Table III for additional data and other compounds prepared by this method.

In **Method B**, the product oiled out of solution and was extracted with two 25-mL portions of $CHCl_3$. The combined extracts were washed with 25 mL of brine, dried ($MgSO_4$), and spin evaporated in vacuo to give a clear syrup, which solidified when left overnight. In **Method C**, DMF was used as the solvent; the crude product was dissolved in a minimum of Me_2CO, diluted with 300 mL of ice–H_2O, and basified to pH 7–8 with 5% $NaHCO_3$. The product was collected and washed with H_2O, and the dissolution–precipitation was repeated twice more when the yellow color of 4-nitrophenol was no longer present. See Table III for compounds prepared by these methods.

3-[4-(Fluorosulfonyl)benzamido]benzyl Bromide. Method D. A mixture of 0.449 g (1.4 mmol) of **22** and 5 mL of 30% anhydrous HBr–AcOH was heated at 100 °C for 10 min. The solution was cooled on an ice bath and then diluted with ice–H_2O. The product was collected, washed with H_2O, dried over $CaSO_4$, and finally over P_2O_5 under low vacuum: yield 0.520 g (96%) of a white powder, which was homogeneous by TLC (B) and gave a positive test for active halide.[29] This material was used without further purification.

5-(3-Ethoxybenzyl)-1-[4-[3-(fluorosulfonyl)benzamido]-benzyl]uracil (13). Method E. A mixture of 0.246 g (1.0 mmol) of **3**, 5 mL of hexamethyldisilazane, and 0.2 mL of chlorotrimethylsilane with protection from moisture was refluxed with stirring for 4 h, during which dissolution occurred. To the cooled solution was added a dispersion of 0.560 g (1.5 mmol) of 4-[3-(fluorosulfonyl)benzamido]benzyl bromide in 5 mL of acetonitrile. The mixture was refluxed with stirring for 40 h, cooled, and spin evaporated in vacuo, and the residue was dissolved in 5 mL of hot EtOH. This solution was cooled and spin evaporated in vacuo, and the residue was triturated with 5 mL of ice–H_2O. The product was collected, washed with H_2O, and recrystallized from EtOH: yield 0.195 g (36%); mp 219–222 °C. Evaporation of the mother liquors afforded an additional 0.132 g (total 61%), mp 213–219 °C. Several addition recrystallizations gave the analytical sample as white granules: mp 220–222 °C; UV (EtOH) λ_{max} 280 nm; UV λ_{max} pH 13, 278 nm; IR (Nujol) 3280 (NH), 1685, 1660, 1600, 1530 (NHC=O, C=N, C=C), 1410, 1210 (SO_2F), 1255 (COC) cm^{-1}.

Irreversible Inhibition of FUDR Phosphorylase. The irreversible assay was carried out on twice the scale used for the reversible assay.[8] Five pairs of tubes were placed in a rack; the back tubes served as zero-time tubes. In each tube was placed 5.00 mL of the assay mix and Me_2SO or a Me_2SO-inhibitor solution, such that the final inhibitor concentration was 20 μM. After 20 min, 3.0 mL of 1-octanol was added to tube 1 of the back (zero time) tubes and mixed on a Vibro Jr. Mixer for 30 s. Then, tubes 2–5 of the back (zero time) tubes and tubes 1–5 of the front tubes were treated similarly. The tubes were centrifuged for 3 min, the 1-octanol layer was removed, and the extraction was repeated with 3.0 mL of fresh 1-octanol. After the second extraction had been centrifuged and removed, 500 μL of the aqueous layer from each tube was transferred to a new set of five paired tubes. (The outside of the pipet was wiped dry when delivering to the new tubes, and care was taken to avoid getting octanol in the pipet.) To each of the five new back (zero time) tubes was added 500 μL of 5% aqueous trichloroacetic acid, and the contents were mixed. To the new front tubes was added 50 μL of 4 mM FUDR at 30-s intervals, and the contents of each tube were mixed after each addition. Then, 50 μL of 4 mM FUDR was added to the new back (zero time) tubes, and the contents were mixed. After the incubation period, 500 μL of 5% aqueous trichloroacetic acid was added to each of the front tubes. All of the tubes were centrifuged for 5 min, and the solutions were then assayed as for the reversible assay.[8]

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Inhibitors of Adenosine Deaminase. Studies in Combining High-Affinity Enzyme-Binding Structural Units. *erythro*-1,6-Dihydro-6-(hydroxymethyl)-9-(2-hydroxy-3-nonyl)purine[1] and *erythro*-9-(2-Hydroxy-3-nonyl)purine[2]

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erythro-1,6-Dihydro-6-(hydroxymethyl)-9-(2-hydroxy-3-nonyl)purine (4) was synthesized as a potential adenosine deaminase inhibitor, which combines in a single molecule two structural moieties, each of which possesses high affinity to a different region of the enzyme, the catalytic region and an auxiliary binding region which is specific for *erythro*-9-(2-hydroxy-3-nonyl)adenine (1). The potency of 4 (K_i = 1.2 × 10^{-5} M) is about one-seventeenth that of *erythro*-9-(2-hydroxy-3-nonyl)purine (**2**; K_i = 6.8 × 10^{-7} M), which contains only one high-affinity moiety. The mutually interfering rather than reinforcing effects of the two moieties may indicate the lack of simultaneous binding and thus provide insight into the relative geometry of the two binding regions of the enzyme.

Potent inhibitors of adenosine deaminases (ADA), which catalyze the hydrolytic N^6-deamination of adenosine, 2′-deoxyadenosine, and related nucleosides, are of interest as possible medicinal agents. Thus, in addition to its potentiating effect on nucleoside-type antitumor or antiviral agents, pentostatin[3] has generated interest for possible utility in immune modulation and lymphocyte control, as in human T-cell malignancies.[4,5]

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(1) [(*R**,*S**)-(±)]-*β*-Hexyl-1,6-dihydro-6-(hydroxymethyl)-*α*-methyl-9*H*-purine-9-ethanol.

(2) (*R**,*S**)-*β*-Hexyl-*α*-methyl-9*H*-purine-9-ethanol.

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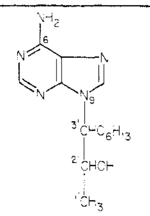
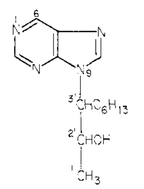
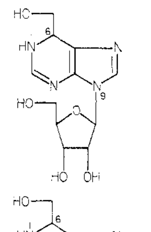
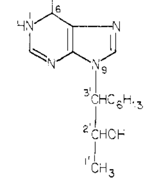
Table I. Competitive Inhibition of Calf Intestinal Adenosine Deaminase (ADA)

no.	structure	K_i, M	K_m/K_i [d,e]
1 [a]		1.3×10^{-9}	1.8×10^4 [f]
2 [a]		6.8×10^{-7}	36
3 [b]		7.0×10^{-7}	35 [g]
4 [c]		1.2×10^{-5}	2.1

[a] Mixture of two isomers: 2'*S*,3'*R* and 2'*R*,3'*S*. [b] Mixture of two isomers: 6*R* and 6*S*. [c] Mixture of four isomers: 2'*S*,3'*R*,6*R*, 2'*R*,3'*S*,6*R*, 2'*S*,3'*R*,6*S*, and 2'*R*,3'*S*,6*S*. [d] K_m for adenosine = 2.5×10^{-5} M. [e] The ratio K_m/K_i may serve as an estimate of the upper limit of REA (relative enzyme affinity) of A-X, an inhibitor, compared to B-Y, the substrate. If moieties X and Y are structurally identical (such as the ribosyl moiety in 3 and in adenosine) and bound identically to the enzyme, the ratio should similarly indicate the REA of the remaining moiety A compared to the corresponding dissimilar moiety B. [f] 1.6×10^4 for human erythrocyte ADA.[9] [g] 19 for human erythrocyte ADA.[9]

Results and Discussion

The potent inhibitory action of pentostatin, coformycin,[6] and 1,6-dihydro-6-(hydroxymethyl)-9-β-D-ribofuranosylpurine (DHMRP, **3**)[7] primarily results from strong binding, of structural groupings analagous to the tetrahedral transition state, to the catalytic site of the enzyme, a region which normally binds the C_6-NH_2 region of adenosine or related substrates.[3,7,8] According to the ratios K_m/K_i (cf. Table I), calculated from reported data, it may be estimated that the heterocyclic moieties in pentostatin and DHMRP (**3**) show a relative enzyme affinity (REA) factor of 2.8×10^6 and 19, respectively, compared to their counterpart (adenine) in the substrates deoxyadenosine and adenosine.[9]

The inhibitory action of *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA, **1**),[10-12] on the other hand, is based on a different mode of binding, since it binds to the enzyme at an auxiliary region considered as closely related to the region which normally binds the ribose portion of adenosine or related substrates. Superficially, the side chain in EHNA appears to show an REA factor of 1.6×10^4, compared to its presumed counterpart (ribose) in adenosine.[9]

By combining in a single molecule two structural moieties each of which possesses high affinity to a different site of an enzyme, an inhibitor of exceptionally high activity could result, provided that simultaneous binding of both moieties could be attained. Compound **4** represents a combination of the catalytic site binding moiety of DHMRP (**3**) and the auxilliary site binding moiety of EHNA (**1**), which might conceivably be about 19 times as active as EHNA.[9]

However, the fact that EHNA (**1**) is not a substrate of ADA indicates that the auxilliary EHNA-specific region of the enzyme might have only a low correspondence in binding geometry with the ribose-binding region, orienting the C-6 amino of EHNA or the transition-state-like portion of **4** away from the catalytic region where deamination occurs. In that event, the two high-affinity moieties of **4** would most likely be unable to reinforce each other in enzyme binding.

In fact, **4** showed a low order of ADA inhibitory activity, consistent with an unsupported footnote disclosing preliminary experiments.[7] On the other hand, compound **2**, which has only one of the high-affinity moieties, is 17 times as active as **4**, being equipotent with the semitight binding inhibitor DHMRP (**3**). The mutually opposing effect clearly indicates that simultaneous binding of the two moieties has not been attained. In addition, the higher activity of EHNA (**1**), relative to that of **2**, indicates an important contribution to binding by the 6-amino group.

Chemistry. Racemic **2** was obtained by palladium-catalyzed hydrogenolytic dechlorination of *erythro*-6-chloro-9-(2-hydroxy-3-nonyl)purine (**5**), which had been prepared according to Schaeffer and Schwender.[10] Subsequent photochemical addition of methanol to **2**, according to the procedure of Connolly and Linschitz,[13] yielded **4**, consisting of two diastereomeric *dl* pairs. While homogeneous on TLC, the two *dl* pairs could be differentiated by the difference in the chemical shift of one of the aromatic protons and were estimated by integration to be present in nearly equal proportions. The compound appeared to be somewhat unstable in neutral aqueous solutions, in accord with the reported susceptibility of the dihydropurine moiety to oxidation and photochemical reactions.[7]

Experimental Section

TLC was performed using EM (E. Merck) silica gel 60 F 254 precoated plates. Column chromatography was performed on EM silica gel 60, particle size 0.040–0.063 nm (230–400 mesh ATSM). Mass spectrum was obtained with a Finnigen 1015 quadrupole spectrometer. Where analyses are indicated by symbols of the

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elements, analytical results were within ±0.4% of the theoretical values.

***erythro*-9-(2-Hydroxy-3-nonyl)purine (2; Mixture of Two Isomers: 2′S,3′R and 2′R,3′S).** Racemic *erythro*-5-amino-4-chloro-6-(2-hydroxy-3-nonyl)pyrimidine (6) [mp 121–122 °C; TLC R_f ($CHCl_3$/MeOH/Et_3N, 86:6:8) ~0.5, yield 1.165 g (4.05 mmol)] was synthesized and treated with triethyl orthoformate according to published procedures.[10] TLC showed the absence of starting material after 1.5 h. After 1 day, the clear solution was evaporated in vacuo to a yellow oil containing crude *erythro*-6-chloro-9-(2-hydroxy-3-nonyl)purine (5). An ice-cold ethanolic solution of the crude product was treated with 550 mg of potassium acetate and hydrogenated in the presence of palladium (from 220 mg of 20% palladium oxide on barium carbonate) at atmosphere pressure for 5 h. After filtering and washing the solid with methanol, the solution was concentrated to a black residue. The residue was chromatographed over 15 g of silica gel packed in chloroform. The column was developed with 50 mL of chloroform and then with chloroform containing methanol in increasing concentration: 50 mL of 1%, 50 mL of 2%, 100 mL of 3% (fractions 21–34), and finally 60 mL of 5% (fractions 35–43). Fractions 23–34 were combined to yield 490 mg (46%) of a viscous residue: TLC R_f 0.34 (7% MeOH in $CHCl_3$); UV (MeOH) λ_{max} 264 nm (log ε 3.86). Anal. $C_{14}H_{22}N_4O{\cdot}0.15H_2O$) C, H, N.

***erythro*-1,6-Dihydro-6-(hydroxymethyl)-9-(2-hydroxy-3-nonyl)purine (4; Mixture of Four Isomers: 2′S,3′R,6R, 2′R,3′S,6R, 2′S,3′R,6S, and 2′R,3′S,6S).** A solution of 244 mg of 2 (0.92 mmol) in 50 mL of dried methanol in a rotating quartz cylinder (15 cm long × 5 cm o.d.) was irradiated with four 15-W G.E. germicidal lamps as described by Connolly and Linschitz[13] under a nitrogen atmosphere for 230 min. The solution was evaporated in vacuo to 285 mg of solid residue, which was then chromatographed over a column of 2 g of silica gel (8.8 × 1.0 cm o.d.). The column was eluted with 6 mL of chloroform and then with chloroform containing increasing amounts of methanol: 27 mL of 2% (fractions 3–15), 15 mL of 4% (fractions 16–22), 15 mL of 3% (fractions 23–27), and 10 mL of 8% (fractions 28–29). The product was obtained from fractions 18–27 as 157 mg (55%) of solid: TLC R_f 0.19 (15% MeOH in $CHCl_3$); mp ~67–72 °C; UV (H_2O) λ_{max} 292 nm (log ε 3.60), 244 (3.37); UV (MeOH) λ_{max} 295 nm (log ε 3.65), 245 (3.40); the UV max at 292 nm of a 0.004% aqueous solution decreased by about 5% after 5 days at room temperature; ^1H NMR ($CDCl_3$, after D_2O exchange), 5.11 (dd, 1 H, $HCCH_2OH$, J = 3.5 and 7.0 Hz), 7.0 (s, 1 H, H-2), 7.16 (s, ~0.45 H) and 7.18 (s, ~0.55 H) (H-8 singlets from each of two *dl* pairs); the NMR sample after D_2O exchange showed extensive decomposition after 1 day; mass spectrum, m/e 294.2 (2.85%), 277.2 (7.37%), 264.3 (18.3%), 263.2 (100%), 219.1 (5.62%), 120.9 (19.46%). Anal. ($C_{15}H_{16}N_4O_2{\cdot}0.13CHCl_3$) C, H, N.

Assays of Adenosine Deaminase Inhibitory Activity. A modification of the procedure of Kalckar[14] was used. Calf intestinal mucosal ADA (EC 3.5.4.4; Sigma Chemical Co., type I) suspended in 3.2 M ammonium sulfate (buffer), 50 μL (125 units), was added to 5 mL of a 0.025 M (pH 8) ammonium acetate buffer, and the solution was dialyzed using Spectra/Por 1 cellulose membrane tubing (Fisher) against 500 mL of the same buffer for 48 h at 5 °C, changing the dialyzing bath every 12 h. Such a preparation, useable over a period of a few weeks when stored at 5 °C, was diluted about 20- to 50-fold with 0.050 M (pH 7.5) phosphate buffer, as needed, to a concentration which would give an uninhibited deamination rate of 1–3 × 10^{-8} M/s with ca. 6 × 10^{-6} M adenosine by the assay procedure below.

The rates of deamination were determined at 25 °C by monitoring for about 3 min the drop in absorbance at 265 nm against six or more varying concentrations of adenosine (8.6 × 10^{-5} to 6.9 × 10^{-6} M) and a fixed concentration of the inhibitor (within ±250% of the eventually determined K_i value) in 0.05 M (pH 7.5) phosphate buffer. The reaction was started by adding 20 μL of the diluted ADA to a premixed solution, prepared about 2 min earlier from solutions of the inhibitor (0.02 mL) and of adenosine (3.02 mL). The initial slopes were converted to "molar concentration per second" rates with a factor of 8100 (confirmed experimentally) as Δε between adenosine and inosine. Subsequent Lineweaver–Burk[15] treatment of the data established the type of inhibition (competitive) and gave the K_i values tabulated in Table I.

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Renin Inhibitors. Substitution of the Leucyl Residues of Leu-Leu-Val-Phe-OCH_3 with 3-Amino-2-hydroxy-5-methylhexanoic Acid

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The 2*S*,3*S* and 2*R*,3*S* diastereoisomers of the hydroxy amino acid 3-amino-2-hydroxy-5-methylhexanoic acid (AHMHA) were synthesized and substituted for the leucyl residues of Leu-Leu-Val-Phe-OCH_3 to yield the following analogues: AHMHA-Leu-Val-Phe-OCH_3, AHMHA-Val-Phe-OCH_3, and Leu-AHMHA-Val-Phe-OCH_3. These analogues were tested in vitro for their ability to inhibit human amniotic renin. All of the analogues were found to inhibit renin to some extent with inhibitory constants in the range of 10^{-3} to 10^{-4} M. The analogues AHMHA-Leu-Val-Phe-OCH_3 and AHMHA-Val-Phe-OCH_3 exhibited competitive inhibition when the 2*S*,3*S* isomer of AHMHA was employed and noncompetitive kinetics when the 2*R*,3*S* isomer of AHMHA was used. For the Leu-AHMHA-Val-Phe-OCH_3 analogues, competitive kinetics were observed regardless of the isomer of AHMHA employed. These latter analogues also proved to be the most active in the above series.

In a previous report[1] I described the synthesis and renin inhibitory activity of several *N*-(α-hydroxyalkanoyl) derivatives of Leu-Val-Phe-OCH_3. These compounds were synthesized in an attempt to mimic the postulated transition state of the renin–angiotensinogen reaction. It was felt that the α-hydroxy moiety of the α-hydroxyalkanoyl residue might simulate the hydroxyl moiety that is thought to be formed when the Leu[10] carbonyl group is converted into a tetrahedral intermediate during the enzymatic reaction. The results of this previous study[1] showed that the replacement of the N-terminal leucyl residue of the known substrate analogue inhibitor Leu-Leu-Val-Phe-OCH_3[2] with various α-hydroxyalkanoyl residues led to

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