Enzyme Inhibitors. 26. Bridging Hydrophobic and Hydrophilic Regions on Adenosine Deaminase with Some 9-(2-Hydroxy-3-alkyl)adenines

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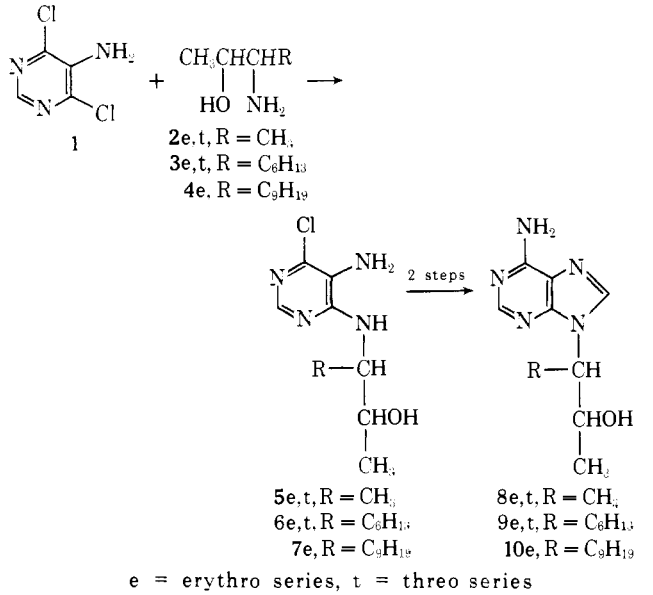
The syntheses of some *erythro*- and *threo*-9-(2-hydroxy-3-alkyl)adenines from 5-amino-4,6-dichloropyrimidine and the appropriate amino alcohols are described. Based on earlier studies, it was predicted and substantiated that the erythro diastereoisomers would be more potent inhibitors of adenosine deaminase than their corresponding threo diastereoisomers. These data support the concept that on adenosine deaminase there is a single binding site for the adenine moiety of the inhibitors and that there is a close spatial relationship of the methyl binding site, the hydroxyl binding site, and the large hydrophobic region utilized by the 9 substituent of the inhibitors.

Earlier studies have shown that calf intestinal mucosal adenosine deaminase possesses several regions which are important for binding the 9 substituent of various 9-substituted adenines.[1-3] These binding areas for the 9 substituent are a large hydrophobic region,[1] a hydroxyl binding site,[2] and a specific methyl binding region.[3] Furthermore, it has been demonstrated that when the 9 substituent of some 9-substituted adenines contains a chiral center, there is a stereoselectivity in the formation of the enzyme-inhibitor complex.[3,4] For example, with some 9-(1-hydroxy-2-alkyl)adenines, the preferred chiral center for EI complex formation has the *R* configuration[4] whereas with 9-(2-hydroxypropyl)adenine, the chiral center with the *S* configuration is bound more tightly to the enzyme than the compound with *R* configuration.[3] With this insight into the types of binding regions and the stereoselectivity of inhibition of adenosine deaminase at two different chiral centers of some 9-substituted adenines, it should be possible to design potent inhibitors of this enzyme. This paper describes the syntheses of some 9-(2-hydroxy-3-alkyl)adenines and their evaluation as inhibitors of adenosine deaminase.

Chemistry. The syntheses of the compounds required for this study were accomplished by the general procedure outlined in Scheme I.[4-6] 5-Amino-4,6-dichloropyrimidine (1) was condensed with an appropriate *erythro*- or *threo*-amino alcohol. The resultant substituted pyrimidine was cyclized with triethyl orthoformate to the 6-chloropurine intermediate which, upon reaction with ammonia, gave the desired 9-(2-hydroxy-3-alkyl)adenine.

Scheme I



e = erythro series, t = threo series

erythro- and *threo*-3-amino-2-butanols[7,8] were prepared from *trans*- and *cis*-2-butenes, respectively. *erythro*-3-Amino-2-nonanol and *erythro*-3-amino-2-dodecanol were prepared by KBH_4 reduction of the corresponding amino ketones, whereas *threo*-3-amino-2-nonanol (**6t**) was synthesized by inversion of the erythro isomer by means of an N → O acyl migration.[9,10]

The stereochemistry of the amino alcohols follows from their methods of synthesis and by the fact that in the pmr spectra, the vicinal spin–spin coupling constant of the two methinyl protons is 3.5 Hz for the erythro isomers and 6.0 Hz for the threo isomer. These pmr results are similar to those found by Portoghese[11] who observed that in the ephedrine isomers, the methinyl proton coupling constants were smaller for ephedrine (erythro) than for Ψ-ephedrine (threo).

Results and Discussion

Earlier studies have established that (*R*)-9-(1-hydroxy-2-alkyl)adenines and (*S*)-9-(2-hydroxypropyl)adenine are better inhibitors of adenosine deaminase than are their corresponding enantiomers.[3,4] If these inhibitors form complexes with the same site on the enzyme,[12] one would predict that in certain inhibitors where the two chiral centers of the 9 substituents were combined, the most active inhibitor of a diastereoisomeric pair would be the one with the erythro configuration. Examination of the data in Table I shows that for some 9-(2-hydroxy-3-alkyl)adenines, the erythro isomers **8e** and **9e** are significantly better inhibitors of adenosine deaminase than are the corresponding threo isomers **8t** and **9t**.

The combined effect of stereochemistry and hydrophobic forces is dramatically shown by these compounds. For example, **10e** will cause 50% inhibition of the enzymic reaction at a concentration of approximately 10 n*M* when the substrate concentration is 6.6×10^{-5} *M*. In terms of free energy, the overall ΔG resulting from stereochemical and hydrophobic forces in a comparison of **10e** with **11** is −5.3 kcal. In terms of the dissociation constant, such a change in free energy would correspond to a K_i of approximately 10^{-4} *M*. Note that this significant contribution to the K_i of **10e** is exclusively from the stereochemical and hydrophobic features found in the 9 substituent of **10e**.

The change in free energy of EI complex formation resulting from the addition of a methyl group to the 9-(1-hydroxy-2-alkyl)adenines (**11–13**) is also given in Table I. Note that for the threo isomers **8t** and **9t**, the change in free energy relative to **11** and **12**, respectively, is minimal, whereas for the erythro isomers **8e**, **9e**, and **10e** the change in free energy is large. In fact, for **9e** and **10e**, the change in free energy is clearly beyond simple hydrophobic trans-

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†This paper is dedicated to Professor Alfred Burger.

Table I. Inhibition of Adenosine Deaminase by Ad-CHR_1CHOHR_2

Compd no.	R_1	R_2	Configuration	$(I/S)_{0.5}$[a]	$\Delta G(CH_3)$,[b] cal
11	CH_3	H	*RS*	1.2[c]	
8e	CH_3	CH_3	Erythro	0.45	−566[d]
8t	CH_3	CH_3	Threo	0.98	−107[d]
12	C_6H_{13}	H	*RS*	0.0062[c]	
9e	C_6H_{13}	CH_3	Erythro	0.00029	−1810[e]
9t	C_6H_{13}	CH_3	Threo	0.0045	−190[e]
13	C_9H_{19}	H	*RS*	0.0030[c]	
10e	C_9H_{19}	CH_3	Erythro	0.00016	−1730[f]

[a]The inhibition index $(I/S)_{0.5}$ is the ratio of the millimolar concentration of the inhibitor for 50% inhibition to the millimolar concentration of the substrate. The concentration of adenosine in all experiments was 0.066 m*M*. [b]Calculated by the equation $\Delta G = -RT \ln [(I/S)_{0.5}$ for A$/(I/S)_{0.5}$ for B], where A and B differ by one methyl group. [c]Data taken from ref 12. [d]Calculation based on compound **11**. [e]Calculation based on compound **12**. [f]Calculation based on compound **13**.

fer forces[13] and probably reflects a special interaction with the methyl binding site on adenosine deaminase resulting in a conformational change in the enzyme.[3,14]

Since both 9e and 10e were very potent inhibitors of adenosine deaminase, it was possible that they would act as pseudoirreversible inhibitors of the enzyme. However, when 9e and 10e were evaluated by the method of Ackermann and Potter,[15] it was found that these inhibitors did not titrate the enzyme but exhibited typical kinetics of reversible inhibition.

In summary, these data offer support to the concept[12] that there is a single binding site on adenosine deaminase for inhibitors which are derivatives of some 9-alkyladenines. It is suggested that the adenine portion of this class of inhibitors all bind to the same site on adenosine deaminase and that the large hydrophobic region, the hydroxyl binding site, and the specific methyl binding site bear a close spatial relationship with the site to which the adenine moiety binds. Thus, the synthesis of compounds which can bridge to all of these binding regions on the enzyme has produced inhibitors of adenosine deaminase with greatly enhanced inhibitory activities.

Experimental Section‡

Method A. ***erythro*-6-Chloro-9-(2-hydroxy-3-butyl)purine.** A mixture of 7.55 g (46.2 mmol) of 4,6-dichloro-5-aminopyrimidine (1), 4.52 g (50.7 mmol) of *erythro*-3-amino-2-butanol (2e),[7,8] 85.0 g (460 mmol) of tributylamine, and 250 ml of 1-pentanol was refluxed for 28 hr under an atmosphere of N_2. The reaction mixture was concentrated *in vacuo* and 50 ml of hexane was added to the mixture. The hexane phase was separated, and the oily residue was dissolved in 10 ml of $CHCl_3$ and placed on a column of neutral alumina (600 g, 35 × 450 mm). The column was eluted with $CHCl_3$ and 20 × 250 ml fractions were collected. The desired material was obtained from fractions 22–42 ($CHCl_3$–MeOH, 50:1). The chromatographically homogeneous *erythro*-5-amino-4-chloro-6-(2-hydroxy-3-butylamino)pyrimidine (5e) would not crystallize; yield 5.06 g (50.6%).

A solution of 4.14 g (19.0 mmol) of 5e, 50 ml of triethyl orthoformate, 15 ml of $CHCl_3$, and 265 mg (2.41 mmol) of $EtSO_3H$ was stirred at room temperature for 45 min. Cooling the reaction mixture at 0° yielded a precipitate, 3.34 g (77.3%), mp 125–129°, which was recrystallized from CCl_4 to give the analytical product: yield 2.90 g (66.8%); mp 129–132°. *Anal.* ($C_9H_{11}ClNO$) C, H, Cl, N.

Method B. ***erythro*-9-(2-Hydroxy-3-butyl)adenine (8e).** A mixture of 1.02 g (4.48 mmol) of *erythro*-6-chloro-9-(2-hydroxy-3-butyl)purine and 50 ml of methanolic NH_3 (20%) was heated at 80° for 17 hr in a steel bomb. The reaction mixture was evaporated *in vacuo* to a residual solid which was extracted with hot Me_2CO. Cooling and addition of hexane to the Me_2CO extract yielded the product: 648 mg (69.7%); mp 165–168°. The material was recrystallized from Me_2CO giving the analytical material: yield 452 mg (48.7%); mp 169–173°. *Anal.* ($C_9H_{13}N_5O$) C, H, N.

***threo*-6-Chloro-9-(2-hydroxy-3-butyl)purine** was prepared by method A from 1 and *threo*-3-amino-2-butanol (2t):[7,8] yield 45%; mp 138–142° ($CHCl_3$–hexane). *Anal.* ($C_9H_{11}ClN_4O$) C, H, Cl, N.

***threo*-9-(2-Hydroxy-3-butyl)adenine** (8t) was prepared by method B from *threo*-6-chloro-9-(2-hydroxy-3-butyl)purine and methanolic NH_3: yield 31%; mp 205–209° (Me_2CO–hexane). *Anal.* ($C_9H_{13}N_5O$) C, H, N.

Method C. 3-Amino-2-nonanone Hydrochloride. A mixture of 3.03 g (19.1 mmol) of 2-amino-1-octanoic acid, 10 ml of pyridine, and 15 ml of Ac_2O was heated on a steam bath for 2.5 hr. The reaction mixture was evaporated *in vacuo*. The mixture was partitioned between $NaHCO_3$ (5%) solution and Et_2O. After the Et_2O extract had been dried with anhydrous $MgSO_4$, it was evaporated *in vacuo* and gave 3.24 g (97.0%) of crude 3-acetamidononan-2-one.

A mixture of 3.24 g of the crude product and 35 ml of concentrated HCl was refluxed for 2 hr and then evaporated *in vacuo*. The crude material after recrystallization from EtOH–Et_2O gave 1.98 g (53.3%), mp 112–114°. Further recrystallizations of the crude product from tetrahydrofuran gave the analytical product, mp 114–117°. *Anal.* ($C_9H_{20}ClNO$) C, H, Cl, N.

Method D. ***erythro*-3-Amino-2-nonanol Oxalate.** To a solution of 14.6 g (75.3 mmol) of 3-amino-2-nonanone hydrochloride in 40 ml of MeOH cooled at 0° was slowly added 8.12 g (150 mmol) of KBH_4. The pH was maintained between pH 5–6 by addition of glacial AcOH during the addition of KBH_4 to the reaction mixture. After the reaction mixture had been stirred at room temperature for 20 hr, the mixture was evaporated *in vacuo* to a residual syrup which was made basic by the addition of NaOH solution (25%). The mixture was extracted with $CHCl_3$ (3 × 100 ml). The $CHCl_3$ extract was dried with anhydrous $MgSO_4$ and evaporated *in vacuo* to give a quantitative yield of the crude liquid product. The crude material was fractionally distilled at reduced pressure to give 10.6 g (88.3%) of pure *erythro*-3-amino-2-nonanol, bp 74–77° (0.08 mm), mp 81–85°.

A portion of the product (232 mg) was converted to the oxalate salt: yield 248 mg (83.3%); mp 101–102° dec. This was recrystallized from *i*-PrOH to give the analytically pure sample: yield 163 mg (54.8%); mp 160–163° dec. *Anal.* ($C_9H_{21}N \cdot 0.5C_2H_2O_4$) C, H, N.

***erythro*-9-(2-Hydroxy-3-nonyl)adenine oxalate (9e)** was prepared by a combination of methods A and B from 1 and 3e. The crude adenine derivative was converted to the oxalate salt, and the salt was recrystallized from *i*-PrOH containing 1% oxalic acid: overall yield 39%; mp 187–190° dec. *Anal.* ($C_{14}H_{23}N_5 \cdot 0.5C_2H_2O_4$) C, H, N.

***erythro*-3-Acetamido-2-nonanol.** To a solution of 18.0 g (113 mmol) of 6e, in 50 ml of THF, was added, at 0°, 12.6 g (124 mmol) of Ac_2O. After the reaction mixture had been stirred at room temperature for 2 hr, cooling and addition of hexane gave a precipitate which was collected by filtration: yield 17.2 g (75.4%); mp 95–97°. Further recrystallizations of this material from THF–hexane gave the analytical sample, mp 95–97°. *Anal.* ($C_{11}H_{23}NO$) C, H, N.

***threo*-3-Amino-2-nonanol Hydrogen Oxalate (3t).** A mixture of 12.2 g (6.07 mmol) of *erythro*-3-acetamido-2-nonanol and 23.8 g (20.0 mmol) of $SOCl_2$ was stirred at 0° for 2 hr and at 100° for 1.5 hr. The reaction mixture was evaporated *in vacuo* to a residual oil which was dissolved in a mixture of 15 ml of *i*-PrOH and 25 ml of concentrated HCl and refluxed for 15 hr. After the reaction mixture had been evaporated *in vacuo* to an oily residue, the crude material was dissolved in $CHCl_3$, washed with 25% NaOH (2 × 10 ml), and dried with anhydrous $MgSO_4$. Evaporation of the $CHCl_3$ solution gave a quantitative yield of 3t. A small portion of the amino alcohol (492 mg, 3.09 mmol) was converted to an oxalate salt which was purified by recrystallization from *i*-PrOH containing 1% oxalic acid: 538 mg (85.6%); mp 134–136°. *Anal.* ($C_9H_{21}NO \cdot C_2H_2O_4$) C, H, N.

***threo*-9-(2-Hydroxy-3-nonyl)adenine oxalate (9t)** was prepared by a combination of methods A and B from 1 and 3t. The

‡The melting points were taken in open capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had ir and uv spectra compatible with their assigned structures and moved as single spots on tlc on Brinkman silica gel. Nmr spectra were determined in DMSO-d_6 on a Varian XL-100 spectrometer (TMS) on saturated solutions of the oxalate salts of the amino alcohols. Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

crude adenine derivative was converted to the oxalate salt in *i*-PrOH containing 1% oxalic acid: yield 46%; mp 208–210°. *Anal.* ($C_{14}H_{23}N_5O \cdot 0.5C_2H_2O_4$) C, H, N.

3-Amino-2-dodecanone hydrochloride was prepared by method C from 2-aminoundecanoic acid: yield 90.6%; mp 114–117° (THF). *Anal.* ($C_{12}H_{26}ClNO$) C, H, Cl, N.

erythro-**3-Amino-2-dodecanol oxalate** (4e) was prepared by method D from 3-amino-2-dodecanone hydrochloride and KBH_4: yield 78.9%; mp 103–110° (*i*-PrOH containing 1% oxalic acid). *Anal.* ($C_{12}H_{27}NO \cdot C_2H_2O_4$) C, H, N.

erythro-**9-(2-Hydroxy-3-dodecyl)adenine oxalate** (10e) was prepared by a combination of methods A and B from 1 and 4e. The crude adenine derivative was converted into the oxalate salt: yield 23%; mp 183–185° (*i*-PrOH containing 1% oxalic acid). *Anal.* ($C_{17}H_{29}N_5O \cdot 0.5C_2H_2O_4$) C, H, N.

Reagents and Assay Procedures. Adenosine deaminase (Type I, calf intestinal mucosa) was purchased from Sigma Chemical Co. The procedure for the assay of reversible inhibitors has previously been described[1-4] and is a modification of the method of Kaplan[16] based on the work of Kalckar.[17]

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Synthesis and Enzymatic Evaluation of Some *N*-Alkyl Branched Chain Piperidine Salts and *N*-Alkyl-3-(*N,N*-diethylcarbamoyl)piperidine Salts as Inhibitors of Acetyl- and Butyrylcholinesterase†

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A kinetic investigation of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) using acetylcholine (ACh) as substrate and *N*-alkyl-3-(*N,N*-diethylcarbamoyl)piperidine (I) and *N*-alkyl branched chain piperidine (II) salts as inhibitors was conducted. Conventional inhibitor binding constants (K_i values) were determined for both series of inhibitors, as well as their effects on the carbamylation of AChE and BuChE by dimethylcarbamyl fluoride (DMCF). Acetylcholinesterase was shown to be more sensitive in terms of affinity, to the transition of a tertiary amine to a quaternary ammonium salt in series II, while the affinity of series I increased with the length of the alkyl chain (C_3–C_{12}) in both enzyme systems. The carbamylation studies revealed a high sensitivity of AChE toward carbamylation by DMCF in the presence of series II, in contrast to BuChE. Affinity correlated with rate enhancement in BuChE, while there was little correlation in AChE. The results were interpreted in terms of the relationship between the anionic and esteratic subsites of these two enzymes, which appears to be of a more dynamic nature in AChE. Examples are presented which demonstrate that affinity data alone are insufficient to characterize binding modes and serve as a basis for structure–activity models.

As part of a kinetic investigation of the binding properties of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), various piperidine analogs, demonstrating varying degrees of hydrophobicity, were synthesized and studied as reversible inhibitors of these enzymes. The principal regions of interest were the anionic sites of these two enzymes and their adjacent hydrophobic areas, since it has been postulated that these areas constitute the major difference between the two enzymes.[1-7] Two series of substituted piperidines were synthesized: series I, *n*-alkyl-3-(*N,N*-diethylcarbamoyl)piperdine hydrobromide salts, and series II, 1-butylpiperidine salts, possessing the butyl grouping in all its isomeric forms. Both the hydrobromide salts and the corresponding methyl quaternary ammonium salts of series II were investigated. These compounds can be expected to bind to the anionic sites of the two cholinesterases and to reflect the topography of the area immediately surrounding these sites. Series I provides continuity with earlier work from these laboratories on BuChE[8-10] and provides a comparison of the importance of hydrophobic interactions in the two enzymes.

Also of interest was to establish the relative binding modes of these two series as well as some other 3-substituted piperidine derivatives.[11] In particular, we were interested in establishing whether or not these various groupings in the 3 position of the ring were binding at the esteratic sites of these enzymes. This latter question was investigated by studying the effects of these reversible inhibitors on the rate of carbamylation of AChE and BuChE by dimethylcarbamyl fluoride (DMCF).

Results and Discussion

It is apparent from the studies with the 1-butylpiperidinium salts that AChE demonstrated a marked response to quaternization of the heterocyclic nitrogen (Table I).

†Dedicated to Professor Alfred Burger.

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