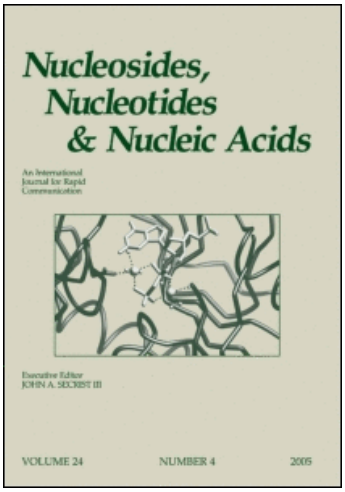

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CARBOCYCLIC SUBSTRATES AND INHIBITORS FOR THE BIFUNCTIONAL LYASE OF PURINE NUCLEOTIDE BIOSYNTHESIS

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CARBOCYCLIC SUBSTRATES AND INHIBITORS FOR THE BIFUNCTIONAL LYASE OF PURINE NUCLEOTIDE BIOSYNTHESIS

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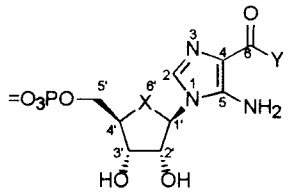
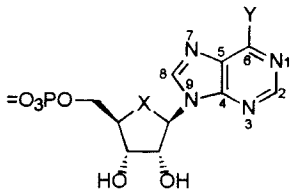
Abstract. The carbocyclic analogs of succinoaminoimidazole carboxamide ribonucleotide (SAICAR) and adenylosuccinate (SAMP) are substrates for the bifunctional lyase of purine biosynthesis, which catalyzes the elimination of fumarate from both SAICAR and SAMP to generate aminoimidazole carboxamide ribonucleotide (AICAR) and AMP, respectively. The glutamate analogs of both ribo- and carbo-SAICAR are inhibitors.

Adenylosuccinate lyase (ASL, EC 4.3.2.2)[1] is unique in that it catalyzes two distinct reactions in the biosynthesis of AMP. These reactions involve the elimination of fumarate, presumably *via* a β-elimination mechanism[2], from SAICAR to yield AICAR in *de novo* purine biosynthesis and from SAMP to yield AMP in the pathway from IMP to AMP. It appears that a single active site is responsible for both conversions[2]. In addition to its biochemical importance, the enzyme is of interest for several clinically relevant reasons. Partial ASL deficiencies have been correlated with cases of severe mental retardation and secondary autistic features[3-7]. Furthermore, ASL is involved in the conversion of the anti-HIV pro-drug ddI to ddATP[8], its active form.

As part of our continuing efforts to enzymologically evaluate carbocyclic nucleotide analogs[9-13], we had developed enantiospecific routes to carbocyclic AICAR (C-AICAR), SAICAR (C-SAICAR), and the glutamate analog (C-GAICAR) of C-SAICAR[14] (FIG. 1). In this report we present the enantiospecific syntheses of carbocyclic adenylosuccinate (C-SAMP), and AMP (C-AMP), a new and convergent synthesis of SAICAR and GAICAR, and the interactions of these compounds with yeast ASL.



AICAR: X=O, Y=NH_2
C-AICAR: X=CH_2, Y=NH_2
SAICAR: X=O, Y=NHCH(COOH)CH_2COOH
C-SAICAR: X=CH_2, Y=NHCH(COOH)CH_2COOH
GAICAR: X=O, Y=NHCH(COOH)$(CH_2)_2$COOH
C-GAICAR: X=CH_2, Y=NHCH(COOH)$(CH_2)_2$COOH



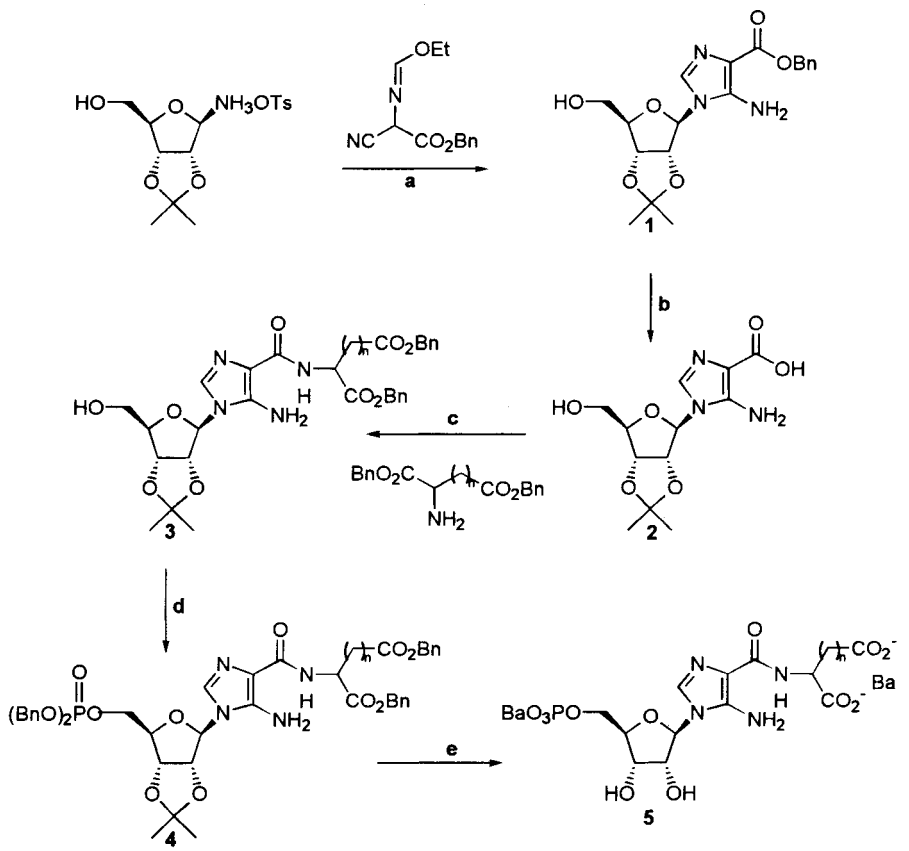
SAMP: X=O, Y=NHCH(COOH)CH_2COOH
C-SAMP: X=CH_2, Y=NHCH(COOH)CH_2COOH
AMP: X=O, Y=NH_2
C-AMP: X=CH_2, Y=NH_2

FIGURE 1

SYNTHESIS

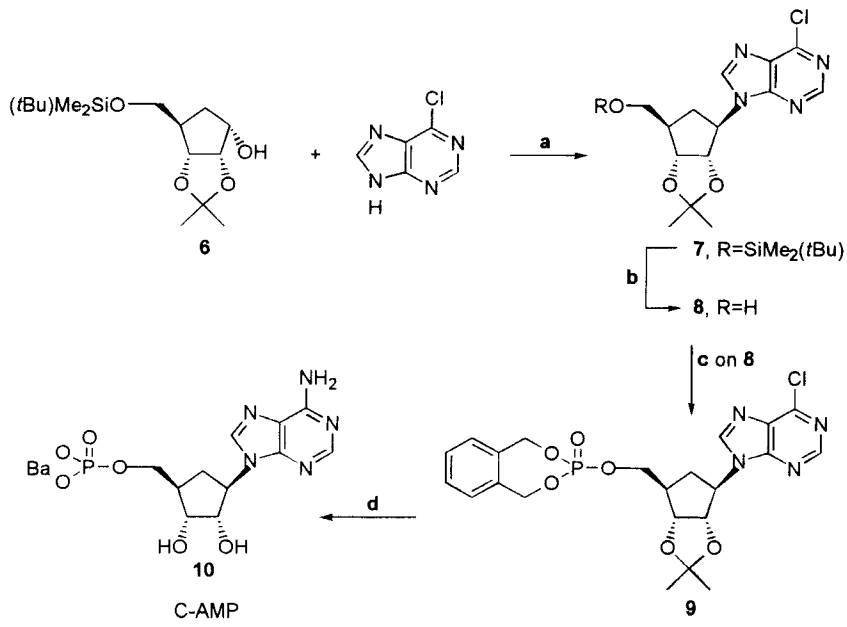
The syntheses of SAICAR and GAICAR (SCHEME 1), which follow from our previous syntheses of C-SAICAR and C-GAICAR[14], employed a key intermediate, **1**, which was obtained from condensation of 2,3-O-isopropylidene-β-D-ribofuranosylammonium tosylate[15] with the formimidate derived from benzyl α-amino-α-cyanoacetate[14]. Acid **2**, which was obtained by hydrogenolysis of **1**, was immediately coupled[16] with either dibenzyl aspartate to afford **3**, n=1, or with dibenzyl glutamate to provide **3**, n=2. Phosphorylation of both to yield **4**, n=1,2, was achieved with tetrabenzyl pyrophosphate[17]. Deprotection of **4**, n=1, by hydrogenolysis followed by treatment with aqueous trifluoroacetic acid, afforded SAICAR (**5**, n=1). The ^{1}H NMR was very similar to that reported[18] for the material prepared enzymatically, indicating that only the β-anomer was formed, and a single phosphorus resonance was observed at δ 6.70. To our knowledge, this is the first reported chemical synthesis of SAICAR. GAICAR (**5**, n=2) was obtained in a similar manner. It displayed a single phosphorus resonance at δ 6.69 and its ^{1}H and ^{13}C NMR were consistent with the proposed structure.

A number of synthetic routes to chiral carbocyclic adenosine (aristeromycin) have been developed[19]. Our synthesis of chiral C-AMP (**10**)



Reagents: **a**, i. CH_3CN, ii. Amberlite IR45, CH_3OH; **b**, H_2, 10% Pd/C, 95% EtOH; **c**, DCC, CF_3-HOBT, CH_3CN; **d**, NaH, tetrabenzyl pyrophosphate, THF; **e**, i. H_2, 10% Pd/C, 95% EtOH, ii. 80% aqueous TFA, iii. $BaBr_2$.

SCHEME 1



Reagents: **a**, Triphenylphosphine, DEAD, THF, benzene; **b**, TBAF, THF; **c**, i. O-Xylene-N,N-diethyl-phosphoramidite, 1H-tetrazole, THF, ii. *m*CPBA; **d**, i. $NH_3(l)$, CH_3OH, ii. H_2, 10% Pd/C, TFA, CH_3OH, iii. $BaBr_2$.

SCHEME 2

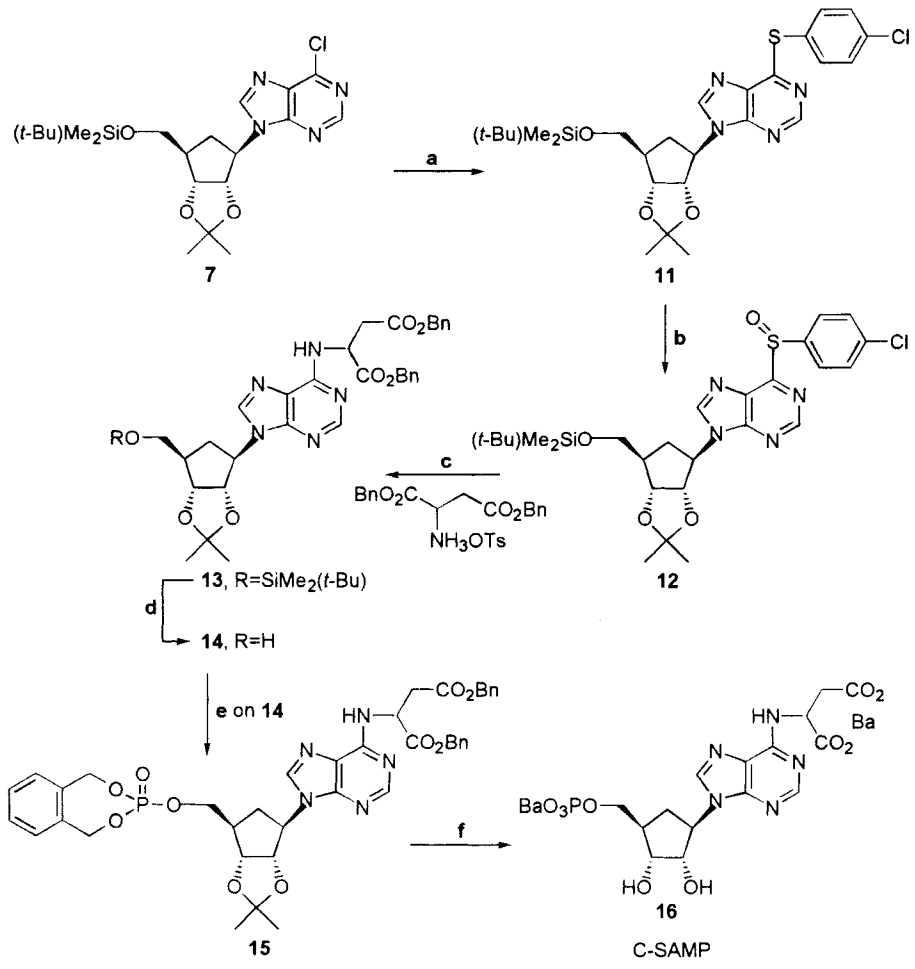
(SCHEME 2) commenced with Mitsunobu coupling[20] of protected alcohol (–)-**6**[14,21] and 6-chloropurine to afford **7**. This approach was chosen because similar coupling of 5-deoxy-**6** with 6-chloropurine afforded the N-9 substituted product exclusively[22] and because **7** was also required for the synthesis of C-SAMP. Selective deprotection of the primary alcohol with tetrabutylammonium fluoride in THF provided **8**. Phosphorylation of **8** was accomplished with O-xylene-N,N-diethylphosphoramidite[23], followed by *in situ* oxidation of the resulting phosphite with *m*-chloroperbenzoic acid, to yield **9**. Treatment of **9** with methanolic ammonia resulted in conversion of the chloro to the amino derivative. Hydrogenolysis, in the presence of trifluoroacetic acid, resulted in removal of the phosphate and isopropylidene protecting groups. C-AMP (**10**) was isolated as

its barium salt[24], with an $[\alpha]_D$ of –7.6 (c=0.51, H_2O) and a λ_{max} at 261 nm (ε=15,180 M^{-1} cm^{-1}, pH 7.4). ^1H and ^{13}C NMR were in agreement with the proposed structure and a single ^{31}P resonance was observed at δ 6.97. (±)-C-AMP, λ_{max} 261 nm (ε=15,100 M^{-1} cm^{-1}, pH 7.0), had been prepared previously[25], however no NMR data were presented.

The chemical synthesis of chiral carbocyclic adenylosuccinate (C-SAMP, **16**, SCHEME 3) was based on the reported[26] synthesis of adenylosuccinate. Thus, sulfide **11** was obtained from **7** upon treatment with 4-chlorothiophenol. Sulfoxide **12** was obtained by oxidation of **11** with *m*-chloroperbenzoic acid. Displacement of the sulfoxide with the tosylate salt of dibenzyl aspartate afforded the completely protected carbocyclic nucleoside **13**. Selective deprotection of the primary alcohol (tetrabutylammonium fluoride in THF), followed by phosphitylation-oxidation with O-xylene-N,N-diethylphosphoramidite-*m*-chloroperbenzoic acid[23], afforded protected carbocyclic nucleotide **15**. Complete deprotection of **15** to yield C-SAMP (**16**) was accomplished by hydrogenolysis in the presence of trifluoroacetic acid. C-SAMP (**16**), isolated as its barium salt, had an $[\alpha]_D$ of –12.6 (c=0.47, H_2O) and a λ_{max} at 270 nm (ε=18,160 M^{-1} cm^{-1}, pH 7.4). ^1H and ^{13}C NMR were in agreement with the proposed structure and a single ^{31}P resonance, at δ 7.23, was observed.

ENZYMOLOGY

Enzymatic evaluation of the ribo- and carbo-nucleotides employed the yeast bifunctional lyase. The cleavage of SAICAR was assayed, as previously described[27], by monitoring the decrease in absorbance at 267 nm minus 320 nm and quantitated using a difference extinction coefficient of 0.7 mM^{-1} cm^{-1}. Cleavage of C-SAICAR[14] (λ_{max} 267, ε=12.4 mM^{-1} cm^{-1}) to C-AICAR[14] (λ_{max} 267, ε=10.6 mM^{-1} cm^{-1}) was assayed in an analogous fashion using a difference extinction coefficient of 1.8 mM^{-1} cm^{-1}. The conversion of SAMP to AMP was measured[27] at 282 nm minus 320 nm, and quantitated with $\Delta\varepsilon$=10 mM^{-1} cm^{-1}. At 280 nm, the extinction coefficient of C-SAMP is 13.3 mM^{-1} cm^{-1}, while that of C-AMP is 3.1 mM^{-1} cm^{-1}. Therefore, the cleavage of C-SAMP to C-AMP was monitored at 280 nm minus 320 nm, using $\Delta\varepsilon$=10.2 mM^{-1} cm^{-1}. Although these



Reagents: **a**, 4-Chlorothiophenol, Et_3N, CH_3OH; **b**, *m*CPBA, CH_2Cl_2; **c**, N,N-Diisopropylethylamine, DMA; **d**, TBAF, THF; **e**, i. O-Xylene-N,N-diethylphosphoramidite, 1H-tetrazole, THF, ii. *m*CPBA; **f**, i. H_2, 10% Pd/C, TFA, CH_3OH, ii. $BaBr_2$.

SCHEME 3

assays suffer from the disadvantage that they monitor loss of substrate rather than formation of product, they are quite reproducible. Moreover, no turnover is observed in the absence of enzyme.

C-SAICAR and C-SAMP were both processed by the yeast lyase. The kinetic constants obtained for these analogs, along with those obtained for the ribose substrates, are included in TABLE 1. The K_m's for all of these substrates were virtually identical, indicating that replacement of the ribose ring oxygen with a methylene group does not adversely affect binding affinity. V_{max}, on the other hand, was significantly affected by this substitution. The V_{max} with C-SAICAR was only approximately 20% of that with SAICAR, while C-SAMP was processed at approximately 30% of the V_{max} obtained with SAMP. Although the site of modification is remote from the substrate cleavage site, its influence on catalytic efficiency is still felt. The diminution in catalytic efficiency observed with the carbocyclic substrates may be due to steric and/or electronic effects. A slight difference in ring conformation resulting from the substitution of methylene for oxygen could perturb the enzyme-substrate interactions optimal for cleavage. It seems less likely that the electronic differences between oxygen and methylene would be transmitted over such a distance.

As expected, neither of the glutamate analogs (GAICAR, C-GAICAR) served as substrates for the lyase. Both, however, were inhibitors of each reaction, competitive against SAICAR and SAMP, respectively, with K_i's less than an order of magnitude higher than the substrate K_m's (TABLE 1). The inhibition constants for GAICAR *versus* SAICAR and SAMP are identical, while those for C-GAICAR show some variation. The value obtained against SAMP is probably the more reliable, given the sensitivity limitations of the SAICAR lyase assay. The inhibition results are consistent with the postulate that a single active site catalyzes both cleavage reactions[2]. For both reactions, the carbocyclic analog was a slightly better inhibitor.

Experimental Section

General

NMR spectra were recorded on a Bruker AC-300 spectrometer. UV spectra were obtained with a Cary 3 spectrophotometer. Optical rotations were

TABLE 1

SAICAR/SAMP LYASE ACTIVITY

Compound	K_m (μM)	V_{max} (μmol/min-mg)	V/K (rel)	K_i (μM)
SAICAR	11 ± 2	0.500 ± 0.020	100	
C-SAICAR	12 ± 3	0.107 ± 0.007	19.6	
GAICAR[a]				99 ± 21
C-GAICAR[a]				53 ± 8
SAMP	12 ± 1	0.280 ± 0.010	100	
C-SAMP	14 ± 1	0.086 ± 0.003	26.3	
GAICAR[b]				99 ± 7
C-GAICAR[b]				83 ± 10

[a] *Versus* SAICAR as varied substrate.

[b] *Versus* SAMP as varied substrate.

measured, using a Rudolph Autopol III polarimeter, at the sodium D line in a 10 cm pathlength cell at 25 °C and concentrations are reported in g/100 mL. Phosphate assays[28] were employed to obtain an independent analysis of the concentrations of nucleotide solutions used for determination of extinction coefficients. TLC was performed with either silica gel plates (Eastman 13181) or, for the phosphomonoesters, cellulose plates (Eastman 13254). Column chromatographic purifications utilized silica gel 60 (70-230 mesh). Solvents were reagent grade and were dried by standard methods[29]. Reactions were run under anhydrous conditions under nitrogen, unless otherwise noted. Adenylosuccinate, yeast adenylosuccinate lyase, and bovine serum albumin were purchased from Sigma and used without further purification.

Enzyme Assays

All assays were performed at 25 °C in 20 mM potassium phosphate, pH 7.4. The lyase was dissolved in 20 mM potassium phosphate, pH 7.4-0.2 mg/mL

BSA. Reaction components were incubated at 25 °C for 5 min, and the reaction was initiated by the addition of a small volume of enzyme to a final volume of 1 mL. Initial velocity data were analyzed[30] according to Equation 1 for determination of the kinetic constants and according to Equations 2 and 3 for competitive and non-competitive inhibition, respectively.

$$v = VS/[K_m + S] \quad (1)$$

$$v = VS/[K_m(1 + I/K_{is}) + S] \quad (2)$$

$$v = VS/[K_m(1 + I/K_{is}) + S(1 + I/K_{ii})] \quad (3)$$

5-Amino-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl) imidazole-4-benzyl carboxylate (1). A freshly prepared[14] solution of benzyl α-amino-α-cyanoacetate (2.12 g, 11.1 mmol) and triethyl orthoformate (7.4 mL, 44.4 mmol) in CH_3CN (40 mL) was refluxed for 1 h. The solvent was evaporated and the residue was dried *in vacuo*. The residue was dissolved in CH_3CN (40 mL), the solution was cooled to 0 °C, and solid ribofuranosylammonium tosylate (2 g, 5.53 mmol) was added. The resulting solution was stirred at room temperature for 14 h. Evaporation of solvent left a residue which was purified on silica gel (CH_3OH in CH_2Cl_2, 0.5→3%) to afford 1.29 g (2.29 mmol, 41.5%) of **1** as the tosylate salt. The free amine (0.839 g, 2.15 mmol, 39%) was obtained after ion exchange with Amberlite IR45 in CH_3OH: TLC (silica, CH_2Cl_2-CH_3OH, 95:5) R_f 0.44; $[\alpha]_D$ –66.3 (c=0.86, CH_3OH); 1H NMR ($CDCl_3$, TMS) δ 7.40-7.20 (m, 5H, Ph), 7.14 (s, 1H, H-2), 5.84 (bs, 2H, $N\underline{H}_2$), 5.54 (d, J=3.0 Hz, 1H, H-1'), 5.38 (s, 2H, $C\underline{H}_2Ph$), 4.96 (dd, J=9.5 Hz, J=6.7 Hz, 2H, H-2', H-3'), 4.25 (d, J=1.3 Hz, 1H, H-4'), 3.78 (ABX, Δδ=0.10 ppm, J_{AB}=11.9 Hz, J_{AX}=2.2 Hz, J_{BX}=1.5 Hz, 2H, H-5'), 1.57 (s, 3H, $C(C\underline{H}_3)_2$), 1.33 (s, 3H, $C(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$) δ 164.0 (C-6), 145.6 (C-5), 136.4 (Ph), 130.2 (C-2), 128.1 (Ph), 128.0 (Ph), 124.4 (Ph), 114.5 (C-4), 111.0 ($\underline{C}(CH_3)_2$), 92.2 (C-1'), 85.3 (C-2'), 82.5 (C-3'), 80.6 (C-4'), 65.4 ($\underline{C}H_2Ph$), 61.3 (C-5'), 27.2 ($C(\underline{C}H_3)_2$).

5-Amino-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl) imidazole-4-dibenzyl aspartyl carboxamide (3, n=1). A solution of **1** (86 mg, 0.22 mmol) in

CH_3OH (2 mL)-95% ethanol (2 mL) was hydrogenated (1 atm.) over 10% Pd/C (20 mg) at room temperature for 16 h. The suspension was filtered through Celite. Solvent was evaporated and the residue was dried *in vacuo*. CF_3-HOBT (59 mg, 0.287 mmol) and CH_3CN (1 mL) were added to the residue, followed by a solution of dibenzyl aspartate (140 mg, 0.45 mmol) in CH_3CN (1 mL) and solid DCC (92 mg, 0.442 mmol). The suspension was stirred at room temperature for 21 h. The suspension was filtered and solvent was evaporated to leave a residue which was purified on silica gel (CH_3OH in CH_2Cl_2, 1→3%) to provide **3, n=1** (47 mg, 0.079 mmol, 36%) as an oil: TLC (silica, CH_2Cl_2-CH_3OH, 97:3) R_f 0.33; $[\alpha]_D$ –25.0 (c=0.54, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 7.47 (d, J=8.6 Hz, 1 H, NH), 7.30-7.10 (m, 10 H, Ph), 7.02 (s, 1 H, H-2), 5.74 (s, 2 H, NH_2), 5.57 (d, J=3.6 Hz, 1 H, H-1'), 5.13 (s, 2 H, CH_2Ph), 5.10-4.90 (m, 5 H, CH_2Ph, H-α, H-2', H-3'), 4.23 (d, J=2.3 Hz, 1 H, H-4'), 3.84 (ABX, Δδ=0.08 ppm, J_{AB}=11.8 Hz, J_{AX}=1.9 Hz, J_{BX}=1.4 Hz, 2 H, H-5'), 3.00 (ABX, Δδ=0.14 ppm, J_{AB}=16.8 Hz, J_{AX}=5.3 Hz, J_{BX}=5.3 Hz, 2 H, H-β), 2.10-1.90 (bs, 1 H, OH), 1.58 (s, 3 H, $C(CH_3)_2$), 1.33 (s, 3 H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 170.8 (α-CO_2), 170.4 (β-CO_2), 164.7 (C-6), 143.1 (C-5), 135.5 (Ph), 135.4 (Ph), 129.2 (C-2), 128.5 (Ph), 128.3 (Ph), 128.1 (Ph), 114.7 (C-4), 113.9 ($C(CH_3)_2$), 92.1 (C-1'), 85.1 (C-2'), 82.6 (C-3'), 80.3 (C-4'), 67.3 (CH_2Ph), 66.8 (CH_2Ph), 61.6 (C-5'), 48.2 (C-α), 37.0 (C-β), 27.3 ($C(CH_3)_2$), 25.2 ($C(CH_3)_2$).

5-Amino-1-(2',3'-O-isopropylidene-4'-dibenzyl phosphoroxymethyl-β-D-ribofuranosyl) imidazole-4-dibenzyl aspartyl carboxamide (4, n=1). To a cold (0 °C), stirred solution of **3** (39 mg, 0.066 mmol) and tetrabenzyl pyrophosphate (42.4 mg, 0.079 mmol) in THF (2 mL), was added NaH (3 mg, 0.072 mmol). Stirring was continued for 1.5 h at 0 °C and 2 h at 25 °C. The solution was cooled to 0 °C, NaH (1 mg, 0.024 mmol) was added, and stirring was continued for 30 min at 0 °C. H_2O (1 drop) was added, solvent was evaporated, and the residue was purified on silica gel (CH_3OH in CH_2Cl_2, 0→3%) to afford **4, n=1** (40 mg, 0.047 mmol, 71%) as an oil: TLC (silica, CH_2Cl_2-CH_3OH, 97:3) R_f 0.38; $[\alpha]_D$ –16.7 (c=0.43, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 7.48 (d, J=8.7 Hz, NH), 7.40-7.20 (m, 20 H, Ph), 5.49 (d, J=3.9 Hz, 1 H,

H-1'), 5.15-4.95 (m, 11 H, C$\underline{H}_2$Ph, N$\underline{H}_2$, H-α), 4.76 (dd, J=6.8 Hz, J=4.4 Hz, 1 H, H-3'), 4.68 (dd, J=6.6 Hz, J=3.3 Hz, 1 H, H-2'), 4.20 (m, 1 H, H-4'), 4.15-4.05 (m, 2 H, H-5'), 3.02 (ABX, Δδ=0.17 ppm, J_{AB}=16.7 Hz, J_{AX}=5.1 Hz, J_{BX}=5.1 Hz, 2 H, H-β), 1.59 (s, 3 H, C(C$\underline{H}_3$)$_2$), 1.30 (s, 3 H, C(C$\underline{H}_3$)$_2$); ^{13}C NMR ($CDCl_3$) δ 170.8 (α-$\underline{C}O_2$), 170.4 (β-$\underline{C}O_2$), 164.4 (C-6), 142.3 (C-5), 135.5-135.4 (4 Ph), 128.8(C-2), 128.7-127.8 (20 Ph), 115.4 (C-4), 115.0 ($\underline{C}(CH_3)_2$), 91.2 (C-1'), 82.8 (C-4'), 79.7 (C-3'), 76.6 (C-2'), 69.8 (C-5'), 67.2 ($CO_2\underline{C}H_2Ph$), 66.7 ($CO_2\underline{C}H_2Ph$), 66.0 (2 $PO_3\underline{C}H_2Ph$), 48.0 (C-α), 37.0 (C-β), 27.1 (C($\underline{C}H_3$)$_2$), 25.2 (C($\underline{C}H_3$)$_2$); ^{31}P NMR ($CDCl_3$, H_3PO_4 (ext.)) δ 0.16.

SAICAR (5, n=1). A solution of **4, n=1** (27 mg, 0.032 mmol) in 80% aqueous alcohol (10 mL), containing TFA (3 drops) and ethylene glycol (1 drop), was hydrogenated (1 atm.) over 10% Pd/C (20 mg) for 16 h. The suspension was filtered through Celite and solvent was evaporated. The residue was dissolved in H_2O (2 mL) and TFA (1 mL) and the resulting solution was stirred at 25 °C for 24 h. After evaporation of solvent, the residue was dissolved in H_2O (0.9 mL), treated with 1 M $BaBr_2$ (0.16 mL, 0.16 mmol), and the pH was adjusted to pH 9. The resulting suspension was kept at 4 °C for 1 h, and the precipitate that formed was removed by centrifugation. Absolute ethanol (5 mL) was added to the supernatant and the suspension that resulted was kept at –20 °C for 30 min. The precipitate was collected by centrifugation, washed with ethanol and ether, and dried *in vacuo* to provide **5, n=1** (19.4 mg, 0.027 mmol, 84%) as a white powder: TLC (cellulose, EtOH-H_2O-HOAc, 7:3:1) R_f 0.53; UV (20 mM, potassium phosphate, pH 7.4) λ_{max} 267 nm (ε=13.3 mM^{-1} cm^{-1}); ^{1}H NMR (D_2O, HOD) δ 7.54 (s, 1 H, H-2), 5.69 (d, J=7.0 Hz, 1 H, H-1'), 4.72 (dd, J=7.1 Hz, J=5.6 Hz, 1 H, H-2'), 4.61 (dd, J=8.5 Hz, J=4.5 Hz, 1 H, H-α), 4.55-4.45 (m, 1 H, H-3'), 4.33 (m, 1 H, H-4'), 4.0 (m, 2 H, H-5'), 2.72 (ABX, Δδ=0.10 ppm, J_{AB}=15.6 Hz, J_{AX}=4.6 Hz, J_{BX}=8.3 Hz), 2 H, H-β); ^{13}C NMR (D_2O, EtOH (ext.)) δ 179.9 (α-$\underline{C}O_2^-$), 179.5 (β-$\underline{C}O_2^-$), 165.9 (C-6), 143.5 (C-5), 132.1 (C-2), 123.2 (C-4), 88.8 (C-1'), 85.5 (C-4'), 72.9 (C-3'), 71.2 (C-2'), 64.1 (C-5'), 52.7 (C-α), 40.8 (C-β); ^{31}P NMR (D_2O, 85% H_3PO_4 (ext.)) δ 6.7; MS (ion spray) MH^+ 726.6, calcd. for $C_{13}H_{15}N_4O_{12}PBa_2$, M 724.9.

5-Amino-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl) imidazole-4-dibenzyl glutamyl carboxamide (3, n=2). A solution of **1** (300 mg, 0.77 mmol) in 1:1 CH_3OH-EtOH (10 mL) was hydrogenated (1 atm.) over 10% Pd/C (50 mg) for 28 h. The mixture was filtered through Celite, solvent was evaporated, and the residue was dried *in vacuo*. CF_3-HOBT (172 mg, 0.847 mmol) and CH_3CN (3 mL) were added to the residue, followed by a solution of dibenzyl glutamate (466 mg, 1.424 mmol) in CH_3CN (3 mL) and solid DCC (482 mg, 2.3 mmol). The suspension was stirred at 25 °C for 25 h. The mixture was filtered, solvent evaporated, and the residue was purified on silica gel (CH_3OH in CH_2Cl_2, 0.25→3%) to provide **3, n=2** (203 mg, 0.333 mmol, 43%) as an oil: TLC (silica, CH_2Cl_2-CH_3OH, 95:5) R_f 0.54; $[\alpha]_D$ –22.9 (c=0.85, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 7.40-7.20 (m, 10 H, Ph), 7.12 (d, J=8.6 Hz, 1 H, NH), 7.03 (s, 1 H, H-2), 5.63 (s, 2 H, NH_2), 5.59 (d, J=3.5 Hz, 1 H, H-1'), 5.20-5.00 (m, 7 H, CH_2Ph, H-2', H-3', H-α), 4.24 (m, 1 H, H-4'), 3.88 (ABX, Δδ=0.09 ppm, J_{AB}=11.7 HZ, J_{AX}=2.2 HZ, J_{BX}=1.8 HZ, 2 H, H-5'), 2.60-1.60 (m, 5 H, OH, H-β, H-γ), 1.58 (s, 3 H, $C(CH_3)_2$), 1.37 (s, 3 H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 172.4 (α-CO_2), 171.7 (β-CO_2), 164.9 (C-6), 142.8 (C-5), 135.5 (Ph), 128.8 (C-2), 128.7-128.2 (Ph), 114.9 (C-4), 114.4 ($C(CH_3)_2$), 91.9 (C-1'), 85.1 (C-2'), 82.8 (C-3'), 80.2 (C-4'), 66.8 (CH_2Ph), 64.4 (CH_2Ph), 61.7 (C-5'), 50.9 (C-α), 30.4 (C-γ), 27.8 (C-β), 27.3 ($C(CH_3)_2$), 24.8 ($C(CH_3)_2$).

5-Amino-1-(2',3'-O-isopropylidene-4'-dibenzyl phosphoroxymethyl-β-D-ribofuranosyl) imidazole-4-dibenzyl glutamyl carboxamide (4, n=2). To a cold (0 °C) solution of **3, n=2** (161 mg. 0.264 mmol) and tetrabenzyl pyrophosphate (185 mg, 0.344 mmol) in THF (8 mL), was added NaH (12.7 mg, 0.53 mmol). The mixture was stirred for 3h at 4 °C, NaH (3 mg, 0.125 mmol) was added, and stirring was continued for 45 min at 0 °C. H_2O (2 drops) was added and solvent was evaporated to yield a residue which was purified on silica gel (CH_3OH in CH_2Cl_2, 0→3%) to afford **4, n=2** (118 mg, 0.136 mmol, 51%) as an oil: TLC (silica, CH_2Cl_2-CH_3OH, 98.5:1.5) R_f 0.26; $[\alpha]_D$ –25.8 (c=0.83, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 7.40-7.25 (m, 20 H, Ph), 7.11 (d, J=8.6 Hz, NH), 7.03 (s, 1 H, H-2), 5.48 (d, J=3.9 Hz, 1 H, H-1'), 5.17 (s, 2 H, CO_2CH_2Ph), 5.10 (s, 2 H,

$N\underline{H}_2$), 5.08 (s, 2 H, $CO_2C\underline{H}_2Ph$), 5.05-4.90 (m, 4 H, $PO_3C\underline{H}_2Ph$), 4.90-4.75 (m, 2 H, H-2', H-α), 4.67 (dd, J=6.7 Hz, J=3.4 Hz, 1 H, H-3'), 4.20-4.15 (m, 1 H, H-4'), 4.15-4.05 (m, 2 H, H-5'), 2.50-2.45 (m, 2 H, H-γ), 2.45-2.25 (m, 1 H, H-β), 2.15-2.00 (m, 1 H, H-β), 1.55 (s, 3 H, $C(C\underline{H}_3)_2$), 1.30 (s, 3 H, $C(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$) δ 172.4 (α-$\underline{C}O_2$), 171.8 (β-$\underline{C}O_2$), 164.5 (C-6), 142.2 (C-5), 135.9 (Ph), 135.5 (Ph), 128.8 (C-2), 128.7-128.1 (Ph), 115.4 (C-4), 115.1 ($\underline{C}(CH_3)_2$), 91.2 (C-1'), 82.8 (C-4'), 79.7 (C-3'), 77.4 (C-2'), 69.8 (C-5'), 67.0 ($CO_2\underline{C}H_2Ph$), 66.4 ($CO_2\underline{C}H_2Ph$), 66.0 ($PO_3\underline{C}H_2Ph$), 65.9 ($PO_3\underline{C}H_2Ph$), 50.8 (C-α), 30.4 (C-γ), 27.9 (C-β), 27.1 ($C(\underline{C}H_3)_2$), 25.2 ($C(\underline{C}H_3)_2$); ^{31}P NMR ($CDCl_3$, H_3PO_4 (ext.)) δ 0.16.

GAICAR (5, n=2). A solution of **4, n=2** (100 mg, 0.115 mmol) in 70% ethanol (9.5 mL) containing TFA (3 drops) was hydrogenated (1 atm.) over 10% Pd/C (40 mg) for 19 h. The mixture was filtered through Celite and solvent was evaporated. The residue was dissolved in H_2O (3 mL) and TFA (1.5 mL) and this solution was stirred at 25 °C for 24 h. Solvent was evaporated, the residue was dissolved in H_2O (3 mL), 1 M Ba Br_2 (0.46 mL, 0.46 mmol) was added, and the pH was adjusted to pH 9. The suspension was kept at 4 °C for 1 h and the precipitate was removed by centrifugation. Absolute ethanol (15 mL) was added to the supernatant and precipitated product was collected by centrifugation after 30 min at –20 °C. The precipitate was washed with ethanol and ether and dried *in vacuo* to provide **5, n=2** (69.5 mg, 0.094 mmol, 82%) as a white powder: TLC (cellulose, EtOH-H_2O-HOAc, 7:3:1) R_f 0.62; $[\alpha]_D$ –14.7 (c=0.55, H_2O); UV (20 mM potassium phosphate, pH 7.4) λ_{max} 270 nm (ε=13.3 mM^{-1} cm^{-1}); ^1H NMR (D_2O, HOD) δ 7.54 (s, 1 H, H-2), 5.70 (d, J=7.1 Hz, H-1'), 4.72 (dd, J=6.8 Hz, J=5.9 Hz, H-2'), 4.46 (m, 1 H, H-α), 4.33 (m, 1 H, H-3'), 4.10-3.90 (m, 3 H, H-4', H-5'), 2.40-2.00 (m, 4 H, H-γ, H-β); ^{13}C NMR (D_2O, EtOH (ext.)) δ 183.0 (α-$\underline{C}O_2^-$), 180.0 (γ-$\underline{C}O_2^-$), 166.1 (C-6), 143.5 (C-5), 132.1 (C-2), 88.8 (C-1'), 85.6 (C-4'), 72.9 (C-3'), 71.2 (C-2'), 62.4 (C-5'), 55.0 (C-α), 34.6 (C-γ), 29.5 (C-β); ^{31}P NMR (D_2O, 85% H_3PO_4 (ext.)) δ 6.69; MS (ion spray) MH^+ 740.7, calcd. for $C_{14}H_{17}N_4O_{12}PBa_2$, M 738.9.

6-Chloro-9-[2',3'-O-isopropylidene-4'-(*tert*-butyldimethylsilyloxymethyl)cyclopentyl]]-9H-purine (7). To a cold (0 °C), stirred solution of **(–)-6**

(1g, 3.3 mmol), triphenylphosphine (2.62 g, 9.9 mmol), and 6-chloropurine (1 g, 6.6 mmol) in THF (10 mL) and benzene (5 mL), was added DEAD (1.57 mL, 1.74 g, 9.9 mmol). Stirred at 0 °C for 2h, followed by 8 h at reflux. Evaporation of solvent left a residue which was purified on silica gel (EtOAc in hexanes, 0→30%) to yield **7** (1.132 g, 2.6 mmol, 78%) as an oil: TLC (silica, ether-hexanes, 2:1) R_f 0.36; $[\alpha]_D$ –21.1 (c=1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, TMS) δ 8.69 (s, 1 H, H-2), 8.15 (s, 1 H, H-8), 4.97 (t, J=6.5 Hz, 1 H, H-2'), 4.84-4.81 (m, 1 H, H-1'), 4.65 (dd, J=7.0 Hz, J=4.2 Hz, 1 H, H-3'), 4.23 (dd, J=9.0 Hz, J=3.0 Hz, 2 H, H-5'), 2.58-2.49 (m, 2 H, H-4', H-6'), 2.43-2.37 (m, 1 H, H-6'), 1.54 (s, 3 H, $C(C\underline{H}_3)_2$), 1.33 (s, 3 H, $C(C\underline{H}_3)_2$), 0.89 (s, 9 H, $SiC(C\underline{H}_3)_3$), 0.05 (s, 6 H, $Si(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$) δ 151.8 (C-4), 151.7 (C-2), 151.2 (C-6), 144.3 (C-8), 132.3 (C-5), 113.3 ($\underline{C}(CH_3)_2$), 83.7 (C-2'), 80.7 (C-3'), 62.7 (2 C, C-1', C-5'), 45.5 (C-4'), 32.9 (C-6'), 27.6 ($C(\underline{C}H_3)_2$), 25.8 ($SiC(\underline{C}H_3)_3$), 25.1 ($C(\underline{C}H_3)_2$), 18.2 ($Si\underline{C}(CH_3)_3$), -5.5 ($Si(\underline{C}H_3)_2$.

6-Chloro-9-(2',3'-O-isopropylidene-4'-hydroxymethylcyclopentyl)-9H-purine (8). To a stirred solution of **7** (0.15 g, 0.34 mmol) in THF (5 mL) was added 1 M TBAF in THF (0.43 mL, 0.43 mmol) and stirring was continued for 2 h at 25 °C. Solvent was evaporated to leave an oil which was purified on silica gel (CH_3OH in CH_2Cl_2, 0→3%) to afford **8** (0.094 g, 0.29 mmol, 85%) as a clear oil: TLC (silica, ether) R_f 0.17; $[\alpha]_D$ –49.1 (c=0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, TMS) δ 8.74 (s, 1 H, H-2), 8.28 (s, 1 H, H-8), 5.03 (dd, J=6.6 Hz, J=6.0 Hz, 1 H, H-2'), 4.93-4.87 (m, 1 H, H-1'), 4.74 (dd, J=6.9 Hz, J=3.4 Hz, 1 H, H-3'), 3.90-3.80 (m, 2 H, H-5'), 3.01 (bs, 1 H, OH), 2.60-2.45 (m, 3 H, H-4', 2 H-6'), 1.58 (s, 3 H, $C(C\underline{H}_3)_2$, 1.32 (s, 3 H, $C(C\underline{H}_3)_2$; ^{13}C NMR ($CDCl_3$) δ 151.7 (C-4), 151.3 (C-2), 151.1 (C-6), 144.6 (C-8), 132.4 (C-5), 113.8 ($\underline{C}(CH_3)_2$), 84.0 (C-2'), 81.8 (C-3'), 63.5 (C-5'), 63.0 (C-1'), 45.5 (C-4'), 33.0 (C-6'), 27.5 ($C(\underline{C}H_3)_2$), 25.1 ($C(\underline{C}H_3)_2$.

6-Chloro-9-[2',3'-O-isopropylidene-4'-(*o*-xylylphosphoroxymethyl)-cyclopentyl]-9H-purine (9). To a stirred solution of **8** (50 mg, 0.154 mmol) in THF (5 mL) was added 1H-tetrazole (20 mg, 0.286 mmol) and O-xylene-N,N-diethylphosphoramidite (55 mg, 0.231 mmol). After 10 h at 25 °C, the reaction was cooled to 0 °C, *m*CPBA (55%, 97 mg, 0.31 mmol) was added, and stirring

was continued for 8 h at 25 °C. Solvent was evaporated, the residue was dissolved in ethyl acetate (10 mL), and this solution was washed with 10% $Na_2S_2O_5$ (10 mL), saturated $NaHCO_3$ (10 mL), H_2O (10 mL), and saturated NaCl (10 mL). After drying (Na_2SO_4), solvent was evaporated to leave an oil which was purified on silica gel (CH_3OH in CH_2Cl_2, 0→2%) to afford **9** (70 mg, 0.138 mmol, 89%) as a clear oil: TLC (silica, CH_2Cl_2-CH_3OH, 19:1) R_f 0.55; $[\alpha]_D$ –15.0 (c=0.01, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 8.66 (s, 1 H, H-8), 8.24 (s, 1 H, H-2), 7.4-7.3 (m, 4 H, Ph), 5.28 (d, J=3.9 Hz, 2 H, C$\underline{H}_2$Ph), 5.23 (d, J=3.9 Hz, 2 H, C$\underline{H}_2$Ph), 5.12 (dd, J=7.0 Hz, J=6.0 Hz, 1 H, H-2'), 5.0-4.85 (m, 1 H, H-1'), 4.77 (dd, J=7.1 Hz, J=4.7 Hz, 1 H, H-3'), 4.37 (t, J=5.8 Hz, 2 H, H-5'), 2.65-2.55 (m, 3 H, H-4', H-6'), 1.57 (s, 3 H, C(C$\underline{H}_3$)$_2$), 1.31 (s, 3 H, C(C$\underline{H}_3$)$_2$); ^{13}C NMR ($CDCl_3$) δ 151.6 (C-4), 151.2 (C-2), 151.1 (C-6), 144.5 (C-8), 135.1 (Ph), 132.3 (C-5), 129.5 (Ph), 128.9 (Ph), 114.2 (C(CH$_3$)$_2$), 83.2 (C-2'), 80.5 (C-3'), 68.5 (2 C, $\underline{C}H_2$Ph), 68.1 (C-5'), 62.2 (C-1'), 44.0 (C-4'), 33.0 (C-6'), 27.4 (C($\underline{C}H_3$)$_2$), 25.0 (C($\underline{C}H_3$)$_2$); ^{31}P NMR ($CDCl_3$, 85% H_3PO_4 (ext.)) δ –0.26.

C-AMP (10). A solution of **9** (0.3 g, 0.59 mmol) in CH_3OH (2 mL) in a bomb was cooled in dry ice and liquid ammonia (~ 5 mL) was added. The bomb was sealed and then heated at 60 °C for 24 h. After cooling (dry ice), the bomb was opened and the ammonia was allowed to evaporate. Solvent was evaporated, the residue was dissolved in CH_3OH (10 mL) and TFA (8 drops), and the solution was hydrogenated (1 atm.) over 10% Pd/C (30 mg) for 18 h. The mixture was filtered through Celite and evaporated. The residue was dissolved in H_2O (1.5 mL), 1 M $BaBr_2$ (1.18 mL, 1.18 mmol) was added, and the pH was adjusted to pH 8.5. Absolute ethanol (13.5 mL) was added and the resulting suspension was kept at –20 °C for 10 h. The precipitate was collected by centrifugation, washed with ethanol and ether, and dried *in vacuo* to afford **10** (0.21 g, 0.43 mmol, 74%) as a powder: TLC (cellulose, EtOH-H_2O-HOAc, 7:3:1) R_f 0.27; $[\alpha]_D$ –7.6 (c=0.51, H_2O); ^1H NMR (D_2O, HOD) δ 8.44 (s, 1 H, H-2), 8.27 (s, 1 H, H-8), 4.56 (dd, J=9.4 Hz, J=5.5 Hz, 1 H, H-1'), 4.23 (dd, J=5.5 Hz, J=3.1 Hz, 1 H, H-3'), 4.0-3.8 (m, 2 H, H-5'), 2.6-2.5 (m, 1 H, H-6'), 2.5-2.35 (m, 1 H, H-4'), 2.0-1.85 (m, 1 H, H-6'); H-2' obscured by HOD; ^{13}C NMR (D_2O, pH 1, EtOH

(ext.)) δ 149.2 (C-6), 148.6 (C-4), 143.5 (C-2), 143.0 (C-8), 118.0 (C-5), 74.9 (C-2'), 71.2 (C-3'), 66.6 (C-5'), 57.0 (C-1'), 42.7 (C-4'), 27.7 (C-6'); ^{31}P NMR (D_2O, 85% H_3PO_4 (ext.)) δ 6.97; MS (ion spray) MH^+ 481.8, calcd. for $C_{11}H_{14}N_5O_6PBa$, M 480.6.

6-(4-Chlorophenylthio)-9-[2',3'-O-isopropylidene-4'-(*tert*-butyldimethylsilyloxymethyl)cyclopentyl]-9H-purine (11). To a stirred solution of **7** (0.4 g, 0.91 mmol) in CH_3OH (10 mL), was added triethylamine (0.18 mL, 1.29 mmol) and 4-chlorothiophenol (0.182 g, 1.26 mmol) and stirring was continued for 1 h at 25 °C. Solvent was evaporated to leave an oil that was dissolved in $CHCl_3$ (40 mL). The $CHCl_3$ solution was washed with saturated $NaHCO_3$ (15 mL), dried over Na_2SO_4, and evaporated to leave an oil which was purified on silica gel ($CHCl_3$) to afford **11** (0.428 g, 0.78 mmol, 86%) as a colorless oil: TLC (silica, ether-hexanes, 2:1) R_f 0.44; $[\alpha]_D$ −11.3 (c=0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, TMS) δ 8.60 (s, 1 H, H-2), 8.07 (s, 1 H, H-8), 7.58 (d, J=9.0 Hz, 2 H, Ph), 7.43 (d, J=9.0 Hz, 2 H, Ph), 5.01 (dd, J=6.7 Hz, J=6.1 Hz, 1 H, H-2'), 4.9-4.8 (m, 1 H, H-1'), 4.67 (dd, J=7.0 Hz, J=4.3 Hz, 1 H, H-3'), 3.80-3.70 (m, 2 H, H-5'), 2.6-2.5 (m, 1 H, H-6'), 2.41-2.29 (m, 2 H, H-4', H-6'), 1.53 (s, 3 H, $C(C\underline{H})_3)_2$), 1.36 (s, 3 H, $C(C\underline{H}_3)_2$), 0.91 (s, 9 H, $SiC(C\underline{H}_3)_3$), 0.07 (s, 3 H, $SiC(C\underline{H}_3)_2$), 0.05 (s, 3 H, $SiC(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$) δ 159.8 (C-6), 151.7 (C-4), 148.9 (C-2), 142.3 (S-Ph), 136.6 (C-8), 135.6 (Cl-Ph), 131.3 (2 C, Ph), 129.3 (2 C, Ph), 125.7 (C-5), 113.3 ($\underline{C}(CH_3)_2$), 83.5 (C-2'), 80.5 (C-3'), 62.5 (C-5'), 62.1 (C-1'), 45.4 (C-4'), 33.0 (C-6'), 27.4 ($C(\underline{C}H_3)_2$), 25.7 (3 C, $SiC(\underline{C}H_3)_3$), 25.0 ($C(\underline{C}H_3)_2$), 18.1 ($\underline{C}(CH_3)_3$), −5.6 (2 C, $Si(\underline{C}H_3)_2$).

6-(4-Chlorophenylsulfinyl)-9-[2',3'-O-isopropylidene-4'-(*tert*-butyldimethylsilyloxymethyl)cyclopentyl]-9H-purine (12). A solution of *m*CPBA (55%, 0.151 g, 0.48 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a cold (−5 °C), stirred solution of **11** (0.25 g, 0.45 mmol) in CH_2Cl_2 (10 mL). After 3 h at −5 °C, the solution was extracted with 10% $Na_2S_2O_5$ (5 mL), saturated $NaHCO_3$ (5mL), H_2O (5 mL), and saturated NaCl (5 mL). After drying (Na_2SO_4), solvent was evaporated to leave an oil which was purified on silica gel (1% EtOH in CH_2Cl_2) to afford **12** (0.2 g, 0.35 mmol, 78%) as a clear oil: TLC (silica, CH_2Cl_2-

CH_3OH, 95:5) R_f 0.50; $[\alpha]_D$ –22.1 (c=0.095, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 9.08 (s, 1 H, H-2), 8.24 (s, 1 H, H-8), 7.95 (d, J=9.5 Hz, 2 H, Ph), 7.43 (d, J=9.5 Hz, 2 H, Ph), 5.05-4.9 (m, 1 H, H-2'), 4.9-4.8 (m, 1 H, H-1'), 4.7-4.6 (m, 1 H, H-3'), 3.77 (ABX, Δδ=0.07 ppm, J_{AB}=10.2 Hz, J_{AX}=4.4 Hz, J_{BX}=3.5 Hz, 2 H, H-5'), 2.7-2.5 (m, 1 H, H-6'), 2.45-2.35 (m, 2 H, H-4', H-6'), 1.56 (s, 3 H, $C(C\underline{H}_3)_2$), 1.30 (s, 3 H, $C(C\underline{H}_3)_2$), 0.89 (s, 9 H, $SiC(C\underline{H}_3)_3$), 0.08 (s, 3 H, $SiC(C\underline{H}_3)_2$), 0.03 (s, 3 H, $SiC(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$) δ 161.9 (C-6), 152.2 (C-4), 145.6 (C-2), 145.4 (C-8), 141.1 (SO-Ph), 137.7 (Cl-Ph), 129.5 (2 C, Ph), 126.5 (3 C, Ph, C-5), 113.7 ($\underline{C}(CH_3)_2$), 83.7 (C-2'), 80.6 (C-3'), 62.7 (C-5'), 62.5 (C-1'), 45.3 (C-4'), 32.8 (C-6'), 27.5 ($C(\underline{C}H_3)_2$), 25.8 (3 C, $SiC(\underline{C}H_3)_3$), 25.1 ($C(\underline{C}H_3)_2$), 18.2 ($Si\underline{C}(CH_3)_3$), –5.5 (2 C, $Si(\underline{C}H_3)_2$).

Dibenzyl-N-[9-(2',3'-O-isopropylidene-4'-(*tert*-butyldimethylsilyloxymethyl)cyclopentyl)-9H-purin-6-yl]-L-aspartate (13). A solution of **12** (174 mg, 0.31 mmol), dibenzyl-L-aspartate toluene-4-sulfonate (0.398 g, 0.82 mmol), and N,N-diisopropylethylamine (0.23 mL, 1.32 mmol) in DMA (5 mL) was heated at 70 °C for 36 h. Solvent was evaporated and the residue was partitioned between $CHCl_3$ (25 mL) and saturated $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was dissolved in ethyl acetate (10 mL) and the solution was washed with 3 M H_3PO_4 (3 × 5 mL). The aqueous layer was extracted with ethyl acetate (15 mL) and the combined organic solution was washed with saturated $NaHCO_3$ (25 mL) and dried (Na_2SO_4). Evaporation of solvent left an oil which was purified by silica gel chromatography (1% EtOH in $CHCl_3$) to yield **13** (105mg, 0.147 mmol, 47%) as a clear oil: TLC (silica, CH_2Cl_2-CH_3OH, 97:3) R_f 0.34; $[\alpha]_D$ –23.1 (c=1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 8.32 (s, 1 H, H-2), 7.83 (s, 1 H, H-8), 7.35-7.2 (m, 10 H, Ph), 6.7 (bs, 1 H, N$\underline{H}$), 5.55-5.35 (bm, 1 H, H-α), 5.15 (s, 2 H, $C\underline{H}_2Ph$), 5.07 (s, 2 H, $C\underline{H}_2Ph$), 5.06-5.0 (m, 1 H, H-2'), 4.8-4.7 (m, 1 H, H-1'), 4.67 (dd, J=4.4 Hz, J= 7.0 Hz, 1 H, H-3'), 3.78 (d, J=4.1 Hz, 2 H, H-5'), 3.16 (ABX, Δδ=0.11 ppm, J_{AB}=16.8 Hz, J_{AX}=5.3 Hz, J_{BX}=4.9 Hz, 2 H, H-β), 2.55-2.3 (m, 3 H, H-4', H-6'), 1.56 (s, 3 H, $C(C\underline{H}_3)_2$), 1.31 (s, 3 H, $C(C\underline{H}_3)_2$), 0.92 (s, 9 H, $SiC(C\underline{H}_3)_3$), –0.08 (s, 6 H, $Si(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$) δ 170.8 ($\underline{C}O_2$), 170.4 ($\underline{C}O_2$), 153.7 (C-6), 152.4 (C-

4), 149.8 (C-2), 139.3 (C-8), 135.4 (Ph), 135.3 (Ph), 128.5-128.1 (Ph), 120.6 (C-5), 113.4 ($\underline{C}(CH_3)_2$), 83.8 (C-2'), 80.6 (C-3'), 67.3 ($\underline{C}H_2Ph$), 66.7 ($\underline{C}H_2Ph$), 62.9 (C-5'), 61.8 (C-1'), 47.2 (C-α), 45.7 (C-4'), 36.8 (C-β), 33.4 (C-6'), 27.5 ($C(\underline{C}H_3)_2$), 25.8 ($SiC(\underline{C}H_3)_3$), 25.1 ($C(\underline{C}H_3)_2$), 18.2 ($Si\underline{C}(CH_3)_3$), −5.5 ($Si(\underline{C}H_3)_2$).

Dibenzyl-N-[9-(2',3'-O-isopropylidene-4'-hydroxymethylcyclopentyl)-9H-purin-6-yl]-L-aspartate (14). To a stirred solution of **13** (90 mg, 0.12 mmol) in THF (5 mL) was added 1 M TBAF in THF (0.16 mL, 0.16 mmol) and stirring was continued at room temperature overnight. Solvent was evaporated and the residue was purified on silica gel (4% CH_3OH in CH_2Cl_2) to afford **14** (40 mg, 0.066 mmol, 55%) as an oil: TLC (silica, CH_2Cl_2-CH_3OH, 95:5) R_f 0.42; $[\alpha]_D$ −27.6 (c=1.02, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 8.32 (s, 1 H, H-2), 7.85 (s, 1 H, H-8), 7.3-7.2 (m, 10 H, Ph), 6.9 (bs, 1 H, N$\underline{H}$), 5.42 (bm, 1 H, H-α), 5.15 (s, 2 H, $C\underline{H}_2Ph$), 5.07 (s, 2 H, $C\underline{H}_2Ph$), 5.05-4.95 (m, 1 H, H-2'), 4.8-4.7 (m, 2 H, H-1', H-3'), 3.9-3.75 (m, 2 H, H-5'), 3.15 (ABX, $\Delta\delta$=0.07 ppm, J_{AB}=16.9 Hz, J_{AX}=5.3 Hz, J_{BX}=4.9 Hz, 2 H, H-β), 2.6-2.4 (m, 3 H, H-4', H-6'), 1.58 (s, 3 H, $C(C\underline{H}_3)_2$), 1.31 (s, 3 H, $C(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$) δ 170.7 ($\underline{C}O_2$), 170.3 ($\underline{C}O_2$), 153.8 (C-6), 152.2 (C-4), 151.4 (C-2), 139.9 (C-8), 135.2 (Ph), 128.4-128.0 (Ph), 120.7 (C-5), 113.0 ($\underline{C}(CH_3)_2$), 84.3 (C-2'), 82.1 (C-3'), 67.3 ($\underline{C}H_2Ph$), 66.7 ($\underline{C}H_2Ph$), 63.7 (C-5'), 62.9 (C-1'), 49.9 (C-α), 45.3 (C-4'), 36.7 (C-β), 33.1 (C-6'), 27.6 ($C(\underline{C}H_3)_2$), 25.0 ($C(\underline{C}H_3)_2$).

Dibenzyl-N-[9-(2',3'-O-isopropylidene-4'-(*o*-xylylphosphoroxymethyl)-cyclopentyl)-9H-purin-6-yl]-L-aspartate (15). A solution of **14** (40 mg, 0.066 mmol), O-xylene-N,N-diethylphosphoramidite (39 mg, 0.16 mmol), and 1H-tetrazole (13 mg, 0.18 mmol) in THF (5 mL) was stirred at 25 °C for 8 h. The solution was cooled (0 °C), *m*CPBA (55%, 136 mg, 0.43 mmol) was added, and stirring was continued for 6 h. Solvent was evaporated and the residue was dissolved in ethyl acetate (10 mL). This solution was washed with 10% $Na_2S_2O_5$ (5 mL), saturated $NaHCO_3$ (5 mL), H_2O (5 mL), and saturated NaCl (5 mL) and dried (Na_2SO_4). Evaporation of solvent left an oil which was purified on silica gel (3% CH_3OH in CH_2Cl_2) to yield **15** (44 mg, 0.056 mmol, 85%) as a clear oil: TLC (silica, CH_2Cl_2-CH_3OH, 95:5) R_f 0.50; $[\alpha]_D$ −12.1 (c=1.01, $CHCl_3$); ^1H NMR

(CDCl$_3$, TMS) δ 8.23 (s, 1 H, H-2), 7.83 (s, 1 H, H-8), 7.5-7.1 (m, 14 H, Ph), 6.7 (bs, 1 H, NH), 5.5-5.4 (bm, 1 H, H-α), 5.4-5.0 (m, 9 H, H-2', POCH$_2$Ph, overlapping 5.14 (s, CH$_2$Ph) and 5.06 (s, CH$_2$Ph)), 4.9-4.7 (m, 2 H, H-1', H-3'), 4.5-4.3 (m, 2 H, H-5'), 3.15 (ABX, Δδ=0.11 ppm, J_{AB}=17.0 Hz, J_{AX}=5.4 Hz, J_{BX}=4.5 Hz, 2 H, H-β), 2.7-2.4 (m, 3 H, H-4', H-6'), 1.56 (s, 3 H, C(CH$_3$)$_2$), 1.30 (s, 3 H, C(CH$_3$)$_2$); ^{13}C NMR (CDCl$_3$) δ 170.7 (CO$_2$), 170.3 (CO$_2$), 153.7 (C-6), 152.2 (C-4), 151.3 (C-2), 139.6 (C-8), 135.3 (Ph), 129.4-128 (Ph), 120.7 (C-5), 113.9 (C(CH$_3$)$_2$), 83.3 (C-2'), 80.6 (C-3'), 68.5 (CH$_2$Ph), 68.2 (CH$_2$Ph), 68.1 (C-5'), 67.3 (CH$_2$Ph), 66.7 (CH$_2$Ph), 61.6 (C-1'), 49.8 (C-α), 44.2 (C-4'), 36.7 (C-β), 33.2 (C-6'), 27.4 (C(CH$_3$)$_2$), 25.1 (C(CH$_3$)$_2$); ^{31}P NMR (CDCl$_3$, 85% H$_3$PO$_4$ (ext.)) δ –0.19.

C-SAMP (16). A solution of **15** (25 mg, 0.032 mmol) in CH$_3$OH (10 mL) and TFA (6 drops) was hydrogenated (1 atm.) over 10% Pd/C (15 mg) at 25 °C for 10 h. The suspension was filtered through Celite and solvent was evaporated to leave a residue which was dried *in vacuo*. The residue was dissolved in H$_2$O (5 mL), 1 M BaBr$_2$ (0.062 mL, 0.062 mmol) was added, and the pH was adjusted to pH 8.5. Addition of absolute ethanol, followed by chilling at –20 °C for 3 h, resulted in the formation of a precipitate. The precipitate was collected by centrifugation, washed with ethanol and ether, and dried *in vacuo* to afford **C-SAMP (16)** (22 mg, 0.03 mmol, 94%) as a white powder: TLC (cellulose, EtOH-H$_2$O-HOAc, 7:3:1) R_f 0.62; $[\alpha]_D$ –12.6 (c=0.47, H$_2$O); ^{1}H NMR (D$_2$O, HOD); δ 8.65 (s, 1 H, H-2), 8.48 (s, 1 H, H-8), 5.2-4.9 (m, H-2', H-α, partially obscured by HOD), 4.8-4.7 (m, 1 H, H-1'), 4.43 (dd, J=5.5 Hz, J=3.0 Hz, 1 H, H-3'), 4.2-4.0 (m, 2 H, H-5'), 3.06 (ABX, Δδ=0.13 ppm, J_{AB}=15.5 Hz, J_{AX}=4.2 Hz, J_{BX}=9.0 Hz, 2 H, H-β), 2.85-2.7 (m, 1 H, H-6'), 2.7-2.5 (m, 1 H, H-4'), 2.2-2.0 (m, 1 H, H-6'); ^{13}C NMR (D$_2$O, EtOH (ext.)) δ 178.9 (2 C, CO$_2^-$), 153.9 (C-6), 152.1 (C-4), 148.3 (C-2), 140.0 (C-8), 118.8 (C-5), 75.2 (C-3'), 71.8 (C-2'), 64.9 (C-5'), 58.8 (C-1'), 53.7 (C-α), 44.5 (C-4'), 39.9 (C-β), 28.5 (C-6'); ^{31}P NMR (D$_2$O, 85% H$_3$PO$_4$ (ext.)) δ 7.23; MS (ion spray) MH$^+$-Ba 595.5, calcd. for C$_{15}$H$_{16}$O$_{10}$N$_5$PBa, 594.5.

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REFERENCES AND NOTES

1. Abbreviations used: ASL, adenylosuccinate lyase; AICAR, amino-imidazole carboxamide ribonucleotide; BSA, bovine serum albumin; C-AICAR, carbocyclic aminoimidazole carboxamide ribonucleotide; CF_3-HOBT, 6-trifluoromethyl-1-hydroxybenzotriazole; *m*CPBA, *meta*-chloro-perbenzoic acid; DEAD, diethyl azodicarboxylate; DCC, dicyclohexyl-carbodiimide; ddATP, 2',3'-dideoxyadenosine triphosphate; ddI, 2',3'-dideoxyinosine; DMA, dimethylacetamide; GAICAR, glutaroamino-imidazole carboxamide ribonucleotide; C-GAICAR, carbocyclic glutaro-aminoimidazole carboxamide ribonucleotide; SAICAR, succinoamino-imidazole carboxamide ribonucleotide; C-SAICAR, carbocyclic succino-amino imidazole carboxamide ribonucleotide; TBAF, tetrabutylammonium fluoride; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; TMS, tetramethylsilane.
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