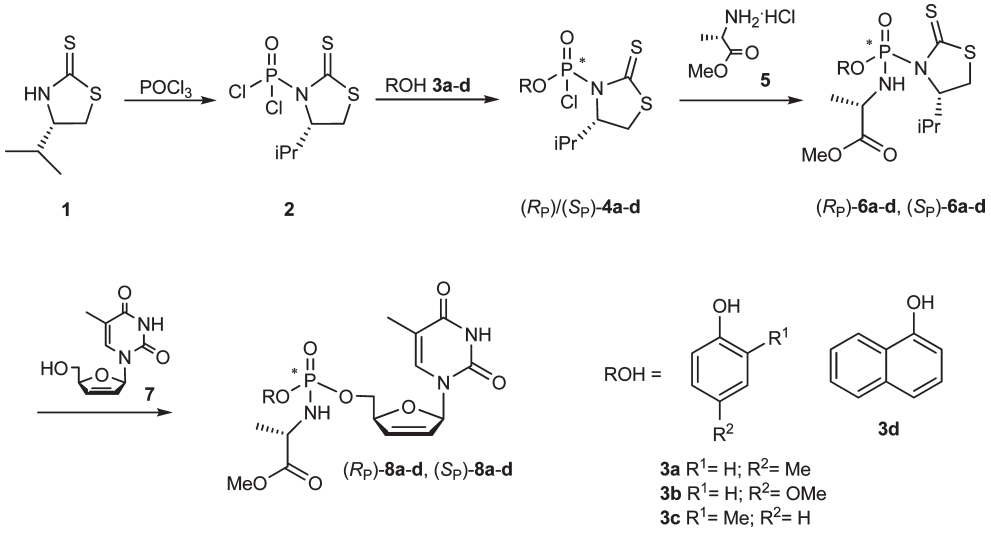
Published on Web 10/14/2010

Scheme 1. General Synthesis Strategy toward Diastereomerically Pure Phosphoramidate Prodrugs (R_P)-**8a**−**d** and (S_P)-**8a**−**d**



Results and Discussion

Chemistry. The diastereoselective synthesis is based on the chiral auxiliary (*S*)-4-isopropylthiazolidine-2-thione **1**. The use of the thiazolidine-2-thione **1** was the result of an in-depth developing process that will be summarized in a further publication. Besides the stereochemical induction, the auxiliary has to fulfill two further important criteria: (a) it should lead to chromatographically separable diastereomers at the phosphordiamidate level; (b) it has to be suitable in the selective replacement by the nucleoside (or an analogue) in the last step of the reaction sequence under mild reaction conditions. Scheme 1 outlines the concept of the synthesis route that was studied using four phenol derivatives **3a**−**d** and the methyl ester of L-alanine **5**.

This approach requires (*S*)-4-isopropylthiazolidine-2-thione **1** as a chiral auxiliary, which was synthesized according to the procedure of Delaunay et al.[14] The chiral auxiliary **1** was reacted in CH_2Cl_2 at 0 °C with 3 equiv of phosphoryl chloride and 1.1 equiv of NEt_3 to give the phosphorodichloridate **2**. ^{31}P NMR spectroscopy of the crude product showed only one phosphorus containing compound (δ = 3.70 ppm). The crude product of phosphorodichloridate **2** was pure enough to be used in the following reaction without further purification. Next, phosphorodichloridate **2** was reacted with phenol derivatives **3a**−**c** and 1-naphthol **3d** to give the chiral phosphorochloridates **4a**−**d** (Figure 1). The diastereomeric ratio of this key step was dependent on the phenol derivatives **3a**−**d** and on the reaction conditions (Table 1).

Phosphorochloridates **4a**−**d** were synthesized by two different methods and were isolated as an up to 17:1 mixture of two diastereomers. According to method 1, acetone and DBU[a] at −91 °C for 25−45 min were used. In method 2 acetone and NEt_3 at −91 °C for 2 h followed by 1 h at room temperature were used in order to complete the reaction. Both methods have an influence on the diastereomeric excess (de) in the cases in which 4-methylphenol **3a** and 4-methoxyphenol **3b** were used. Method 2 gave better diastereomeric excess of phosphorochloridates **4a**,**b** than method 1. In contrast, the use of 2-methylphenol **3c** and 1-naphthol **3d** led to significantly lower diastereoselectivity. Most striking, in the case of 1-naphthol **3d** the diastereoselectivity was completely lost when method 2 was used. The conversion of **2** varied between a moderate 64% to an excellent 93% independent of the method. Compounds **4** were found to be too unstable to be purified or separated into the diastereomers by chromatography.

[a] Abbreviations: DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene.

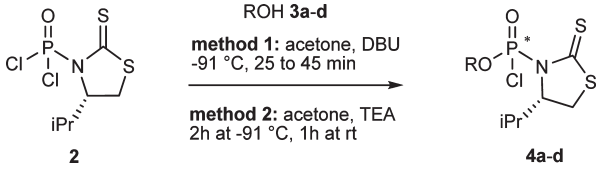


Figure 1. Synthesis of aryl-[(*S*)-4-isopropylthiazolidine-2-thione]-phosphorochloridate derivatives **4a**−**d**.

Table 1. ^{31}P NMR Chemical Shifts and Diastereomeric Excess of **4a**−**d**

		method 1		method 2	
compd	δ (^{31}P) (ppm)[a]	conversion of **2** (%)[b]	% de[b]	conversion of **2** (%)[b]	% de[b]
4a	−1.58, *−1.41*	93	81	77	88
4b	−1.03, *−0.88*	83	72	83	89
4c	−2.16, *−1.66*	64	51	66	28
4d	−1.39, *−1.28*	79	36	90	3

[a] ^{31}P NMR chemical shifts of major diastereomers are in italic font. [b] Conversion and de determined by ^{31}P NMR of the crude mixture.

The synthesis of phosphordiamidates (R_P)- and (S_P)-**6a**−**d** was carried out with L-alanine methyl ester hydrochloride **5** and the diastereomerically enriched mixture of phosphorochloridates **4a**−**d** obtained according to method 1. This L-amino acid was used because previously L-alanine had been identified as being the most effective amino acid to be used in the phosphoramidate approach.[15]

The synthesis required 1 equiv of phosphorochloridate **4a**−**d**, 1 equiv of L-alanine methyl ester hydrochloride **5**, and 3 equiv of NEt_3 in CH_2Cl_2. After the addition of NEt_3 at 0 °C the reaction mixture was stirred for 16 h at room temperature (Figure 2).

Table 2 summarizes the conversion of starting materials **4** and the diastereomeric excess of the phosphordiamidates **6a**−**d**. Again, the results demonstrate that the aromatic residue attached to the phosphorus atom in the first reaction

played an important role with regard to the observed diastereoselectivity. In the case of phosphordiamidates **6a**,**b** the determined diastereomeric excess was identical to that of the precursors **4a**,**b**. However, 2-methoxyphenol and 1-naphthol phosphordiamidate derivatives **6c**,**d** were obtained in even lower diastereoselectivity than phosphorochloridate **4c**,**d**. Nevertheless, at this stage the phosphordiamidates **6a**−**d** were found to be sufficiently stable to be purified and separated in the stereochemically pure diastereomers by column chromatography. The diastereomeric excess of **6a**−**d** was determined by ^{31}P NMR and found to be ≥95%. The diastereomers were first assigned as the "major" and the "minor" diastereomer, which differ in the configuration at the phosphorus atom. Interestingly, except compound **6c** the "minor" diastereomers **6a**,**b**,**d** were crystalline compounds, whereas all "major" diastereomers were obtained as oils. X-ray crystal structure analyses of the minor diastereomers **6a**,**b**,**d** proved the S_P-configuration at the phosphorus atom (Figure 3).[16] Therefore, the "major" diastereomer must have the R_P-configuration.

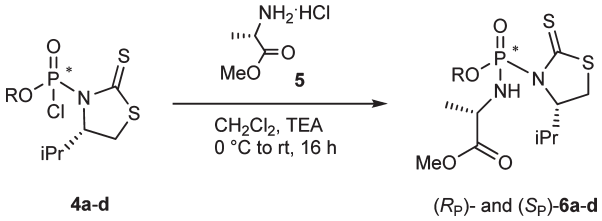


Figure 2. Synthesis of aryl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate derivatives **6a**−**d**.

Table 2. ^{31}P NMR Chemical Shifts and Diastereomeric Excess of **6a**−**d**

compd	δ (^{31}P) (ppm)[a]	conversion of **4** (%)[b]	% de[b,c]
6a	*−0.86*, 1.43	78	81
6b	*−0.54*, 1.82	60	72
6c	*−0.96*, 0.66	54	30
6d	*−0.32*, 1.21	62	28

[a] ^{31}P NMR chemical shifts of the major diastereomers are in italic font. [b] Conversion and de determined by ^{31}P NMR of the crude mixture. [c] The starting materials are the phosphorochloridate derivatives **4a**−**d** obtained with method 1.

Phosphordiamidates (R_P)-**6a**−**d** or (S_P)-**6a**−**d** were reacted separately with 3′-deoxy-2′,3′-didehydrothymidine (d4T, stavudin, Zerit) **7** to give phosphoramidate prodrugs (S_P)-**8a**−**d** and (R_P)-**8a**−**d**, respectively (Figure 4). The reactions were carried out in a 1:1 mixture of THF/CH_3CN, with 1 equiv of phosphordiamidate derivatives **6a**−**d**, 1.5 equiv of d4T **7**, and 3 equiv of *tert*-butylmagnesium chloride. The Grignard reagent was used in analogy to Uchiyama's method.[17]

Table 3 shows the isolated yields and the diastereomeric purity of phosphoramidate prodrugs (S_P)-**8a**−**d**. Taking the generally accepted addition−elimination mechanism for

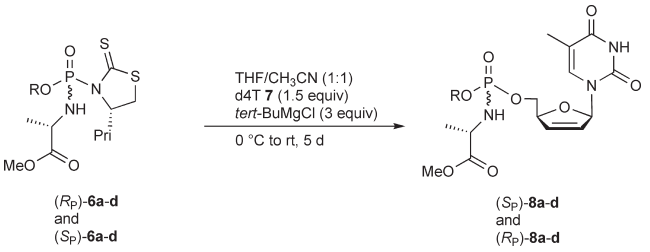


Figure 4. Synthesis of phosphoramidate prodrugs (S_P)- and (R_P)-**8a**−**d**.

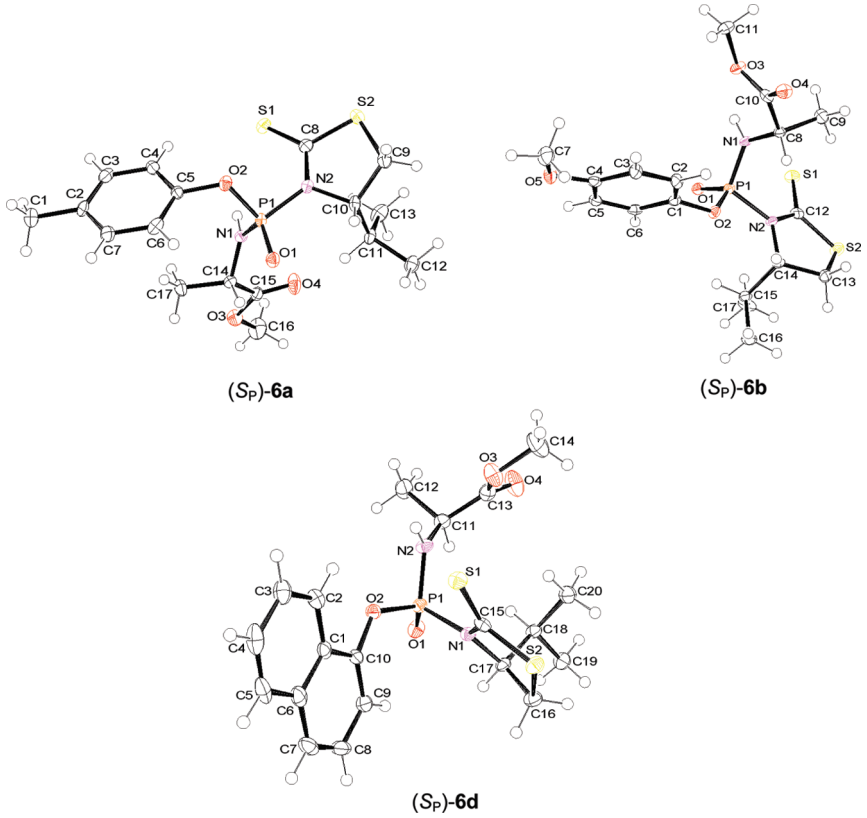


Figure 3. ORTEP plot of phosphordiamidate derivatives (S_P)-**6a**,**b**,**d**.

Table 3. Yields and Diastereomeric Excess of Phosphoramidate Prodrugs (S_P)- and (R_P)-**8a**−**d**

compd	δ (^{31}P) (ppm)	yield[a] (%)	% de[b,c]	compd	δ (^{31}P) (ppm)	yield[a] (%)	% de[b,d]
(S_P)-**8a**	3.25	13	≥95	(R_P)-**8a**	2.59	22	≥95
(S_P)-**8b**	3.59	14	≥95	(R_P)-**8b**	2.96	38	≥95
(S_P)-**8c**	3.01	7	94	(R_P)-**8c**	2.65	30	≥95
(S_P)-**8d**	3.27	16	≥95	(R_P)-**8d**	2.87	29	≥95

[a] Isolated yields. [b] de determined by ^{31}P NMR after purification. [c] The starting materials are the phosphordiamidate derivatives (R_P)-**6a**−**d** with ≥95% diastereomeric excess. [d] The starting materials are the phosphordiamidate derivatives (S_P)-**6a**−**d** with ≥95% diastereomeric excess.

Table 4. Antiviral Activity of Phosphoramidates **8a**−**d**

	EC$_{50}$ (μM)[a]				
	CEM/0[c]				
compd	HIV-1 (III$_B$)	HIV-2 (ROD)	CEM/TK$^-$[d] HIV-2 (ROD)	C3H/3T3 MSV	CC$_{50}$ (μM)[b] CEM/0
(S_P)-**8a**	0.12 ± 0.049	0.14 ± 0.014	0.032 ± 0.022	1.8 ± 0.6	79 ± 6.4
(R_P)-**8a**	0.82 ± 0.0	0.46 ± 0.30	2.1 ± 1.7	1.8 ± 1.2	> 250
(S_P)-**8b**	0.15 ± 0.014	0.14 ± 0.085	0.048 ± 0.0085	2.0 ± 0.0	77 ± 7.8
(R_P)-**8b**	1.1 ± 0.34	0.86 ± 0.049	1.9 ± 2.1	5.3 ± 5.7	235 ± 21
(S_P)-**8c**	1.3 ± 0.21	1.1 ± 0.44	1.9 ± 1.2	9.8 ± 7.0	> 250
(R_P)-**8c**	1.3 ± 0.14	1.3 ± 0.14	2.9 ± 2.3	4.0 ± 2.7	> 250
(S_P)-**8d**	0.15 ± 0.0071	0.16 ± 0.0071	0.13 ± 0.0	0.53 ± 0.13	70 ± 1.4
(R_P)-**8d**	0.12 ± 0.049	0.45 ± 0.52	0.25 ± 0.12	0.72 ± 0.11	107 ± 5.7
d4T **7**	0.84 ± 0.078	0.75 ± 0.49	132 ± 4.9	2.5 ± 1.4	> 250

[a] Antiviral activity in T-lymphocytes: 50% effective concentration. [b] Cytostatic activity: 50% cytostatic concentration. [c] Wild-type CEM/0 cells. [d] Thymidine kinase-deficient CEM TK$^-$ cells.

substitutions at the phosphorus atom into consideration, the stereochemistry in (R_P)-**6** should be inverted to give (S_P)-**8**. This conclusion is supported by studies with phosphoramidates of BVdU.[11] The diastereomeric excess was determined by ^{31}P NMR after column chromatography and found to be equal to or even greater than 95%. In the case of phosphoramidate prodrug (S_P)-**8c** the diastereomeric excess was 94%. Therefore, the values correspond to ≥95% de of the starting material (R_P)-**6a**−**d**. The diastereomeric excess was not determined by ^{31}P NMR of the crude mixture because of the low yields of (S_P)-**8a**−**d**. For comparison the diastereomeric mixture of **8a** was synthesized, according to the published procedure, in moderate yield of 37% in a 1:1.1 ratio.[4] This diastereomeric mixture of phosphoramidate **8a** appears as only one spot on the TLC plate and was found to be inseparable by column chromatography.

Table 3 also summarizes the isolated yields and the diastereomeric purity of phosphoramidate prodrugs (R_P)-**8a**−**d**. The diastereomeric excess was again determined by ^{31}P NMR after column chromatography and corresponded to ≥95% de of the starting material (S_P)-**6a**−**d**. Compared to the reaction using (R_P)-**6**, it is obvious that the reaction of (S_P)-**6** led to twice as high chemical yields for unknown reasons.

Although the reactions gave convincing stereoselectivities, the synthesis of phosphoramidate prodrugs **8a**−**d** is still associated with moderate chemical yields. One reason for the moderate yields is that the starting materials **6a**−**d** and **7** are not completely converted. Even after 5 days the starting materials still remained in considerable amounts in the reaction mixture besides the formed product. It seems that the reaction went to an equilibrium.

Therefore, this fact clearly indicates that the chemical yield of the reaction can be improved. In order to investigate the influence of the base on the reaction, the Grignard reagent *tert*-butylmagnesium chloride was replaced by sodium hydride or DBU. These experiments were done with phosphordiamidate derivative (R_P)-**6a** to get phosphoramidate prodrug **8a** (Figure 4, R = 4-Me-Ph). However, the replacement of the Grignard reagent by NaH or DBU led to significant loss of stereoselectivity at the phosphorus atom. Although the starting material (R_P)-**6a** had a de of ≥95%, the phosphoramidate prodrug **8a** was isolated as a 1:0.6 mixture of the diastereomers without improving the chemical yield (7% and 20%, respectively). Most striking, the complexation of sodium hydride with 15-crown-5 led to a complete racemization at the phosphorus atom (28% chemical yield).

Antiviral Evaluation. (R_P)- and (S_P)-phosphoramidates **8a**−**d** were evaluated for their anti-HIV activity in vitro. All compounds showed activity against HIV-1 and HIV-2 in wild-type CEM/0 cell cultures within the same concentration range as the parental d4T (Table 4). It is also interesting to notice that the S_P and R_P diastereomers fully keep their antiviral potential in thymidine kinase-deficient CEM cell cultures whereas the parental d4T loses its activity by more than 100-fold. This observation points to the efficiency of the prodrugs to intracellularly release d4TMP. Thereby, the need of the presence of TK for drug activation is circumvented.

The anti-HIV activity data of diastereomers **8a**,**b** proved the importance of the diastereoselective synthesis. Diastereomers (S_P)-**8a**,**b** were found to be more active than (R_P)-**8a**,**b** (65-fold in the case of **8a** and 40-fold for **8b** against HIV-2 in CEM/TK-deficient cells). There seems to be a tendency of higher activity in HIV-2 (ROD) infected wild-type CEM cells (~3-fold) of (S_P)-**8a** and (S_P)-**8b** but a lower activity of (R_P)-**8a** and (R_P)-**8b** against HIV-2 in CEM TK$^-$ than in CEM/0 cell cultures. The reason for this observation is unclear. Since CEM TK$^-$ represents a clone derived from CEM/0, it cannot be excluded that uptake or processing of certain diastereomers is more or less efficient than in the wild-type cells than the other diastereomers. However, it remains to be proven whether the observed differences are significant or not. Earlier studies in our laboratories showed that the more active derivative of the separated diastereomers of 3-methyl-*cyclo*Sal-d4TMP (EC$_{50}$ = 0.06 μM) was endowed with an antiviral potency of the same order of magnitude as compounds (S_P)-**8a** and (S_P)-**8b**.[18] Interestingly, the 3-methyl-*cyclo*Sal compound

with the lower antiviral activity lost only 5- to 10-fold of the antiviral activity compared to the more active diastereomer. Surprisingly, the diastereomers **8c**,**d** showed almost identical antiviral values. Since compounds **8** vary only in the attached phenol, there should be a difference in the activation of these compounds. Two possible options can play a role: (i) the ortho-methyl phenyl or the naphthyl substituent interferes differently with the activating enzymes (e.g., carboxyesterases) and/or the particular phenyl/naphthyl substituents induce an altered compound conformation that significantly influences the cellular uptake of the compounds. Differences in the activation process of different phosphoramidates of d4T[19] and other nucleoside analogues[20,21] have already been reported, and thus, this may support the first option. Currently, incubation experiments in CEM-cell extracts are performed to address this possibility. The diastereomeric preference of S_P versus R_P for **8a** and **8b** also seems cell-type-specific, since this effect was not observed for their inhibitory activity against Moloney sarcoma virus (MSV) induced transformation of murine C3H/3T3 cells (Table 4).

Conclusion

A diastereoselective synthesis of phosphoramidate prodrugs **8a**−**d** of d4TMP is described. The synthetic route uses (*S*)-4-isopropylthiazolidine-2-thione **1** as chiral auxiliary, which is converted in three steps into the key intermediates **6a**−**d**. These compounds were obtained in up to 81% de. By column chromatography the diastereomeric purity was increased to ≥95%. X-ray analysis of three different intermediates **6** proved that the R_P diastereomer was obtained preferentially. The phosphordiamidate derivatives (R_P)-**6a**−**d** and (S_P)-**6a**−**d** were reacted separately with d4T **7** to give phosphoramidate prodrugs (S_P)-**8a**−**d** and (R_P)-**8a**−**d** as almost diastereomerically pure compounds (≥95% de). The antiviral tests of diastereomers **8a**,**b** showed significantly different antiviral potencies in CEM/TK-deficient cells and thus verify the importance of the phosphorus configuration on the eventual antiviral activity.

The obtained results proved that the diastereoselective synthesis of phosphoramidate prodrugs **8a**−**d** has been achieved leading to almost diastereomerically pure compounds although the chemical yields of **8a**−**d** require further improvement. Further optimization of this step is now in progress.

Experimental Section

All experiments were conducted under scrupulously dry conditions and under nitrogen atmosphere. Solvents CH_2Cl_2, CH_3CN, and NEt_3 were distilled from CaH_2 and stored over molecular sieves. Et_2O and THF were distilled from sodium or potassium benzophenone and stored over molecular sieves. Acetone was distilled from phosphorus pentoxide and stored over molecular sieves. DBU was distilled under nitrogen and stored over molecular sieves. Petroleum ether 50−70, EtOAc, CH_2Cl_2, and CH_3OH employed in chromatography were distilled before use. For column chromatography, silica gel 60, 230−400 mesh, was used. Thin layer chromatography was performed on precoated aluminum plates 60 F_{254} with 0.2 mm layer of silica gel containing a fluorescence indicator. NMR spectra were recorded on a 400 or 500 MHz spectrometer (AMX 400, 400, or DRX 500). All ^1H and ^{13}C NMR chemical shifts are quoted in ppm and were calibrated on solvent signals. ^{31}P NMR chemical shifts are quoted in ppm using H_3PO_4 as external reference. High resolution mass spectra were obtained with a VG Analytical VG/70-250F spectrometer (FAB, matrix was *m*-nitrobenzyl alcohol). HR-ESI spectra were obtained with an Agilent Technologies ESI-TOF 6224 spectrometer. Analytical HPLC was carried out on a VWR-Hitachi LaChromElite HPLC system consisting of a VWR-Hitachi L-2130 pump, autosampler, and a VWR-Hitachi UV detector L-2455. The column used was a Nucleodur C18 Isis, 5 μm (Macherey-Nagel). Elution was performed using a water/acetonitrile (Sigma-Aldrich, HPLC grade) eluent: 0−80% CH_3CN (0−60 min), 80−0% CH_3CN (60−65 min), 0% CH_3CN (65−70 min), flow rate 0.5 mL/min. UV detection was at 265 nm. The purity of phosphoramidate prodrugs **8** was checked by means of HPLC and was in all cases ≥95%.

Preparation of [(*S*)-4-Isopropylthiazolidine-2-thione]phosphorodichloridate 2. A solution of (*S*)-4-isopropylthiazolidine-2-thione **1** (0.80 g, 4.96 mmol) and phosphoryl chloride (1.39 mL, 14.8 mmol) in CH_2Cl_2 (12 mL) was cooled to 0 °C. NEt_3 (0.76 mL, 5.45 mmol) in CH_2Cl_2 (12 mL) was added dropwise. Following the addition, the reaction mixture was allowed to warm to room temperature and stirred for 16 h. The solvent was removed under reduced pressure, and the residue was suspended in CH_2Cl_2 (1 mL) and Et_2O (15 mL). The precipitated triethylammonium chloride was filtered under nitrogen and washed with Et_2O (5 mL). The solvent of the filtrate was removed under reduced pressure to give the crude product **2** as an oil. Further purification was not possible because of high reactivity, but the ^{31}P NMR spectrum showed only one signal at 3.70 ppm.

General Procedure A: Preparation of Aryl-[(*S*)-4-isopropylthiazolidine-2-thione]phosphorochloridates 4. Method 1. A solution of [(*S*)-4-isopropylthiazolidine-2-thione]phosphorodichloridate (**2**, 1.0 equiv) and the phenol or 1-naphthol derivative (**3**, 1.0 equiv) in acetone was cooled to −91 °C. DBU (1.0 equiv) was added dropwise. After 25−45 min (dependent on **3**) at −91 °C the reaction was quenched with saturated ammonium chloride solution and extracted with CH_2Cl_2 three times. The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (petroleum ether 50−70/EtOAc 2:1).

Method 2. A solution of [(*S*)-4-isopropylthiazolidine-2-thione]-phosphorodichloridate (**2**, 1.0 equiv) and the respective phenol derivative (**3**, 1.0 equiv) in acetone was cooled to −91 °C. NEt_3 (1.0 equiv) was added dropwise. The reaction mixture was stirred 2 h at −91 °C, followed by 1 h at room temperature. The reaction was quenched with saturated ammonium chloride solution and extracted with CH_2Cl_2 three times. The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (petroleum ether 50−70/EtOAc 2:1).

General Procedure B: Preparation of Aryl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidates 6. A suspension of the aryl-[(*S*)-4-isopropylthiazolidine-2-thione]phosphorochloridate derivative (**4**, 1.0 equiv) and L-alanine methyl ester hydrochloride **5** (1.0 equiv) in CH_2Cl_2 was cooled to 0 °C. NEt_3 (3.0 equiv) was added dropwise. Following the addition, the reaction mixture was allowed to warm to room temperature and stirred for 16 h. The reaction was quenched with saturated ammonium chloride solution and extracted with CH_2Cl_2 three times. The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (petroleum ether 50−70/EtOAc 2:1).

General Procedure C: Preparation of 5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(aryl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidates 8. A solution of d4T **7** (1.5 equiv) in THF/CH_3CN (1:1) was cooled to 0 °C. *tert*-Butylmagnesium chloride (3.0 equiv, 1.7 M solution in THF) was added dropwise. Following the addition, the reaction mixture was allowed to warm to room temperature and stirred for 30 min. A solution of aryl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate **6** (1 equiv) in THF/CH_3CN (1:1) was added to the nucleoside suspension at 0 °C. Following the addition, the reaction mixture was allowed to warm to room temperature and stirred for 5 days. The reaction was quenched with saturated ammonium chloride solution and

extracted with CH_2Cl_2 three times. The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH 39:1). The product was freeze-dried.

5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(4-methylphenyl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidate (S_P)-8a. General procedure C was used with 4-methylphenyl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate ((R_P)-**6a**, 0.17 g, 0.4 mmol), d4T **7** (0.13 g, 0.6 mmol), *tert*-butylmagnesium chloride (0.70 mL, 1.2 mmol), and 5.3 mL of THF/CH_3CN. The product (S_P)-**8a** (0.02 g, 13%) was obtained as a colorless foam. ^{1}H NMR [ppm] (400 MHz, $CDCl_3$): δ = 8.52 (brs, 1H), 7.32 (brs, 1H), 7.09 (d, 2H), 7.06−7.01 (m, 3H), 6.37−6.33 (m, 1H), 5.87 (d, 1H), 5.03 (brs, 1H), 4.41−4.26 (m, 2H), 4.02−3.91 (m, 1H), 3.70 (s, 3H), 3.63 (t, 1H), 2.30 (s, 3H), 1.81 (s, 3H), 1.31 (d, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 3.25.

5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(4-methylphenyl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidate (R_P)-8a. General procedure C was used with 4-methylphenyl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate ((S_P)-**6a**, 0.20 g, 0.4 mmol), d4T **7** (0.16 g, 0.7 mmol), *tert*-butylmagnesium chloride (0.87 mL, 1.4 mmol), and 6.5 mL of THF/CH_3CN. The product (R_P)-**8a** (0.05 g, 22%) was obtained as a colorless foam. ^{1}H NMR [ppm] (400 MHz, $CDCl_3$): δ = 8.16 (brs, 1H), 7.26−7.25 (m, 1H), 7.04−7.02 (m, 4H), 7.02−6.98 (m, 1H), 6.30−6.26 (m, 1H), 5.92−5.88 (m, 1H), 5.02−4.97 (m,1H), 4.29−4.24 (m, 2H), 4.05−3.92 (m, 1H), 3.71 (s, 3H), 3.58 (t, 1H), 2.31 (s, 3H), 1.86 (d, 3H), 1.36 (d, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 2.59.

5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(4-methoxyphenyl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidate (S_P)-8b. General procedure C was used with 4-methoxyphenyl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate ((R_P)-**6b**, 0.10 g, 0.23 mmol), d4T **7** (0.08 g, 0.35 mmol), *tert*-butylmagnesium chloride (0.41 mL, 0.69 mmol), and 3.1 mL of THF/CH_3CN. The product (S_P)-**8b** (0.02 g, 14%) was obtained as a colorless foam. ^{1}H NMR [ppm] (400 MHz, $CDCl_3$): δ = 8.68 (s, 1H), 7.32 (d, 1H), 7.10−7.05 (m, 2H), 7.04−7.01 (m, 1H), 6.84−6.79 (m, 2H), 6.37−6.33 (m, 1H), 5.90−5.86 (m, 1H), 5.05−5.00 (m, 1H), 4.40−4.26 (m, 2H), 4.02−3.91 (m, 1H), 3.77 (s, 3H), 3.70 (s, 3H), 3.64 (t, 1H), 1.82 (d, 3H), 1.30 (d, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 3.59.

5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(4-methoxyphenyl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidate (R_P)-8b. General procedure C was used with 4-methoxyphenyl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate ((S_P)-**6b**, 0.11 g, 0.26 mmol), d4T **7** (0.08 g, 0.38 mmol), *tert*-butylmagnesium chloride (0.45 mL, 0.77 mmol), and 3.4 mL of THF/CH_3CN. The product (R_P)-**8b** (0.04 g, 38%) was obtained as a colorless foam. ^{1}H NMR [ppm] (400 MHz, $CDCl_3$): δ = 8.48 (brs, 1H), 7.25−7.24 (m, 1H), 7.14−7.08 (m, 2H), 7.02−6.98 (m, 1H), 6.85−6.79 (m, 2H), 6.29−6.26 (m, 1H), 5.92−5.88 (m, 1H), 5.02−4.96 (m, 1H), 4.29−4.23 (m, 2H), 4.03−3.92 (m, 1H), 3.77 (s, 3H), 3.71 (s, 3H), 3.65 (t, 1H), 1.86 (d, 3H), 1.36 (d, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 2.96.

5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(2-methylphenyl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidate (S_P)-8c. General procedure C was used with 2-methylphenyl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate ((R_P)-**6c**, 0.13 g, 0.31 mmol), d4T **7** (0.10 g, 0.46 mmol), *tert*-butylmagnesium chloride (0.54 mL, 0.92 mmol), and 4.1 mL of THF/CH_3CN. The product (S_P)-**8c** (0.01 g, 7%) was obtained as a colorless foam. ^{1}H NMR [ppm] (400 MHz, $CDCl_3$): δ = 8.36 (brs, 1H), 7.29−7.28 (m, 1H), 7.24−7.21 (m, 1H), 7.20−7.17 (m, 1H), 7.15−7.10 (m, 1H), 7.08−7.05 (m, 1H), 7.04−7.02 (m, 1H), 6.39−6.36 (m, 1H), 5.93−5.90 (m, 1H), 5.07−5.02 (m, 1H), 4.39−4.28 (m, 2H), 4.05−3.95 (m, 1H), 3.72 (s, 3H), 3.60 (t, 1H), 2.26 (s, 3H), 1.74 (d, 3H), 1.34 (d, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 3.01, 2.65 (dr = 44.7:1, 95% de).

5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(2-methylphenyl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidate (R_P)-8c. General procedure C was used with 2-methylphenyl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate ((S_P)-**6c**, 0.15 g, 0.36 mmol), d4T **7** (0.12 g, 0.54 mmol), *tert*-butylmagnesium chloride (0.64 mL, 1.08 mmol), and 4.8 mL of THF/CH_3CN. The product (R_P)-**8c** (0.05 g, 30%) was obtained as a colorless foam. ^{1}H NMR [ppm] (400 MHz, $CDCl_3$): δ = 8.52 (brs, 1H), 7.29−7.26 (m, 1H), 7.22 (d, 1H), 7.21−7.16 (m, 1H), 7.16−7.10 (m, 1H), 7.09−7.03 (m, 1H), 7.02−6.98 (m, 1H), 6.29−6.25 (m, 1H), 5.93−5.87 (m, 1H), 5.02−4.95 (m, 1H), 4.25 (dd, 2H), 4.07−3.95 (m, 1H), 3.71 (s, 3H), 3.70−3.69 (m, 1H), 2.28 (s, 3H), 1.86 (d, 3H), 1.38 (d, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 2.65.

5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(1-naphthyl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidate (S_P)-8d. General procedure C was used with 1-naphthyl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate ((R_P)-**6d**, 0.14 g, 0.31 mmol), d4T **7** (0.10 g, 0.46 mmol), *tert*-butylmagnesium chloride (0.54 mL, 0.92 mmol), and 4.1 mL of THF/CH_3CN. The product (S_P)-**8d** (0.02 g, 19%) was obtained as a colorless foam. ^{1}H NMR [ppm] (400 MHz, $CDCl_3$): δ = 8.06−8.00 (m, 2H), 7.87−7.83 (m, 1H), 7.69−7.65 (m, 1H), 7.55−7.49 (m, 2H), 7.48−7.44 (m, 1H), 7.39 (t, 1H), 7.29−7.27 (m, 1H), 7.04−7.01 (m, 1H), 6.38−6.34 (m, 1H), 5.90−5.87 (m, 1H), 5.09−5.05 (m, 1H), 4.48−4.34 (m, 2H), 4.09−3.98 (m, 1H), 3.63 (s, 3H), 3.58 (t, 1H), 1.62 (d, 3H), 1.26 (d, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 3.27.

5′-*O*-(3′-Deoxy-2′,3′-didehydrothymidinyl)-*O*-(1-naphthyl)-*N*-[(*S*)-methoxyalaninyl]phosphoramidate (R_P)-8d. General procedure C was used with 1-naphthyl-*N*-[(*S*)-alaninyl]-[(*S*)-4-isopropylthiazolidine-2-thione]phosphordiamidate ((S_P)-**6d**, 0.19 g, 0.43 mmol), d4T **7** (0.14 g, 0.64 mmol), *tert*-butylmagnesium chloride (0.75 mL, 1.28 mmol), and 5.7 mL of THF/CH_3CN. The product (R_P)-**8d** (0.06 g, 29%) was obtained as a colorless foam. ^{1}H NMR [ppm] (400 MHz, $CDCl_3$): δ = 8.75 (brs, 1H), 8.15−7.99 (m, 1H), 7.92−7.77 (m, 1H), 7.72−7.60 (m, 1H), 7.59−7.45 (m, 3H), 7.44−7.33 (m, 1H), 7.31−7.19 (m, 1H), 7.07−6.93 (m, 1H), 6.34−6.20 (m, 1H), 5.97−5.83 (m, 1H), 5.09−4.93 (m, 1H), 4.43−4.22 (m, 2H), 4.17−3.97 (m, 1H), 3.96−3.79 (m, 1H), 3.65 (s, 3H), 1.79 (s, 3H), 1.40−1.28 (m, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 2.87.

Antiretroviral Evaluation. Human immunodeficiency virus type 1 (HIV-1) was originally obtained from a persistently HIV-infected H9 cell line, as described previously, and was kindly provided by Dr. R. C. Gallo (then at the National Institutes of Health, Bethesda, MD). Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells. HIV-2 (strain ROD) was kindly provided by Dr. L. Montagnier (then at the Pasteur Institute, Paris, France), and virus stocks were prepared from the supernatants of HIV-2-infected MT-4 cells. CEM cells were obtained from the American Type Culture Collection (Rockville, MD). CEM cells were infected with HIV as previously described.[15,18] Briefly, 4×10^5 CEM cells/mL were infected with HIV-1(III_B) or HIV-2(ROD) at ~100 $CCID_{50}$ (50% cell culture infective dose) per mL of cell suspension. The thymidine kinase-deficient CEM cell cultures were also infected with HIV-2(ROD). Then 100 μL of the infected cell suspensions was transferred into 96-well microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 4−5 days, giant cell formation was recorded microscopically in the HIV-infected cell cultures. The 50% effective concentration is defined as the compound concentration required to inhibit virus-induced cytopathicity by 50%. The 50% cytostatic concentration is defined as the compound concentration required to inhibit CEM cell proliferation by 50%, as derived by counting the cell numbers in the presence of different compound concentrations by use of a Coulter particle counter ZI (Analysis, Gent, Belgium). For Moloney sarcoma virus (MSV) assays, C3H/3T3 cells were seeded at 20 000 cells/mL into wells of tissue culture cluster plates (48 wells/plate). Following a 24 h incubation period, cell cultures were infected with 80 focus-forming units of MSV during 120 min, whereafter the culture medium was replaced by 1 mL of fresh medium containing appropriate concentrations of the test compound. After 6 days, transformation of the cells was examined

microscopically. The EC_{50} was defined as the compound concentration required to inhibit MSV-induced cell transformation by 50%.

Acknowledgment. We thank Isabelle Nevoigt for solving the X-ray structures and Leen Ingels and Lizette van Berckelaer for excellent technical assistance. C.M. thanks the University of Hamburg, Germany, for support. J.B. received a grant from Katholieke Universiteit Leuven, Belgium (Grant GOA 10/14).

Supporting Information Available: ^{1}H, ^{31}P, and ^{13}C NMR spectroscopic data; mass spectrometric data; melting points of phosphordiamidates (S_P)-**6a**−**c**; analytical HPLC data of phosphoramidate prodrugs **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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