levels. Prolactin secretion seems to be regulated by central as well as by peripheral GABA receptors, the latter receptors being located directly on the anterior pituitary.[92] Activation of the central and peripheral GABA receptors stimulates and inhibits, respectively, prolactin release,[92] suggesting that GABA agonists like P4S and isoguvacine, which do not pass the BBB, have therapeutic interest in this clinical situation.

In agreement with the involvement of GABA in feeding behavior, THIP (po) has anorexigenic actions stronger than those of cocaine (po) but ten times weaker than those of *d*-amphetamine.[93] This action of THIP may be relevant for the treatment of human obesity.

GABA Uptake Inhibitors as Potential Antiepileptics. The pharmacology of a number of GABA uptake inhibitors on single cells in vivo has been investigated using microelectrophoretic techniques. In experiments where inhibitor and GABA were administered simultaneously to cells in the spinal cord or in the cerebellum of cats,[94-96] all types of inhibitors, namely, DABA (neuronal) THPO (glial), and nipecotic acid, and guvacine (glial/neuronal) (Figure 2) enhanced the depressant action of GABA on neuronal firing. However, marked differences between different types of inhibitors were observed after intracerebroventricular (icv) injection into mice.[73] DABA and ACHC provoked generalized seizures, whereas THPO and nipecotic acid protected the animals against audiogenic seizures, and systemic administration of THPO or prodrugs of nipecotic acid (Figure 4) effectively protected the animals against seizures.[62]

Why are inhibitors of neuronal GABA uptake convulsants and selective glial uptake inhibitors anticonvulsants? Intramuscular injections of THPO or nipecotic acid ethyl ester into mice elevate the concentration of GABA in the nerve terminals of the brain,[74] probably because blockade of the glial uptake system results in a preferential reuptake of synaptically released GABA into the nerve terminals. This increase of the releasable pool of GABA may facilitate the GABA neurotransmission process and in this way produce anticonvulsant effects.[62]

The neuronal GABA uptake process seems to be coupled to the release system by an as yet unknown mechanism, and ACHC and related amino acids have been shown to be effective inducers of GABA release.[64] Thus, the convulsant effects of ACHC and DABA (icv) may be the consequence of interruption of GABA-mediated inhibition by depletion of GABA from the terminals. Nipecotic acid also has some effect on neuronal GABA uptake (Figure 2), although weaker than the effect on the glial system.[33] Nipecotic acid does not, however, stimulate GABA release from synaptosomes in vitro, suggesting that the mechanism of interaction of this uptake inhibitor with the neuronal transport carrier is different from that of ACHC and DABA.[64] Such a difference may contribute to the difference between the pharmacology of these amino acids and nipecotic acid. In any case, the present investigations have brought glial GABA uptake inhibitors into focus as potential antiepileptic drugs.

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Communications to the Editor

Adenosine Deaminase Inhibitors. Conversion of a Single Chiral Synthon into *erythro*- and *threo*-9-(2-Hydroxy-3-nonyl)adenines

Sir:

Adenosine deaminase (ADA) inhibitors are known to enhance the cytotoxic activity of a variety of adenosine analogues. Among these are adenosine arabinoside (*ara*-A), 8-azaadenosine, and formycin.[1]

Coformycin (CF) and 2′-deoxycoformycin (2′-dCF) are two naturally occurring nucleoside antibiotics which are the most potent inhibitors of the enzyme identified to date (K_i = 10^{-11} to 10^{-12} M).[2,3] Among the synthetic compounds, (±)-*erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) was rationally designed and synthesized by Schaeffer and Schwender[4] and was found to be less active than the above antibiotics ($K_i = 4 \times 10^{-9}$ M).[5] However, reactivation of inhibited ADA has been observed to be much faster for (±)-EHNA than for CF and 2′-dCF.[6] It is this property that has been cited recently as being of potential importance in viral chemotherapy.[7] Prompted by these reports, the synthesis of the title compounds (1–4) was undertaken to identify the most active inhibitor and to examine the biological activity of these isomers vis-à-vis

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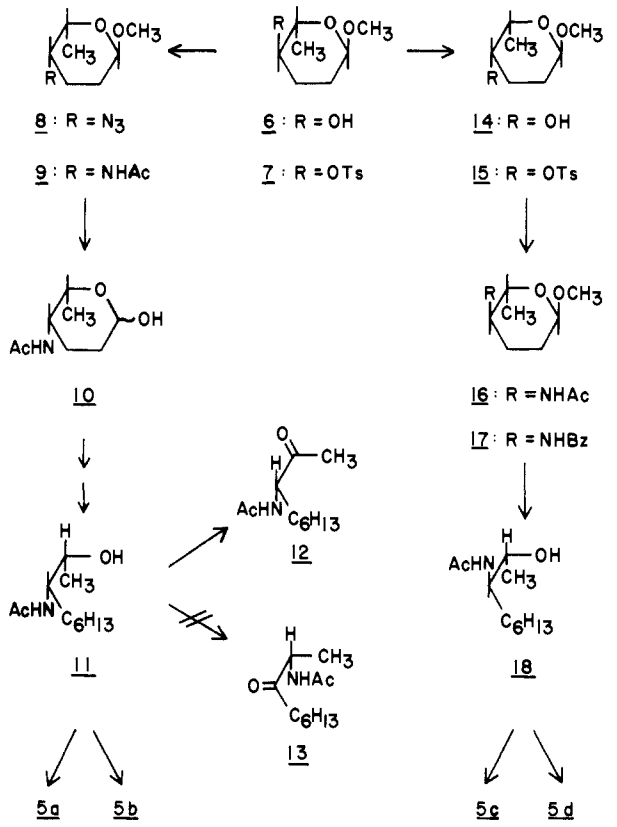
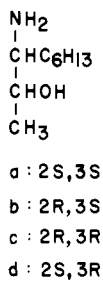
Scheme I



their effect on purine metabolism.[8]

Starting from a sugar-derived chiral substrate, the preparation of four key amino alcohols (**5a–d**), their incorporation into the adenine nucleus, as well as the ADA inhibitory activity of 1–4 are reported.

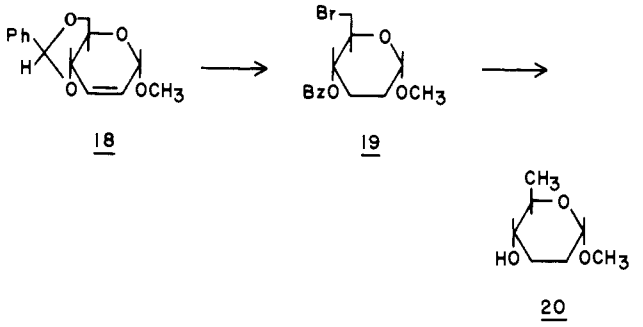


5 a : 2S,3S
b : 2R,3S
c : 2R,3R
d : 2S,3R

Retrosynthetic analysis led to methyl α-L-amicetoside (**6**)[9] and methyl α-rhodinoside (**14**),[10] two trideoxyhexopyranosides that can be obtained from L-rhamnose in high yields. Scheme I depicts the synthetic routes to the amino alcohols (**5a–d**), where reactions involving chiral centers have an unambiguous stereochemical outcome.

Tosylation of **6**, followed by azide displacement, furnished the azido compound 8 ($[\alpha]_D$ −71.2° (*c* 0.99, EtOH)), which was catalytically reduced over 5% Pd/C and acetylated to give the acetamido derivative **9** ($[\alpha]_D$ −114.3° (*c*, 1.02, EtOH)) in overall yields of 73% from **6**. That no rearrangement to a furanose occurred during azide displacement, similar to that reported for L-rhamnose-4-sulfonates,[11] was proven by chromic acid oxidation of the acetamido alcohol **11** to the corresponding methyl ketone **12**. The methyl group in this molecule appeared as a singlet at δ 2.2, as compared to a doublet (J = 6 Hz) at δ 1.2 for that in the parent alcohol **11**. Had a rearrangement taken place a hexyl ketone (**13**) would have been obtained whose 1-methyl group would have remained a doublet. The synthesis was then continued by adding a three-carbon unit via a Wittig reaction of the trideoxyhexopyranose **10**, obtained by acid hydrolysis from **9**, to give an isomeric mixture of olefins in 50–60% yields after column chromatography on silica gel. This mixture was reduced (5% Pd/C) to afford the acetamido alcohol **11** in quantitative yield.[12]

Scheme II



Choice of the hydrolytic conditions of the acetamido group in **11** furnished either of the target amino alcohols. Hydrolysis with 1 N HCl or hydrazine gave the (−)-threo isomer **5a**, while treatment with thionyl chloride prior to acid hydrolysis resulted in inversion of configuration at C-2 by an SN_i mechanism, via an oxazoline intermediate,[13] to provide the (−)-erythro isomer **5b**.

The remaining amino alcohols **5c** and **5d** were prepared in an identical fashion starting with **14** ($[\alpha]_D$ −116.9° (*c* 1.02, $CHCl_3$)). This compound was obtained from **7** in a two-step sequence which involved an inversion at C-4. A SN_2 displacement of the C-4 tosylate group with sodium benzoate was followed by base hydrolysis of the resulting benzoate ester to furnish **14**. This inversion also occurred without rearrangement and was proven by converting the tosylate **15** to the known benzamide **17**.[14] In addition to the proof of ring size, the latter sequence of reactions provided evidence for the stereochemical assignments at the chiral centers in question, since the physicochemical constants of synthetic **17** ($[\alpha]_D$ −140.2° (*c* 0.5, EtOH), lit.[14] $[\alpha]^{24}_D$ −139° (*c* 0.5, EtOH); mp 137–138 °C, lit.[14] mp 136–140) matched those of **17** obtained from natural sources.[14]

The four chiral adenines (1–4) were prepared following the published synthesis of the racemic material by condensation of **5** and 5-amino-4,6-dichloropyrimidine, ring formation with triethyl orthoformate, and chloride displacement with liquid ammonia.[4]

An equally attractive synthesis of **5** was also accomplished starting with methyl α-D-amicetoside (**20**), the enantiomer of **6** which is derived from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside via the hexeno-

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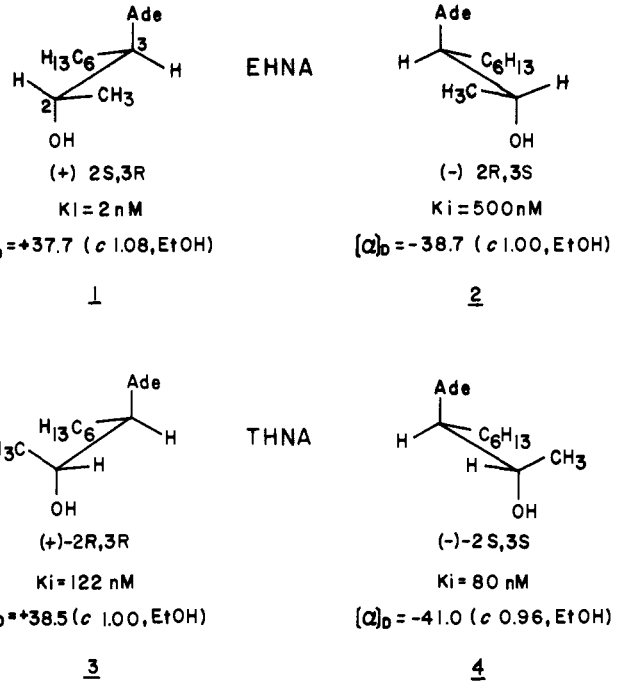
Chart I



pyranoside[15] 18 followed by catalytic reduction, and formation of the 6-deoxy derivative (**20**) according to known methods (Scheme II).[16]

Inhibition of human erythrocytic ADA was performed on all four isomers (**1**–**4**), and the K_i values obtained are listed in Chart I. The most active compound was found to be (+)-*erythro*-9-[2(*S*)-hydroxy-3(*R*)-nonyl]adenine (**1**).[17] Its enantiomer was the least active, while the two threo isomers, where only one chiral center is inverted, had reduced but comparable activities. Their effect on purine metabolism as well as their chemotherapeutic activity are currently under investigation.

It is worth noting that the synthetic methods described herein are versatile, utilize inexpensive starting materials, and offer the opportunity to incorporate the amino alcohols **5** into other heterocycles. Of special interest will be future attempts to attach **5d** to the aglycon of coformycin.[3] Furthermore, the Wittig reaction can be modified to introduce functional groups on the lipophilic hydrocarbon portion of **5**. Such groups could play an important role either as ligands, which might aid in the purification of ADA, or as probes of the hydrophobic center of the enzyme, resulting in a more potent inhibitor.

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(18) Chargé de Recherches, INSERM, France.

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Inhibition of Thymidylate Synthetase by 5-Alkynyl-2′-deoxyuridylates[1]

Sir:

Thymidylate (dTMP) synthetase (EC 2.1.1.45) catalyzes the conversion of 2′-deoxyuridylate (dUMP) and 5,10-methylenetetrahydrofolate (CH_2-H_4folate) to 2′-deoxythymidylate (dTMP) and 7,8-dihydrofolate. This enzyme represents the sole de novo pathway for dTMP synthesis and has received much attention as a target for inhibitors with potential chemotherapeutic value. The catalytic mechanism and inhibition of this enzyme have recently been reviewed.[2,3] One class of potent inhibitors of this enzyme is 5-substituted dUMP's, which act as mechanism-based inhibitors. An early event in the normal enzymatic reaction involves nucleophilic attack of a cysteine thiol of the enzyme at the 6 position of dUMP to form 5,6-dihydropyrimidine intermediates which are covalently bound to the enzyme during the remaining catalytic sequence. The 5-substituted dUMP's, which are mechanism-based inhibitors of this enzyme, undergo similar nucleophilic attack at the 6 position; subsequently, the analogue either remains attached to the enzyme or a moiety at the 5 position of the inhibitor is activated so that it may covalently interact with the enzyme.

β,γ-Acetylenic carbonyl compounds have received much attention as suicide inactivators of enzymes.[4,5] The acetylenic functional group is normally inert toward nucleophiles, but enzyme-catalyzed generation of a carbanion at the α carbon can result in isomerization to a conjugated allene; the latter is a powerful Michael acceptor, and if a nucleophile of the enzyme is juxtaposed to the reactive β carbon, covalent bond formation can occur. As suggested by other workers,[4,6] since the initial covalent bond formation between dTMP synthetase and 5-substituted dUMP's generates a transient carbanion at the 5 position, 5-alkynyl-dUMP's are potential suicide inactivators of this enzyme. 5-Ethynyl-dUrd (EdUrd) has recently been

(1) Abbreviations used: E, 5-ethynyl; HOP, 5-(3-hydroxypropynyl); HOB, 5-(4-hydroxybutynyl); H, hexynyl; PhE, 2-phenylethynyl. All other abbreviations are those recommended by IUPAC.
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