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Synthesis of Acyclic Nucleoside and Nucleotide Analogues from Amino Acids: A Convenient Approach to a PMEA–PMPA Hybrid

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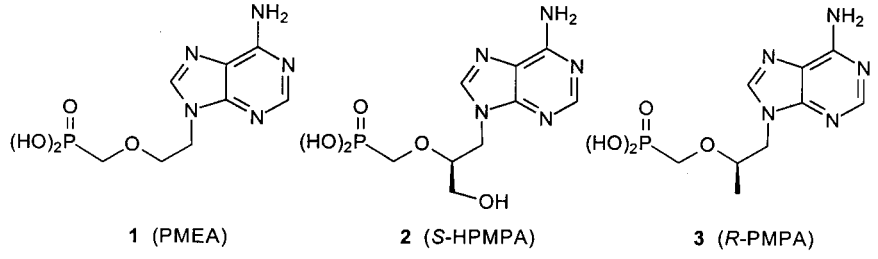
Abstract—Nonracemic amino alcohols derived from common amino acids have been used to assemble acyclic nucleoside and nucleotide analogues with control of absolute stereochemistry. Both (*R*)- and (*S*)-2-amino-1-propanol, readily available from D- or L-alanine, were used to prepare the nucleoside analogues (*R*)- and (*S*)-9-[1-methyl-2-hydroxyethyl]adenine, and then the nucleotide analogues (*R*)- and (*S*)-9-[1-methyl-2-phosphonomethoxyethyl]adenine. In a similar fashion, the CBz derivative of L-threonine was used to prepare first (1*R*,2*R*)-9-[1-hydroxymethyl-2-hydroxypropyl]adenine, and then the bis phosphonomethoxy derivative. The bis phosphonate derived from threonine represents a unique structural hybrid of PMEA and PMPA, both of which have well established antiviral activity.

Interest in nucleoside phosphonates has been stimulated by a series of studies on the antiviral activity of acyclic nucleotide analogues.[1,2] One prototypical compound, 9-(2-phosphonylmethoxyethyl)adenine (PMEA, **1**), has impressive activity against retroviruses in vitro[3] and potent activity against HIV. These discoveries encouraged preparation of a family of related compounds[4] including, most notably, the 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl) or HPMP series and the 9-(*R*)-(2-phosphonylmethoxypropyl) or PMP series, represented by the adenine derivatives *S*-HPMPA (**2**) and *R*-PMPA (**3**), respectively (Fig. 1). Extensive studies have revealed a broad spectrum of potent antiviral activity for these compounds, and different modes of action (and profiles of antiviral activity) despite the strong similarity of structures.[4]

It has been recognized for some time that the absolute stereochemistry of HPMPA and PMPA significantly affects their in vitro antiviral potency.[5] For example, a change in absolute stereochemistry at the single asymmetric center of PMPA has been reported to result in approximately a 45-fold change in some EC_{50} values, which may be because the enantiomers differ in their ability to serve as substrates for AMP kinase.[6] But whatever the reason for the different biological activity of these enantiomers, these findings place a premium on synthetic methods that allow preparation of such compounds with control of their absolute stereochemistry. In this paper we report the use of amino alcohols readily available in nonracemic form through reduction of common amino acids to assemble new nonracemic nucleoside analogues, and the further elaboration of some of these compounds into acyclic nucleotide analogues of the phosphonomethoxy category.

As a first demonstration of this approach, the amino alcohol obtained by reduction of D-alanine, (*R*)-2-amino-1-propanol



1 (PMEA) **2** (*S*-HPMPA) **3** (*R*-PMPA)

Figure 1.

Keywords: nucleosides; nucleotides; phosphonic acids and derivatives.

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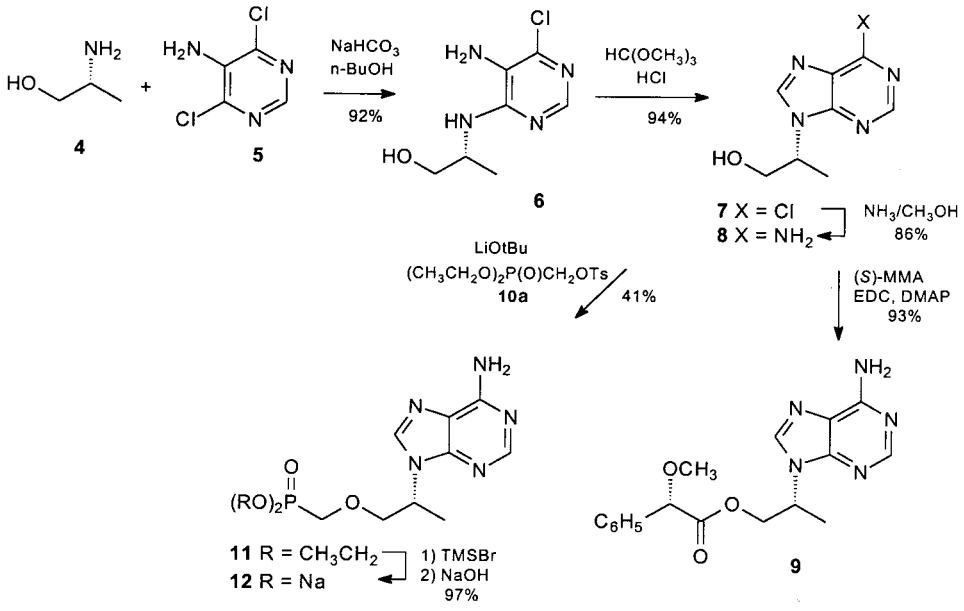


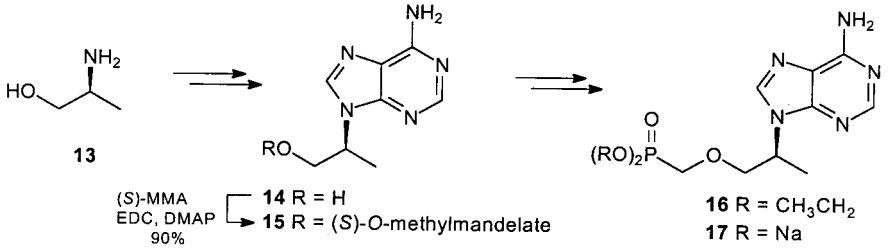
Scheme 1.

(**4**), was converted into the corresponding nonracemic adenine derivative **8** through minor variations on the classic Montgomery sequence[7] (Scheme 1). Condensation of 5-amino-4,6-dichloropyrimidine (**5**) with amine **4** in refluxing 1-butanol provided the intermediate pyrimidine **6**.[8] Closure of the imidazo ring was brought about by reaction with trimethyl orthoformate yielding purine **7** without protection of the primary hydroxyl group, and a final reaction with methanolic ammonia gave the adenine derivative **8** in an overall yield of 74% for the 3-step sequence. The enantiomeric purity of the final product was established as >92% through condensation with (*S*)-*O*-methylmandelic acid (MMA) and analysis of the ^{1}H NMR spectra of the resulting ester **9**.

Addition of a phosphonomethoxy group to compound **8** was best accomplished through reaction with tosylate **10a** and Li*O*t*Bu* in DMF,[9] which consistently gave phosphonate **11** in ~40% yield. Efforts to improve this yield through use of excess reagents or by reaction with the triflate analogue (**10b**) of phosphonate **10a**[10] went unrewarded. Hydrolysis of the ethyl esters in phosphonate **11** was accomplished under standard conditions[11] to give the desired phosphonic acid as its sodium salt (**12**) in nearly quantitative yield.

A parallel series of reactions (Scheme 2) was used to prepare the enantiomeric acyclic nucleoside **14** from (*S*)-2-amino-1-propanol (**13**). After an ee >92% was established by preparation of the (*S*)-*O*-methylmandelate derivative **15**, the alcohol **14** was converted to the phosphonate ester **16** and then to the sodium salt **17** through reactions parallel to those used to prepare compound **12**. In both enantiomeric series, the ee of the product follows directly from that of the starting amino alcohol, and no evidence of racemization was observed.

In theory, the synthetic sequences described above could be applied with an amino alcohol derived from any amino acid.



Scheme 2.

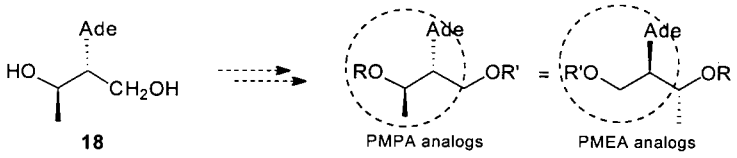
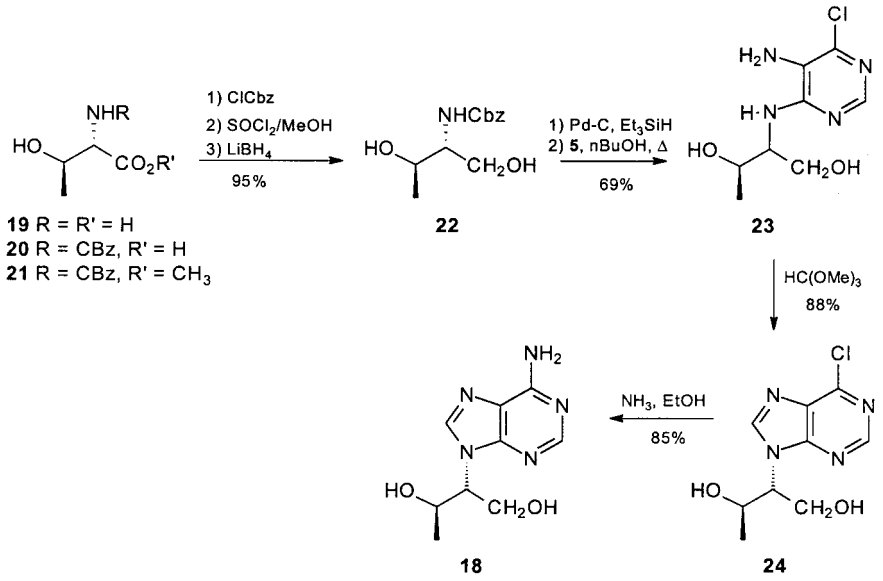


Figure 2.

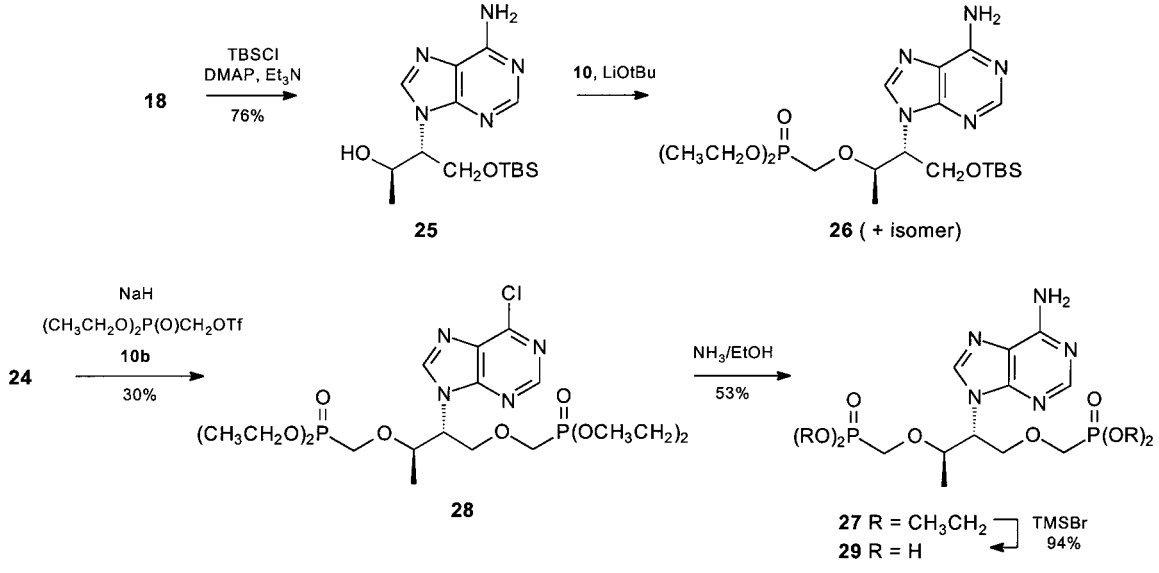


Scheme 3.

While there are a number of such targets that might be interesting, one that we found particularly intriguing was the acyclic nucleoside **18**. This compound, which can be viewed as a derivative of D-threonine, could be further elaborated into nucleotide analogues that can be considered members of either the PMPA or PMEA series (Fig. 2) and might combine the varied aspects of their biological activities. Thus, preparation of compound **18** became the immediate goal.

For preparation of compound **18**, commercial threonine (**19**) was first protected as its Cbz derivative (**20**) and then converted to the methyl ester (**21**) and reduced under standard conditions to obtain the *N*-protected amino alcohol **22** (Scheme 3). The CBz group of compound **22** was cleaved through hydrogenolysis upon treatment with 5% formic acid in methanol in the presence of palladium black, or more conveniently by passing a solution of the protected amine **22** in HCO_2H/CH_3OH through a column of palladium black.[12] However, this procedure gave the formate salt of the desired amine sometimes accompanied by the formamide derivative. Use of triethylsilane as the hydrogen donor[13] with palladium black in ethanol resulted in a product more readily isolated. After the silane reduction, the initial product was treated directly with compound **5** to obtain the substituted pyrimidine **23**. Compound **23** was converted to the desired purine **24** by reaction with trimethyl orthoformate under standard conditions. Both preparation of the pyrimidine **23** and the purine **24** could be accomplished in good yield without protection of the hydroxyl groups. A final reaction of compound **24** with ethanolic ammonia gave the new adenine-derived diol **18** as a single diastereomer in nonracemic form.

Selective conversion of the diol **18** into a phosphonomethoxy derivative apparently would benefit from



Scheme 4.

protection of one of the two hydroxyl groups. Selective protection of the primary alcohol was readily achieved upon treatment of diol **18** with TBSCl/DMAP/Et_3N in DMF. The site of silylation was easily established from the ^1H NMR spectrum of the product (**25**): when this spectrum was recorded in $(CD_3)_2SO$, a clean doublet observed at δ 5.18 reflected the presence of the free secondary hydroxyl group. Unfortunately, treatment of compound **25** with LiOtBu and phosphonate **10a** gave a mixture of products that proved difficult to separate rather than solely the desired product **26**. Based on the NMR spectra of the mixture it appeared that rearrangement of the TBS group prior to alkylation had resulted in formation of a mixture of two regioisomeric phosphonomethoxy products.

Instead of pursuing other routes to the mono phosphonoalkylated products, compound **18** was treated with an excess of base and phosphonate **10a** in an effort to obtain the bis phosphonate **27** (Scheme 4). However, this approach gave only traces of the desired product by ^{31}P NMR, which may be consistent with literature reports on the difficulty of this type of reaction.[9] Fortunately similar treatment of the chloropurine derivative **24** with phosphonate **10b** resulted in formation of the bis phosphonate **28** in modest yield. This chloropurine could be converted to the desired adenine derivative **27** by treatment with ethanolic ammonia. Hydrolysis of the phosphonate esters of compound **27** was readily accomplished by treatment with TMSBr and collidine, affording the target bis phosphonate **29**.

In conclusion, these investigations have demonstrated that nonracemic amino alcohols can be employed to construct nucleoside analogues with control of the absolute stereochemistry. Phosphonomethylation of the alcohol or diol affords the corresponding nucleotide analogues. This strategy has provided a convenient route to a novel hybrid of the anti-viral agents PMPA and PMPA, the bis phosphonate **29**. Expansion of this strategy to preparation of related compounds and determination of the biological activity of these new compounds will be pursued in due course.

Experimental

Immediately prior to use, tetrahydrofuran (THF) was distilled from sodium/benzophenone, and both acetonitrile and dichloromethane were distilled from calcium hydride. Dimethylformamide (DMF) was distilled from calcium carbonate under reduced pressure and stored over magnesium sulfate under argon. All reactions in nonaqueous media were conducted under a positive pressure of argon. Flash chromatography was performed with Merck grade 62 Å silica gel (40 μm) and preparative layer chromatography was performed on plates of silica gel 60 F_{254}. Preparative reverse phase MPLC separations were conducted with a C18 column. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were recorded at 300 MHz for ^1H (75 MHz for ^{13}C) with $CDCl_3$ as solvent and $(CH_3)_4Si$ (^1H) or $CDCl_3$ (^{13}C, 77.0 ppm) as internal standards unless otherwise noted. All ^{31}P NMR chemical shifts are reported in ppm relative to 85% H_3PO_4 (external standard). High-resolution mass spectra were obtained at the University of Iowa Mass Spectrometry Facility. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

(*R*)-2-[(5-Amino-6-chloropyrimidin-4-yl)amino]-1-propanol (6). A solution of (*R*)-2-amino-1-propanol (**4**, 0.96 g, 12.8 mmol) and 5-amino-4,6-dichloropyrimidine (**5**, 2.10 g, 12.8 mmol) in *n*-butanol (30 mL) was treated with sodium bicarbonate (1.24 g, 14.8 mmol) and heated at reflux. After 48 h, the solution was concentrated in vacuo and the residue was washed with chloroform (3×20 mL). The remaining residue was dissolved in ethyl acetate and the solution was filtered through silica gel. The filtrate was concentrated in vacuo to afford an analytically pure sample of pyrimidine **6** (2.39 g, 92%): mp 77°C; ^1H NMR (DMSO) δ 7.71 (s, 1H), 6.49 (br d, J=7.4 Hz, 1H, exch. with D_2O), 5.04 (br s, 2H, exch. with D_2O), 4.73 (dd, J=4.9, 4.9 Hz, 1H, exch. with D_2O), 4.16 (m, 1H), 3.47 (m, upon D_2O addition: dd, J=10.7, 5.3 Hz, 1H), 3.35 (m, upon D_2O addition: dd, J=10.7, 5.8 Hz, 1H), 1.15 (d, J=6.8 Hz, 3H); ^{13}C NMR (DMSO) δ 151.6, 145.6, 136.8, 123.4, 64.1, 48.3, 17.2. Anal. Calcd for $C_7H_{11}ClN_4O$: C, 41.49; H, 5.47; N, 27.65; Cl, 17.50. Found: C, 41.61; H, 5.46; N, 27.57; Cl, 17.44.

(*R*)-9-(1-Methyl-2-hydroxyethyl)6-chloropurine (7). A solution of compound **6** (2.05 g, 10 mmol) in trimethylorthoformate (40 mL) was stirred under argon as conc. HCl (0.7 mL, 23.0 mmol) was added. The resulting solution was stirred for 6 h at room temperature and then concentrated in vacuo. The residue was crystallized from acetone, providing an analytically pure sample of purine **7** (2.03 g, 94%): mp 203–204°C; ^1H NMR (DMSO) δ 8.76 (s, 1H), 8.74 (s, 1H), 5.04 (dd, J=5.7, 5.7 Hz, 1H exch. with D_2O), 4.76 (m, 1H), 3.85 (m, upon D_2O addition: dd, J=11.4, 7.4 Hz, 1H), 3.74 (m, upon D_2O addition: dd, J=11.4, 4.6 Hz, 1H), 1.54 (d, J=7 Hz, 3H); ^{13}C NMR (DMSO) δ 154.2, 151.1, 148.8, 146.6, 131.0, 63.2, 53.9, 16.2. Anal. Calcd for $C_8H_9ClN_4O$: C, 45.19; H, 4.27; N, 26.35; Cl, 16.67. Found: C, 45.29; H, 4.24; N, 26.31; Cl, 16.58.

(*R*)-9-(1-Methyl-2-hydroxyethyl)adenine (8). A solution of the chloropurine **7** (1.02 g, 4.8 mmol) in methanol (40 mL) was saturated with ammonia at 0°C in a pressure flask. The pressure flask was sealed, warmed to 65–70°C in an oil bath, and held at that temperature for 48 h. After the flask had cooled to room temperature, the solvent was removed in vacuo and the residue was washed with chloroform to remove the side product, (*R*)-9-[1-methyl-2-hydroxyethyl]6-methoxypurine (0.12 g, 12%). The remaining residue was crystallized from methanol, providing the desired product **8** (0.80 g, 86%): mp 212°C; ^1H NMR (DMSO) δ 8.15 (s, 1H), 8.12 (s, 1H), 7.15 (br s, 2H, exch. with D_2O), 5.04 (dd, J=5.5, 5.5 Hz, 1H, exch. with D_2O), 4.60 (m, 1H), 3.80 (m, upon D_2O addition: dd, J=11.4, 7.0 Hz, 1H), 3.69 (m, upon D_2O addition: dd, J=11.4, 4.7 Hz, 1H), 1.48 (d, J=6.8 Hz, 3H); ^{13}C NMR (DMSO) δ 155.9, 152.0, 149.4, 139.7, 119.0, 63.4, 52.6, 16.7. Anal. Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25.; Found: C, 49.75; H, 5.75; N, 36.16.

(*R*)-9-[1-Methyl-2-(*S*)-(α-methoxy)phenylacetoxyethyl]-adenine (9). A solution of the alcohol **8** (8.6 mg, 40 μmol), EDC (43 mg, 200 μmol), DMAP (29 mg, 230 μmol), and (*S*)-(α-methoxy)phenylacetic acid (9.6 mg, 60 μmol) in

dichloromethane (1 mL) was stirred at room temperature. After 48 h, water (1 mL) was added and the mixture was stirred until both phases were clear. The product was extracted into dichloromethane (4×20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by preparative layer chromatography ($CHCl_3$) to provide the desired product **9** (14 mg, 93%). Integration of the resonances for the methoxy hydrogens at $\delta\sim3.35$ ppm established a diastereomeric ratio of 92:8. For the major diastereomer: ^{1}H NMR δ 8.32 (s, 1H), 7.44 (s, 1H), 7.33 (m, 5H), 5.61 (br s, 2H, exch. with D_2O), 4.81 (m, 1H), 4.68 (s, 1H), 4.57 (dd, J=11.6, 6.8 Hz, 1H), 4.49 (dd, J=11.6, 4.2 Hz, 1H), 3.33 (s, 3H), 1.55 (d, J=7.2 Hz, 3H).

(*R*)-9-[1-Methyl-2-((diethylphosphono)methoxy)ethyl]-adenine (11). A slurry of nucleoside **8** (85 mg, 0.44 mmol) in DMF (0.5 mL) was treated with lithium *tert*-butoxide (0.65 mL, 1.0 M in THF) over 5 min. The thick slurry was stirred an additional 5 min before the addition of phosphonate **10a** (160 mg, 0.73 mmol) in THF (0.5 mL). The resulting mixture was stirred at room temperature and became homogeneous after 2 h, but stirring was continued for an additional 48 h. The solution was quenched by addition of 50% acetic acid, the product was extracted into dichloromethane (4×75 mL), and the extract was dried over sodium sulfate. After concentration in vacuo, the resulting oil was purified by column chromatography (10% MeOH/$CHCl_3$) to afford a pure sample of compound **11** (63 mg, 41%): ^{1}H NMR δ 8.27 (s, 1H), 7.93 (s, 1H), 6.39 (br s, 2H, exch. with D_2O), 4.85 (m, 1H), 4.00–3.70 (m, 8H), 1.59 (d, J=7.0 Hz, 3H), 1.21 (t, J=7.0 Hz, 6H); ^{13}C NMR δ 155.6, 152.5, 149.6, 139.4, 119.4, 74.8 (d, J_{CP}=9.9 Hz), 65.2 (d, J_{CP}=166 Hz), 62.3 (d, J_{CP}=6.5 Hz), 50.5, 17.0, 16.3 (d, J_{CP}=5.6 Hz); ^{31}P NMR δ 20.1. Anal. Calcd for $C_{13}H_{22}N_5O_4P$: C, 45.48; H, 6.46; N, 20.39. Found: C, 45.23; H, 6.40; N, 20.19.

(*R*)-9-(1-Methyl-2-phosphonomethoxyethyl)adenine sodium salt (12). A solution of phosphonate **11** (74 mg, 0.2 mmol) in acetonitrile (20 mL) was treated with TMSBr (0.3 mL, 2.2 mmol) and the reaction mixture was stirred at room temperature for 48 h. The solution was concentrated in vacuo and the resulting residue was dissolved in water (20 mL) and stirred for 2 h. The aqueous solution was washed with dichloromethane (2×20 mL) and concentrated in vacuo. The glassy solid was dissolved in 5 mL water, adjusted to pH 7 by addition of 2 M NaOH, and then concentrated to a white solid. The solid was purified by reverse phase chromatography to afford the desired product **12** (69 mg, 97%): ^{1}H NMR (D_2O–DMSO) δ 8.27 (s, 1H), 8.11 (s, 1H), 7.17 (br s, 2H, exch. with D_2O), 4.76 (m, 1H), 3.95 (dd, J=10.3, 7.1 Hz, 1H), 3.81 (dd, J=10.3, 4.6 Hz, 1H), 3.35 (d, J_{PH}=8.3 Hz, 2H), 1.46 (d, J=6.8 Hz, 3H); ^{13}C NMR (D_2O) δ 155.3, 152.0, 148.8, 141.2, 118.5, 74.2 (d, J_{CP}=9.7 Hz), 68.7 (d, J_{CP}=153 Hz), 51.1, 16.4; ^{31}P NMR (D_2O–DMSO) δ 13.5; ES LRMS calcd for $C_9H_{13}N_5O_4PNa_2$ $(M+H)^+$ 332.1, found 332.1.

(*S*)-9-[1-Methyl-2-(*S*)-(α-methoxy)phenylacetoxyethyl]-adenine (15). A solution of the alcohol **14** (12 mg, 60 μmol), prepared by a series of reactions parallel to those described for the *R* enantiomer **8**, EDC (54 mg, 280 μmol), DMAP (33 mg, 270 μmol), and (*S*)-(α-methoxy)-phenylacetic acid (13 mg, 80 μmol) in dichloromethane (2 mL) was stirred at room temperature. After 48 h, water (1 mL) was added and the mixture was stirred until both phases were clear. The product was extracted into dichloromethane (4×20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by preparative layer chromatography ($CHCl_3$) providing the desired product **15** (19 mg, 90%). Integration of the methoxy resonances at $\delta\sim3.33$ ppm established a diastereomeric ratio of 96:4 (92% ee). For the major diastereomer: ^{1}H NMR δ 8.31 (s, 1H), 7.51 (s, 1H), 7.30 (m, 5H), 5.54 (br s, 2H, exch. with D_2O), 4.91 (m, 1H), 4.68 (dd, J=11.6, 6.7 Hz, 1H), 4.67 (s, 1H), 4.32 (dd, J=11.6, 3.7 Hz, 1H), 3.36 (s, 3H), 1.45 (d, J=7.1 Hz, 3H).

***N*-Carbobenzyloxy-L-threonine (20).** A solution of L-threonine (**19**, 2.77 g, 23.3 mmol) and sodium bicarbonate (4.89 g, 58.2 mmol) in 50 mL water was cooled in an ice bath as benzylchloroformate (4.0 mL, 28.0 mmol) was added. The ice bath was removed and the solution was stirred at room temperature while the evolution of carbon dioxide was monitored. The aqueous solution was then washed with ether and acidified to pH 2 by addition of HCl. The milky white solution was extracted with ethyl acetate, dried over sodium sulfate, and concentrated in vacuo to afford the desired product, identical to an authentic sample (5.83 g, 99%): ^{1}H NMR (DMSO) δ 7.37 (s, 5H), 6.96 (d, J=8.7 Hz, 1H, exch. with D_2O), 5.06 (s, 2H), 4.08 (m, 1H), 3.95 (dd, J=8.8, 3.5 Hz, 1H), 1.09 (d, J=6.4 Hz, 3H).

***N*-Carbobenzyloxy-L-threonine methyl ester (21).** A solution of acid **20** (5.83 g, 23.0 mmol) in methanol (100 mL) was cooled in an ice bath and thionyl chloride (2.1 mL, 28.8 mmol) was added dropwise. After the addition was complete, the ice bath was removed and the solution was allowed to warm to room temperature and stirred for 24 h. The methanol was removed in vacuo and the residue was dissolved in chloroform. The resulting solution was washed with saturated sodium bicarbonate, dried over sodium sulfate, and concentrated in vacuo to yield the desired product **21** identical to an authentic sample (5.97 g, 97%): ^{1}H NMR δ 7.36 (s, 5H), 5.57 (br d, J=8.3 Hz, 1H, exch. with D_2O), 5.14 (s, 2H), 4.34 (m, 2H), 3.77 (s, 3H), 2.00 (br d, J=5.2 Hz, 1H, exch. with D_2O), 1.25 (d, J=6.3 Hz, 3H).

(2*R*,3*R*)-2-Carbobenzyloxyamino-1,3-butanediol (22).[14] A solution of ester **21** (5.69 g, 21.3 mmol) in anhydrous THF (50 mL) was cooled in an ice bath as lithium borohydride (0.60 g, 27.7 mmol) was added. After hydrogen evolution ceased, the ice bath was removed and the flask was heated at reflux for 12 h. After the reaction flask had cooled to room temperature, the THF was removed in vacuo, methanol (30 mL) was added and the solution was stirred for 24 h. The solution was neutralized by addition of HCl and concentrated in vacuo. The residue was dissolved in chloroform and that solution was washed with brine, dried over sodium sulfate, and concentrated in vacuo to afford the desired product **22** (5.04 g, 99%). ^{1}H NMR δ 7.35 (s, 5H), 5.59 (br d, 1H, exch. with D_2O), 5.10 (s, 2H), 4.12 (m, 1H), 3.79 (s, 2H), 3.57 (m, 1H), 2.93 (br s, 2H, exch. with D_2O), 1.19 (d, J=6.5 Hz, 3H); ^{13}C NMR δ

157.1, 136.2, 128.5 (2), 128.1, 128.0 (2), 68.2, 66.9 (–CH_2– from DEPT), 64.3 (–CH_2– from DEPT), 56.2, 20.1.

(2*R*,3*R*)-2-[(5-Amino-6-chloropyrimidin-4-yl)amino]-1,3-butanediol (23). A solution of diol **22** (2.70 g, 11.3 mmol) in ethanol (30 mL) was treated with 10% Pd–C (0.36 g, 0.34 mmol), triethylamine (0.56 g, 5.5 mmol), and triethylsilane (4.01 g, 34.5 mmol). The reaction mixture was heated at reflux until the starting material could not be detected by TLC (~3 h). After the solution cooled to room temperature, the Pd–C was removed by filtration and the filtrate was concentrated to afford a clear oil. This oil was treated with pyrimidine **5** (1.82 g, 11.1 mmol) and sodium bicarbonate (1.15 g, 13.7 mmol) in 1-butanol (20 mL) and heated at reflux. After 48 h, the solution was allowed to cool to room temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate and filtered through a short plug of silica gel to provide the pure product **23** (1.82 g, 69%): ^{1}H NMR (DMSO) δ 7.69 (s, 1H), 6.29 (d, J=8.2 Hz, 1H, exch.), 5.11 (s, 2H, exch. with D_2O), 4.62 (m, 2H, exch. with D_2O), 4.12 (m, 1H), 3.98 (m, 1H), 3.56 (m, upon D_2O addition: dd, J=10.9, 6.1 Hz, 1H), 3.45 (m, upon D_2O addition: dd, J=10.9, 6.3 Hz, 1H), 1.03 (d, J=6.4 Hz, 3H); ^{13}C NMR (DMSO) δ 152.5, 145.5, 137.0, 123.4, 64.5, 60.2, 57.4, 20.0; ESI HRMS calcd for $C_8H_{13}N_4O_2Cl$ $(M+H)^+$ 233.0805, found 233.0800.

(1*R*,2*R*)-9-[1-Hydroxymethyl-2-hydroxypropyl]-6-chloropurine (24). A solution of pyrimidine **23** (0.81 g, 3.5 mmol) in trimethylorthoformate (20 mL) was treated with HCl (0.3 mL, 9.8 mmol). The resulting solution was stirred at room temperature for 5 h and then concentrated in vacuo. The residue was crystallized from acetone to afford the analytically pure product **24** (0.74 g, 88%): ^{1}H NMR (DMSO) δ 8.76 (s, 1H), 8.65 (s, 1H), 5.08 (d, J=4.7 Hz, 1H, exch. with D_2O), 4.98 (dd, J=5.5 Hz, 1H, exch. with D_2O), 4.53 (m, 1H), 4.22 (m, 1H), 3.96 (m, 1H), 3.87 (m, 1H), 1.01 (d, J=6.2 Hz, 3H); ^{13}C NMR (DMSO) δ 152.9, 151.2, 148.7, 147.1, 130.5, 64.4, 63.6, 60.4, 20.7. Anal. Calcd for $C_9H_{11}N_4O_2Cl$: C, 44.55; H, 4.57; N, 23.09; Cl, 14.61. Found: C, 44.96; H, 4.64; N, 22.69; Cl, 14.28.

(1*R*,2*R*)-9-[1-Hydroxymethyl-2-hydroxypropyl]adenine (18). A solution of the chloropurine **24** (0.81 g, 3.3 mmol) in ethanol (50 mL) in a pressure flask was saturated with ammonia at 0°C. The pressure flask was sealed and warmed to 65–70°C in an oil bath, and maintained at that temperature for 48 h. After the flask had cooled to room temperature, the solvent was removed in vacuo and the product was crystallized from methanol to obtain the desired product **18** (0.63 g, 85%) as a white crystals: mp 206°C; $[\alpha]_D^{25}$=+17.6° (c=0.5, CH_3OH); ^{1}H NMR (DMSO) δ 8.12 (s, 1H), 8.05 (s, 1H), 7.16 (s, 2H, exch. with D_2O), 5.07 (d, J=5.1 Hz, 1H, exch. with D_2O), 4.97 (dd, J=5.3 Hz, 1H, exch. with D_2O), 4.38 (m, 1H), 4.18 (m, 1H), 3.84 (m, 2H), 0.95 (d, J=6.5 Hz, 3H); ^{13}C NMR (DMSO) δ 155.9, 152.0, 150.2, 140.5, 118.4, 64.5, 62.0, 60.7, 20.6. Anal. Calcd for $C_9H_{13}N_5O_2$: C, 51.92; H, 5.81; N, 26.91. Found: C, 51.85; H, 5.86; N, 26.83.

(2*R*,3*R*)-9-[1-*t*-Butyldimethylsilyloxymethyl-2-hydroxypropyl]adenine (25). A solution of the adenine derivative **18** (0.305 g, 1.4 mmol), TBSCl (0.21 g, 1.4 mmol), DMAP (16 mg, 0.13 mmol), and triethylamine (0.7 mL, 5.0 mmol) in DMF (5 mL) was stirred at room temperature for 72 h. The DMF solution was diluted by addition of water (30 mL) and the product was extracted into dichloromethane (5×30 mL), dried over sodium sulfate, and purified by column chromatography (10% MeOH/$CHCl_3$) to afford the desired product **25** (0.349 g, 76%). ^{1}H NMR (DMSO) δ 8.10 (s, 1H), 8.06 (s, 1H), 7.15 (s, 2H, exch. with D_2O), 5.18 (d, J=5.4 Hz, 1H exch.), 4.43 (m, 1H), 4.21 (m, 1H), 4.00 (m, 2H) (s, 3H), 0.98 (d, J=6.4 Hz, 3H), 0.69 (s, 9H), −0.09 (s, 3H), −0.18 (s, 3H).

(1*R*,2*R*)-9-[1-((Diethylphosphono)methoxy)methyl-2-((diethylphosphono)methoxy)propyl]-6-chloropurine (28). To a stirred suspension of NaH (49 mg, 1.23 mmol) in anhydrous THF (3 mL) was added the chloropurine **24** (60 mg, 0.25 mmol) at −15°C under argon. After 5 min, the solution was treated with (diethoxyphosphono)methyl triflate (**10b** 233 mg, 0.75 mmol) in anhydrous THF (2 mL). The solution was allowed to warm to 0°C and stirred for 2 h. The resulting solution was quenched by addition of water, the product was extracted into dichloromethane, and this solution was dried over sodium sulfate. After concentration in vacuo, the oil was purified by column chromatography (5% MeOH/CH_2Cl_2) to afford pure compound **28** (40 mg, 30%): $[\alpha]_D^{27}$=+6.8° (c=0.9, CH_3CH_2OH); ^{1}H NMR (DMSO) δ 8.78 (s, 1H), 8.66 (s, 1H), 4.90 (m, 1H), 4.18 (m, 2H), 3.79 (m, 13H), 1.13 (d, J=6.0 Hz, 3H), 1.06 (m, 12H); ^{13}C NMR (DMSO) δ 152.7, 151.5, 149.0, 146.6, 130.5, 75.4 (d, J=12.4 Hz), 70.9 (d, J=10.7 Hz), 64.0 (d, J=161.5 Hz), 61.7 (d, J=163.7 Hz), 61.6 (d, J=6.5 Hz, 3C), 61.5 (d, J=6.0 Hz), 59.0, 16.14 (d, J=5.1 Hz), 16.10 (d, J=5.4 Hz, 3C), 15.8; ^{31}P NMR (DMSO) δ 23.7, 23.3; ESI HRMS calcd for $C_{19}H_{33}N_4O_8P_2Cl$ $(M+H)^+$ 543.1540, found 543.1537.

(1*R*,2*R*)-9-[1-((Diethylphosphono)methoxy)methyl-2-((diethylphosphono)methoxy)propyl]adenine (27). A solution of compound **28** (41 mg, 0.07 mmol) in ethanol (10 mL) in a pressure flask was saturated with ammonia at 0°C. The pressure flask was sealed, warmed to 70–80°C in an oil bath, and maintained at that temperature for 2 days. After the flask had cooled to room temperature, the solvent was removed in vacuo and the oil was purified by column chromatography (10% MeOH/CH_2Cl_2) to afford pure compound **27** (19 mg, 53%) and recovered compound **28** (4 mg): $[\alpha]_D^{25}$=+7.4° (c=1.2, CH_3CH_2OH); ^{1}H NMR δ 8.33 (s, 1H), 8.10 (s, 1H), 5.76 (s, 2H), 4.86 (m, 1H), 4.08 (m, 10H), 3.78 (m, 5H), 1.31 (t, J=7.0 Hz, 6H), 1.27 (t, J=7.0 Hz, 6H), 1.14 (d, J=6.2 Hz, 3H); ^{13}C NMR δ 155.5, 153.0, 151.0, 140.9, 119.0, 76.1 (d, J=11.7 Hz), 72.2 (d, J=10.3 Hz), 65.5 (d, J=166.3 Hz), 63.1 (d, J=169.0 Hz), 62.69 (d, J=6.0 Hz), 62.67 (d, J=6.0 Hz), 62.63 (d, J=6.0 Hz), 62.58 (d, J=6.0 Hz), 16.71 (d, J=6.6 Hz), 16.68 (d, J=6.6 Hz), 16.62 (d, J=5.6 Hz, 2C), 16.0; ^{31}P NMR δ 21.2, 20.7; ESI HRMS calcd for $C_{19}H_{35}N_5O_8P_2$ $(M+H)^+$ 524.2039, found 524.2036.

(1*R*,2*R*)-9-[1-(Phosphonomethoxy)methyl-2-(phosphonomethoxy)propyl]adenine (29). To a solution of compound **27** (19 mg, 0.036 mmol) in CH_2Cl_2 (2 mL) was added TMSBr (0.2 mL, 0.15 mmol) at room temperature. After being stirred for 18 h at room temperature, the solvent was removed and the reaction mixture was diluted with

CH_3OH and concentrated in vacuo. The residue was washed with CH_2Cl_2 several times to give the desired phosphonic acid **29** (14 mg, 94%): $[\alpha]_D^{25}=+7.4°$ (c=0.12, CH_3H_2OH); ^1H NMR (D_2O) δ 8.63 (s, 1H), 8.43 (s, 1H), 4.93 (m, 1H), 4.21 (m, 3H), 3.78 (m, 3H), 3.57 (m, 1H), 1.19 (d, J=6.2 Hz, 3H); ^{13}C NMR (D_2O) δ 152.9, 152.2, 147.0, 146.9, 120.3, 79.0 (d, J=12.6 Hz), 74.1 (d, J=11.7 Hz), 68.8 (d, J=168.2 Hz), 66.7 (d, J=170.8 Hz), 62.1, 18.0; ^{31}P NMR (D_2O) δ 21.9, 21.1; ESI HRMS calcd for $C_{11}H_{19}N_5O_8P_2$ $(M-H)^-$ 410.0631, found 410.0619.

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