Synthesis of Pyrimidin-2-one Nucleosides as Acid-Stable Inhibitors of Cytidine Deaminase

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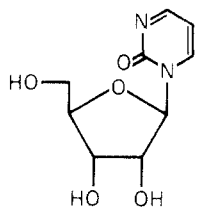
One of the problems encountered in the use of tetrahydrouridine (THU, **2**) and saturated 2-oxo-1,3-diazepine nucleosides as orally administered cytidine deaminase (CDA) inhibitors is their acid instability. Under acid conditions these compounds are rapidly converted into inactive ribopyranoside forms. A solution to this problem was sought by functionalizing the acid-stable but less potent CDA inhibitor 1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**1**) with the hope of increasing its potency to the level achieved with THU. The selection of the hydroxymethyl substituent at C-4, which led to the synthesis of 4-(hydroxymethyl)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**10**), 3,4-dihydro-4-(hydroxymethyl)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**7**), and 3,4,5,6-tetrahydro-4-(dihydroxymethyl)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**28**) was based on the transition-state (TS) concept. The key intermediate precursor, 4-[(benzoyloxy)methyl]-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**24**), was obtained via the classical Hilbert–Johnson reaction between 2-methoxy-4-[(benzoyloxy)methyl]pyrimidine (**20**) and 2,3,5-tri-*O*-benzoyl-1-D-ribofuranosyl bromide (**21**). Deprotection of **24** afforded compound **10**, while its sodium borohydride reduction products afforded compounds **7** and **28** after removal of the blocking groups. Syntheses of 3,4-dihydro-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**9**) and 3,6-dihydro-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**8**), which lack the hydroxymethyl substituent, was accomplished in a similar fashion. The new compounds bearing the hydroxymethyl substituent were more acid stable than THU, and their CDA inhibitory potency, expressed in terms of K_i values, spanned from 10^{-4} to 10^{-7} M in a manner consistent with the TS theory. Compound **7**, in particular, was superior to its parent **1** and equipotent to THU ($K_i = 4 \times 10^{-7}$ M) when examined against mouse kidney CDA. The superior acid stability of this compound coupled to its potent inhibitory properties against CDA should provide a means of testing oral combinations of rapidly deaminated drugs, viz. *ara*-C, without the complications associated with the acid instability of THU.

During the past several years, our laboratory has developed two distinct classes of cytidine deaminase (CDA) inhibitors represented by ribofuranoside nucleosides of 2-oxopyrimidine and 2-oxo-1,3-diazepine.[1-4] These two structural prototypes are represented by compounds **1** and **3**, shown in conjunction with the standard and most commonly used CDA inhibitor, tetrahydrouridine (THU, **2**).[5,6]



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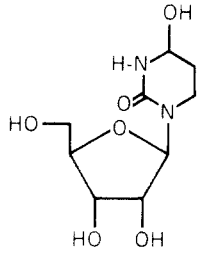

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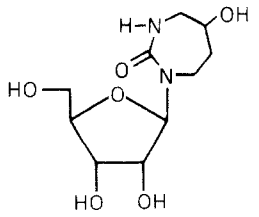

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The results with these and similar compounds indicate that while a more potent inhibition of CDA is achieved by the seven-membered ring nucleosides,[4] an intrinsic acid instability, which is also typical of THU, rapidly transforms these materials into inactive ribopyranoside nucleosides. This renders them useless as oral CDA inhibitors.[7] The aromatic 2-oxopyrimidine riboside **1**, although 10-fold and 100-fold less potent than THU and **3**, respectively, remained unchanged under acid conditions. In addition, **1** appears to be the only CDA inhibitor that is endowed with antitumor activity.[1,8] Such antitumor effect, however, is unrelated to CDA inhibition as demonstrated by the total lack of cytotoxicity displayed by THU and the more potent diazepinone nucleosides.[9,10] The exact mechanism of the antitumor activity of **1** is not completely understood, although formation of the corresponding nucleotide in vivo is strongly suspected.

Based on these premises, we decided to investigate the possibility of designing compounds that would combine in one molecule the superior CDA inhibitory potency of

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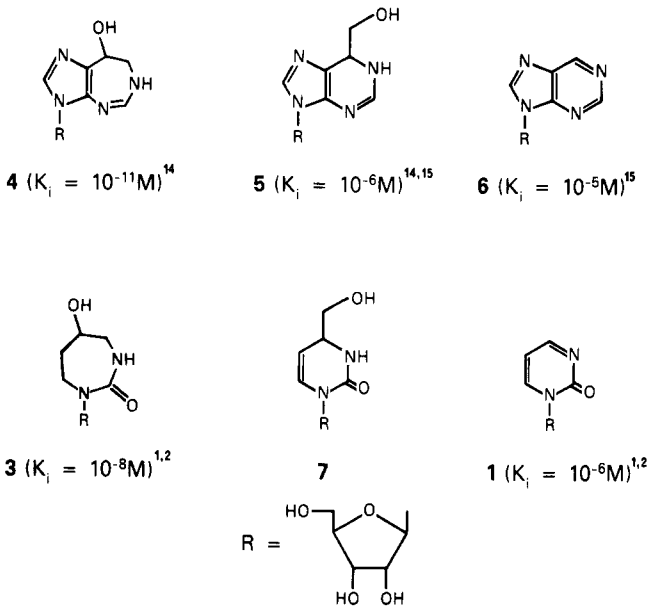
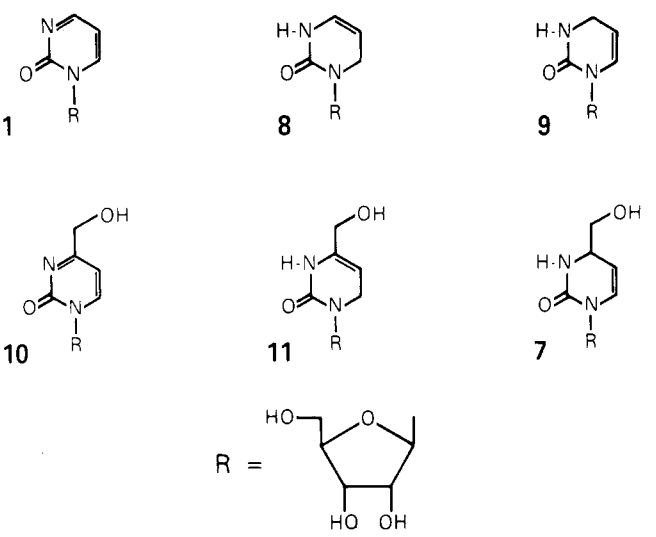


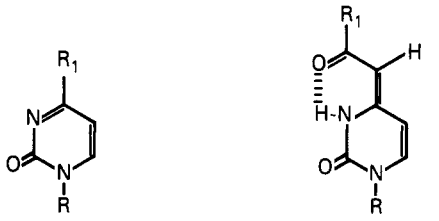
Figure 1. Structure–activity relationships of transition-state inhibitors for adenosine deaminase (ADA) and cytidine deaminase (CDA).

the seven-membered ring diazepinone nucleosides with the acid stability and antitumor properties of the aromatic six-membered 2-oxopyrimidine nucleoside **1**. At the same time, it was also of interest to study the extent to which the six-membered aglycon ring in **1** allowed itself to be altered in order to resemble more closely the transition state of the cytidine deamination reaction.[13]

With the well-studied purine aminohydrolase, adenosine deaminase (ADA), the enzyme's affinity for purine nucleoside inhibitors is as shown in Figure 1. Provided that a similar structure–activity correlation is maintained between the two aminohydrolases ADA and CDA, compound **7** would be expected to have a K_i value between 10^{-6} and 10^{-8} M (Figure 1). This expected increase in potency appeared attractive enough to justify synthesis of **7** and a series of related 2-oxopyrimidine nucleosides (**8–11**). The study of this selected group of compounds was also of interest in order to understand the relative importance of the hydroxymethyl substituent at C-4 in modulating CDA activity (compound **1** vs. **10**, **8** vs. **11**, and **9** vs. **7**). The preparation of **1** has been described previously,[1,17–22] and in this work we wish to report the syntheses of compounds **7–11** and their evaluation as CDA inhibitors.

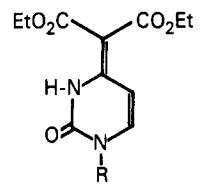
Chemistry. There are few reports in the literature dealing with pyrimidine nucleosides having a carbon substituent at C-4. The simplest carbon-substituted compound, 4-methyl-1-β-D-ribofuranosyl-2-pyrimidinone (**12a**), has been reported and found to be an inferior inhibitor of bacterial CDA relative to the parent riboside **1**.[23,24] In addition, both 4-pyrimidinecarboxylic acid **12b** and the very unstable 4-cyano-1-β-D-ribofuranosyl-2-pyrimidinone **12c** have been reported.[23,25] Somewhat closer in their structural analogy to the intended series, compounds **12d**, **13**, and **14** have been prepared by Yamane et al.[26] via the sulfur extrusion reaction of the corresponding alkylthio derivatives. In this type of compounds, the increased acidity of the exocyclic protons favors the more highly conjugated forms **13** and **14** as demonstrated by UV and ^{1}H NMR measurements.[26]





12a, R_1 = CH_3
b, R_1 = CO_2H
c, R_1 = CN
d, R_1 = CH_2CO_2Et

13a, R_1 = CH_3
b, R_1 = Ph



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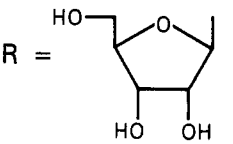

Our approach to the target compounds required first obtaining the pyrimidine base with a hydroxymethyl substituent at C-4 and using it to form the nucleoside by

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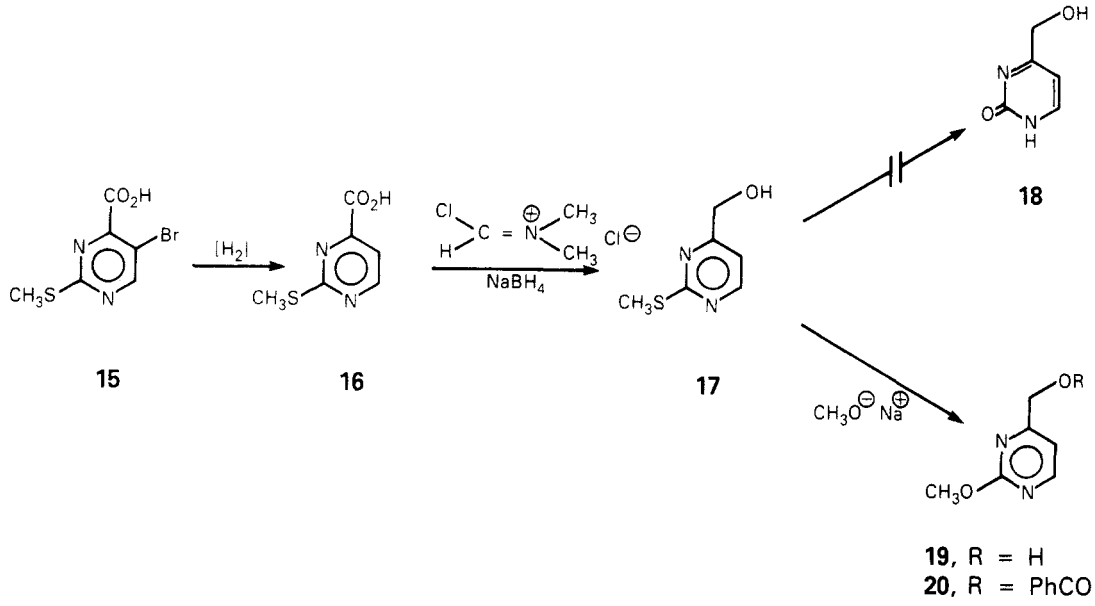
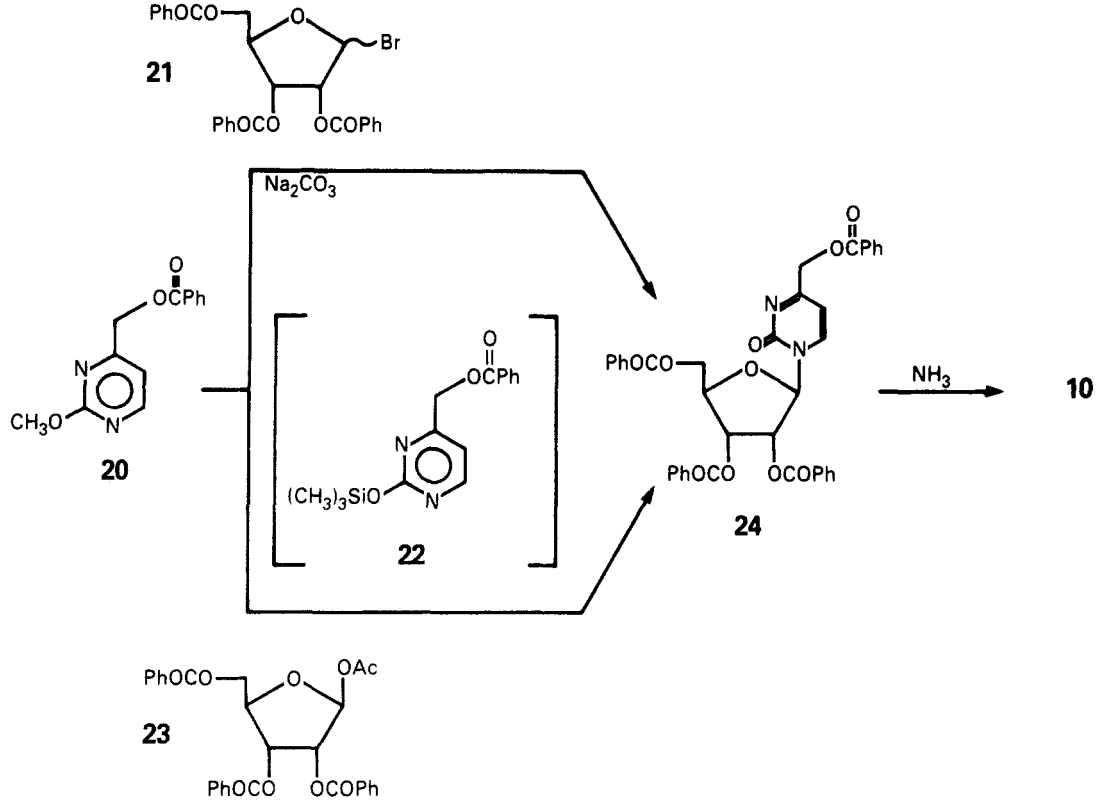
Scheme I



Scheme II



a standard coupling procedure. As exemplified in Scheme I, the known 5-bromo-2-(methylthio)pyrimidine-4-carboxylic acid (**15**)[27] was first catalytically debrominated with hydrogen over Pd and $BaSO_4$ to afford **16**. Reduction of **16** following Fujisawa's procedure (*N,N*-dimethylchloromethyleniminium chloride and $NaBH_4$)[28] produced the desired alcohol **17** in good yield. Transformation of **17** to the desired 2-oxopyrimidine **18** unfortunately failed under either acidic or basic conditions even after oxidation of the methyl sulfide to the sulfone.[29] However, compound **17** could be treated with sodium methoxide to afford 2-methoxy-4-(hydroxymethyl)pyrimidine **19**, which, after protection of its free hydroxyl group as the benzoate ester (**20**), was suitable for condensation with the halogenose sugar **21** by the classical Hilbert–Johnson procedure.[30]

As indicated in Scheme II, this reaction proceeded as expected and compound **24** was obtained in 63% yield. Subsequently, in an effort to simplify the procedure, the heterocycle **20** was pretreated with trimethylsilyl iodide to cleave the ether bond and to generate in situ the silylated base **22**.[31] Following the addition of the sugar acetate

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Scheme III

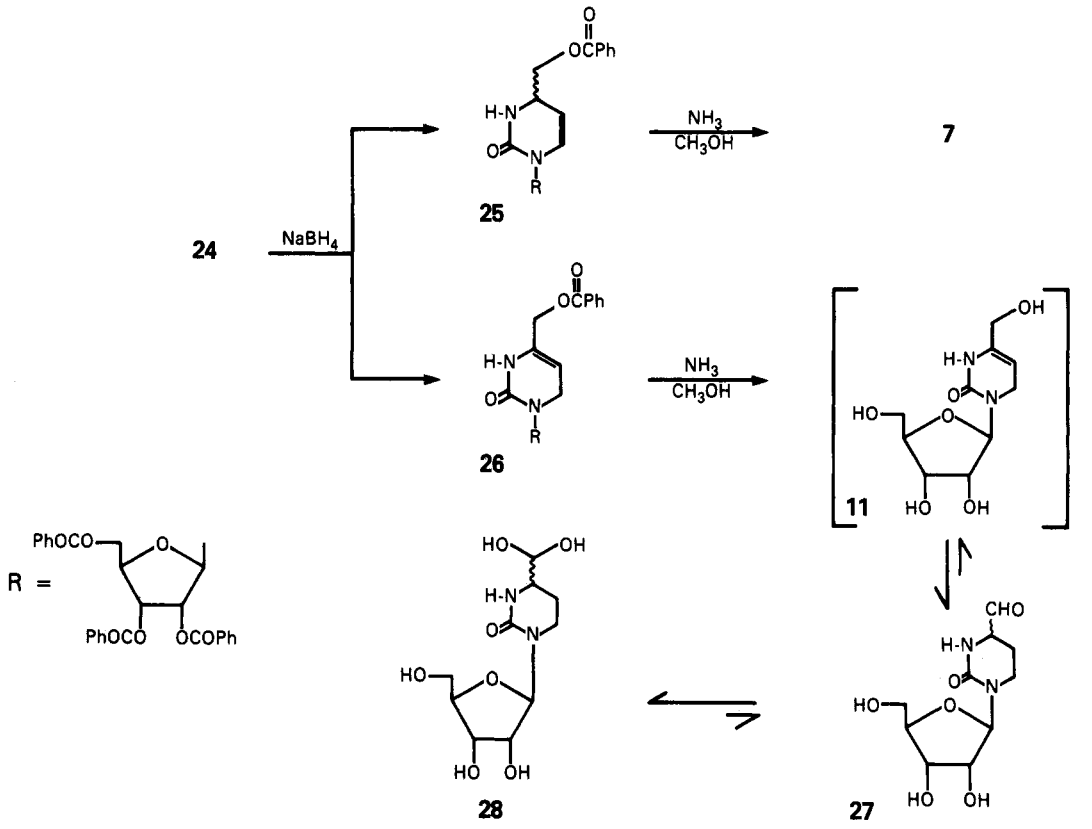

Table I. High-Resolution ^{13}C NMR Analysis for C-6

compd no.	C-6, ppm	coupling const,[a] Hz			
		$^1J_{C\text{-}6,H\text{-}6}$	$^2J_{C\text{-}6,H\text{-}5}$	$^3J_{C\text{-}6,H1'}$	$^3J_{C\text{-}6,H\text{-}4}$
20[b]	159.7	181.0	3.4		
24[b]	143.7	182.0	3.2	5.0	
29[b]	143.6	181.7	3.2	5.4	6.2
1[c]	146.2	185.9	2.3	5.0	6.5

[a] All coupling constants are reported as absolute values. [b] In $CDCl_3$ with $CDCl_3$ as internal reference. [c] In D_2O with 1,4-dioxane as internal reference.

23 and $SnCl_4$ as a Lewis acid catalyst,[32] the reaction proceeded as expected but afforded only 18% of the desired product **24**. Removal of the benzoate groups in **24** with saturated methanolic ammonia produced the desired compound **10** contaminated with some colored byproducts that could be removed by reversed-phase chromatography. The instability of compound **10** under basic conditions might be explained in terms of the increased acidity of the exocyclic methylene protons, which favors tautomerization to form a highly reactive aldehyde. Support for this assumption comes from a similar transformation discovered for a partially reduced pyrimidine analogue (compound **11**, Scheme III). Purified compound **10**, however, was relatively stable in aqueous solution and, as inferred from its ^1H NMR spectrum, exists exclusively as depicted by structure **10**.

Proof of the site of *N*-ribosylation was obtained through ^{13}C NMR long-range coupling and ^1H NOE experiments. The C-6 resonance in compound **24** (δ 143.7) was split into a doublet of doublet of doublets by H-6, H-5, and H-1′, as expected for N-1 ribosylation (Table I). For comparison, equivalent studies performed on compounds **20**, **29**, and **1** corroborated our structural assignment. Unfortunately compound **10** was not stable in solution for the duration of these experiments and therefore was not included. In the NOE experiments performed with compound **10**, the only significant (1.03 or greater) interactions of the pyrimidine protons with the sugar protons were reciprocal interactions of H-6 and H-1′ (1.03 and 1.05) and the enhancement (1.03) of the H-6 resonance upon irradiation of H-2′. In addition to the appearance of the anomeric signal as a singlet at δ 5.92, the interaction of H-6 and H-2′ supports a β-configuration for compound **10**. A similar study of compound **1** showed enhancement of H-6 by irradiation of H-1′, H-2′, H-3′, and H-5′b (1.03, 1.03, 1.06, and 1.03). Irradiation of H-6 enhanced H-1′ (1.04) and H-3′ (1.03). Involvement of H-3′ in the enhancements of this compound and not in **10** may reflect a change in either the glycosidic torsion angle or in the conformation of the sugar.

The generation of the other target compounds (**7** and **11**) required the partial reduction of the pyrimidine ring of **24** as described in Scheme III. This borohydride reduction proceeded in good yield giving equal amounts of the two isomeric dihydropyrimidinones **25** and **26**, which were separated by preparative thin-layer chromatography and characterized by ^1H NMR spectroscopy. A salient feature in the NMR spectra of these compounds was the appearance of an exchangeable NH proton; in **25** the signal was a doublet, whereas in **26** it appeared as a singlet. Following the standard procedure, compound **25** was subsequently deblocked to give target compound **7**. However, when a similar deblocking procedure was applied to the isomeric **26**, the reaction gave exclusively **28**, the hydrated form of aldehyde **27**. Structure **27** was probably generated through a base-catalyzed double-bond isomerization followed by ketalization from **11**, as discussed earlier for **10**. However, in this case, the resulting aldehyde was more stable and preferentially converted to **28** as indicated by the spectral data (see the Experimental Section).

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Scheme IV

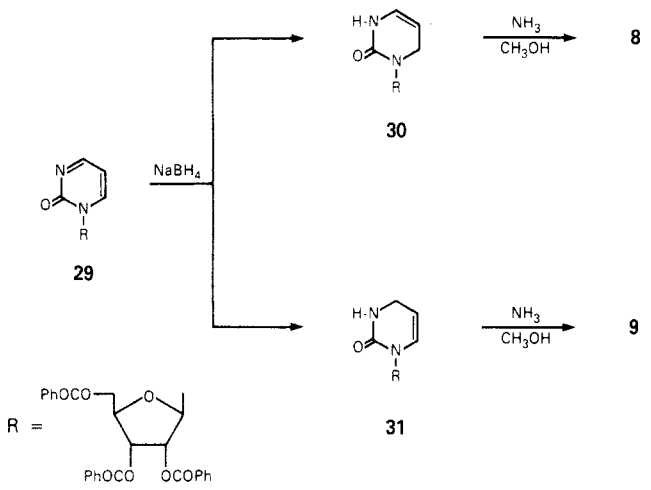

Table II. Cytidine Deaminase Inhibition of Selected 2(1*H*)-Pyrimidinone Nucleosides

compd no.	mouse kidney K_i,[a] M	compd no.	mouse kidney K_i,[a] M
1	2×10^{-6}	7	4×10^{-7}
8	4×10^{-6}	28	2×10^{-5}
9	4×10^{-6}	2[b]	4×10^{-7}
10	1×10^{-4}		

[a] Experimental conditions for these experiments are identical with those described in ref 4. [b] THU.

The stereochemistry at C-4 for compounds **7** and **28** is undefined, and therefore these compounds are mixtures of diastereoisomers. All efforts to develop a chromatographic system to separate these compounds into their components, either at the stage of protected derivatives (compound **25**) or as deblocked final products (compounds **7** and **28**), proved unsuccessful.

The two remaining target compounds (**8** and **9**) were similarly prepared by the partial reduction of **29**.[4] Separation of isomers **30** and **31** by preparative thin-layer chromatography, followed by removal of the blocking groups, afforded the desired products **8** and **9** in good yield (Scheme IV).

Biological Results. The activities of the target compounds against mouse kidney CDA are shown in Table II. As can be seen, partial reduction of the ring had little effect on the activity of the parent compound **1**, since both isomers **8** and **9**, which differ only in the position of the double bond, were nearly equipotent to **1**. The K_i value of 4×10^{-6} M was also identical to that obtained for the completely reduced pyrimidine analogue reported previously by us.[1,4] Incorporation of the hydroxymethyl group at C-4 on the aromatic 2-oxopyrimidine ring, as in **10**, caused a reduction in potency as indicated by the 50-fold increase in the K_i value for this compound. However, the same substituent in the partially reduced analogue **7** produced an increase in potency of 5-fold with respect to the parent riboside **1**. The activity of **7** is indistinguishable from that of THU (**2**, Table I). Compound **28**, which carries an extra hydroxyl group, displayed significantly lower potency than either **7** or **1**. Since the presence or absence of a double bond is not essential for activity (vide supra), the extra hydroxyl group appears to be unnecessary and detrimental. It should be recalled that another intended target was compound **11**. Unfortunately, this compound rapidly tautomerized to the aldehyde **27**, which subsequently hydrated to give the moderately active **28**. The last compound included in Table II is THU, which was used as a positive control.

The two most interesting compounds from this study, namely **7** and **10**, were additionally tested for cytotoxicity in vitro against murine L1210 leukemia cells. Compound **7** was virtually nontoxic up to 0.1 mM, and compound **10** produced only a 25% reduction of cell growth at the same concentration. The compounds were also tested as uridine–cytidine kinase inhibitors, but again only **10** inhibited phosphorylation of uridine at a very high concentration (45%, 1 mM).

Discussion

Concerning the structure–activity relationships in this series, the compounds can be divided into two categories: (a) the nucleosides with no hydroxymethyl groups and (b) the substituted nucleosides with a hydroxymethyl group at C-4. In the first category, the order of potency for the inhibition of CDA appears to be THU (**2**) > **1** = **8** = **9**. According to transition-state (TS) theory, the resemblance of THU to the tetrahedral intermediate in the enzymatic deamination of cytidine would explain its greater affinity for the enzyme.[13] Therefore, if "likeness" to the TS is the determinant factor, the order of activity observed for this group can be explained on that basis. For compound **1**, the well-documented tendency of 2-oxopyrimidines to form 3,4-hydrates[33] would result in the generation of a structure similar to the TS tetrahedral intermediate. The lower potency of **1** relative to **2** is reasonable, since hydration is an equilibrium process, and the concentration of the "active" hydrated intermediate would only be a fraction of the total number of molecules of **1** present at the catalytic site.

The unsubstituted, partially reduced compounds **8** and **9** are biologically indistinguishable as CDA inhibitors. Of these two structures, only **8** has the potential to be hydrated by a two-step process that would generate some amount of THU in the equilibrium mixture. Conversely, compound **9**, although incapable of forming a hydrated species, possesses a tetrahedral carbon at C-4 that appears to be essential for good activity. The fact that **1**, **8**, and **9** are equipotent as CDA inhibitors may be a coincidence.

The order of potency for the hydroxymethyl-substituted compounds was **7** > **28** > **10**. The results from **10** and **7** imply that the essential tetrahedral carbon is C-4 on the pyrimidine ring and not the carbon on the side chain. Furthermore, these results indicate that substitution of 2-oxopyrimidines at C-4, as in **10**, may sterically hinder hydration at that position preventing this carbon atom from acquiring a tetrahedral geometry. The intermediately active compound **28**, although tetrahedral at C-4, is probably overcrowded due to the extra hydroxyl group, which might prevent a close association with the enzyme. The most potent compound, **7**, has both a tetrahedral carbon atom at C-4 plus a single hydroxyl group that could position itself in a comparable steric location as in the TS. A similar argument has been advanced by Wolfenden et al. to explain the activity of compound **5** as an ADA inhibitor in the purine series.[15]

Compound **7** is, as is THU, a mixture of diastereoisomers that was not resolved into its components. While the aminal structure in THU prevents the separation of diastereoisomers, compound **7** has the potential to be resolved into its components. A definite advantage of compound **7** with respect to both THU and **3** is that it is much more stable in the presence of acid. Under pH conditions that isomerized both THU and **3** more than 90% to their respective ribopyranosides,[7] **7** remained 84% as the intact ribofuranoside as judged from NMR studies. Furthermore, compound **7**, as opposed to THU, is UV active and

therefore more easily detected in HPLC studies.

In conclusion, guided by some structural considerations from TS theory, we have designed and synthesized a C-4-substituted 2-oxopyrimidine nucleoside (7) that is superior in potency to the parent compound 1 as a CDA inhibitor. The activity of this compound fell within the expected range on the basis of model predictions (Figure 1), and it equaled the standard CDA inhibitor, THU, in its potency against CDA. Compound 7 is superior to THU with regard to stability and UV activity, properties that could make it a good candidate for oral combination studies with readily deaminated antitumor drugs. This compound, however, lacked the antitumor properties of its progenitor 1, which suggests that CDA inhibitory activity and cytotoxicity are two divergent properties from a structural point of view. This lack of cytotoxicity, nevertheless, is a welcome property in studies aimed at determining the effects of pure CDA inhibition on drugs susceptible to in vivo deamination. Combination studies of 7 and several selected antitumor agents by the oral route are currently being investigated in our laboratory.

Experimental Section

All chemical reagents are commercially available and were purchased from Aldrich Chemical Co. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were measured with a Perkin-Elmer 727B spectrometer as KBr pellets. Proton and ^{13}C NMR spectra were recorded on a Varian XL-200 instrument. Chemical shifts are given as δ values with reference to Me_4Si. For ^{13}C NMR the peak positions were referenced to $CDCl_3$ or to dioxane. Decoupling was accomplished by modulated square-wave irradiation. Fully coupled spectra were Fourier transformed using a manually optimized resolution enhancement parameter. NOE experiments were conducted on dilute samples in D_2O with a decoupler power of 0.032 W. The decoupler offset parameter was arrayed for irradiation of those protons of interest and for points approximately 4 ppm upfield and downfield of the extreme resonances in the spectrum. These off-resonance irradiations were used as references for the measurements of enhancement due to on-resonance irradiation and as references for internal consistency. The integrations of the off-resonance spectra were consistent within 1% in all cases. Integrations were obtained from a digital printout. Specific rotations were measured in a 1-dm cell with a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Columns for chromatography were packed with either silica gel (Bio-Sil A, 200–400 mesh, Bio-Rad Laboratories) or bonded phase octadecyl (C_{18}) from J. T. Baker Chemical Co. and eluted with the solvents indicated in the individual experiments. Preparative TLC chromatography was performed on plates coated with silica gel GF (2000 μm) obtained from Analtech, Inc. Positive-ion fast atom bombardment (FAB) mass spectra were obtained on a VG 7070E mass spectrometer that was equipped with a FAB ion source. The sample was dissolved in a glycerol matrix, and ionization was effected by a beam of xenon atoms derived by neutralizing xenon ions accelerated through 8.6 kV. Cytidine deaminase was measured by use of a mouse kidney preparation with the same methodology as reported previously by us.[4]

5-Bromo-2-(methylthio)pyrimidine-4-carboxylic Acid (15). This compound was prepared from 2-methyl-2-thiopseudourea sulfate and mucobromic acid according to ref 27.

2-(Methylthio)pyrimidine-4-carboxylic Acid (16). Compound **15** (4 g, 16 mmol) was suspended in methanol (200 mL), and after the addition of 2 g of potassium hydroxide and 2 g of 5% Pd on $BaSO_4$, the mixture was hydrogenated in a Parr hydrogenator at 35 psi. The reaction mixture was filtered through a bed of Celite, which was subsequently washed with warm methanol. The combined filtrate was concentrated under vacuum and acidified to pH 1 with concentrated hydrochloric acid. The precipitate formed was recrystallized from ethanol to give 2.7 g (98%) of product: mp 210 °C; NMR (Me_2SO-d_6) δ 2.57 (s, 3 H), 7.70 (d, 1 H, J = 5 Hz), 8.90 (d, 1 H, J = 5 Hz); IR (KBr) 3100, 2550, 1705, 1575 cm^{-1}. Anal. ($C_6H_6N_2O_2S$) C, H, N, S.

4-(Hydroxymethyl)-2-(methylthio)pyrimidine (17). DMF (3.1 mL, 40 mmol) in anhydrous methylene chloride (60 mL) was reacted with oxalyl chloride (10 mL) at 0 °C under stirring for 1 h. After removal of the solvents under reduced pressure, the generated *N,N*-dimethylchloromethyleniminium chloride was treated with a mixture of anhydrous acetonitrile (60 mL) and tetrahydrofuran (100 mL) and cooled to 0 °C. This mixture was reacted with compound **16** (9.18 g, 40 mmol), which was added in portions during the course of 1 h at 0 °C and under constant stirring. After the reaction mixture was cooled to −78 °C, 20 mL of a 2 M solution of $NaBH_4$ in DMF was added and the temperature was allowed to rise slowly to −20 °C during the course of 3 h. The reaction was quenched with 2 N HCl and most of the organic solvents removed on a rotary evaporator. Water was added to the residue, and the pH was adjusted to pH 11 with 1 N NaOH. Following extraction with chloroform, the organic layer was dried ($MgSO_4$), reduced in volume, and flashed chromatographed through a silica gel column with ethyl acetate–hexane (2:3) to give 4.23 g (68%) of compound **17**: mp 120–121 °C (acetone–hexanes); NMR ($CDCl_3$) δ 2.53 (s, 3 H), 4.20 (s, 1 H, D_2O exchanged), 4.60 (s, 2 H), 7.27 (d, 1 H, J = 5 Hz), 8.40 (d, 1 H, J = 5 Hz); IR (KBr) 3300, 2950, 2910, 1580, 1570, 1365 cm^{-1}. Anal. ($C_6H_8N_2OS$) C, H, N, S.

4-(Hydroxymethyl)-2-methoxypyrimidine (19). A solution of compound **17** (1.56 g, 10 mmol) in absolute methanol (50 mL) was treated with 10 mL of 25% sodium methoxide in methanol and refluxed under nitrogen for 12 h. The solvent was removed, and an equivalent amount of concentrated hydrochloric acid was carefully added to neutralize the base. Following an exhaustive extraction with chloroform the organic extract was dried ($MgSO_4$) and column chromatographed (silica gel, ethyl acetate) to give 0.99 g (71%) of **19**: mp 115.5–116.5 °C (ethyl acetate–hexane); NMR ($CDCl_3$) δ 3.45 (br s, 1 H, D_2O exchanged), 4.00 (s, 3 H), 4.67 (s, 2 H), 6.90 (d, 1 H, J = 5 Hz), 8.37 (d, 1 H, J = 5 Hz); IR (KBr) 3280, 3000, 2900, 1570, 1380 cm^{-1}. Anal. ($C_6H_8N_2O_2$) C, H, N.

4-[(Benzoyloxy)methyl]-2-methoxypyrimidine (20). A solution of compound **19** (2.21 g, 15.8 mmol) in anhydrous methylene chloride (80 mL) was treated with 4-(dimethylamino)pyridine (1.95 g, 15.8 mmol) and triethylamine (2.4 mL) followed by benzoyl chloride (1.85 mL, 15.9 mmol) and allowed to react for 12 h at room temperature. After the addition of ice–water, the reaction mixture was extracted with chloroform and the organic extract was successively washed with 2% hydrochloric acid and water. The chloroform layer was dried ($MgSO_4$) and reduced to dryness. The residue was purified through column chromatography (silica gel, ethyl acetate–hexanes, 3:7) to give 3.75 g (98%) of compound **20**: mp 76.5–77.5 °C (acetone); NMR ($CDCl_3$) δ 4.00 (s, 3 H), 5.40 (s, 2 H), 6.97 (d, 1 H, J = 5 Hz), 7.20–8.20 (m, 5 H), 8.40 (d, 1 H, J = 5 Hz); IR (KBr) 2980, 1730, 1590, 1570, 1380 cm^{-1}. Anal. ($C_{13}H_{12}N_2O_3$) C, H, N.

4-[(Benzoyloxy)methyl]-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (24). Method A. Anhydrous sodium carbonate (0.636 g, 6 mmol) was added to a solution of **20** (0.366 g, 1.5 mmol) in 25 mL of methylene chloride, and the mixture was stirred at room temperature for 5 min. Immediately afterward, 5 mmol of 2,3,5-tri-*O*-benzoyl-1-D-ribofuranosyl bromide (**21**)[34] dissolved in anhydrous acetonitrile (30 mL) was added, and the reaction mixture was stirred at room temperature for 24 h under nitrogen. The reaction mixture was filtered, concentrated, and column chromatographed (silica gel, ethyl acetate–hexanes, 3:7) to give 0.63 g (63%) of compound **24**: mp 106–108 °C (acetone–hexanes); NMR ($CDCl_3$) δ 4.65–4.95 (m, 3 H), 5.25 (s, 2 H), 6.31 (d, 1 H, J = 7 Hz), 6.45 (d, 1 H, J = 3.8 Hz), 7.30–7.70 (7, 12 H), 7.90–8.15 (m, 9 H); IR (KBr) 3065, 1725, 1670, 1600, 1450 cm^{-1}. Anal. ($C_{38}H_{30}N_2O_{10}$) C, H, N.

Method B. A solution of compound **20** (0.224 g, 1 mmol) in anhydrous acetonitrile (10 mL) was reacted with 0.32 mL (2.2 mmol) of iodotrimethylsilane and stirred under nitrogen at 70–75 °C overnight. While the mixture turned black, TLC analysis (silica gel, ethyl acetate) revealed the complete absence of starting material. After the solvents were removed in vacuo, the residue

(34) Stevens, J. D.; Fletcher, H. G., Jr. *Synth. Proced. Nucleic Acid Chem.* **1968**, *1*, 532.

was treated with a solution of bis(trimethylsilyl)trifluoroacetamide (BSTFA, 2 mL) in 10 mL of anhydrous acetonitrile and the resultant mixture gently heated for 1 h. The solvent and excess of BSTFA were removed, and the residue was dissolved in 10 mL of 1,2-dichloroethane. This solution was reacted with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**23**, 0.252 g, 0.5 mmol) dissolved in 5 mL of dry 1,2-dichloroethane and treated with 0.5 mL of $SnCl_4$. The reaction continued at room temperature and under nitrogen for 2 days. After the addition of a saturated solution of sodium bicarbonate, the reaction mixture was filtered through a bed of Celite and the organic layer separated and dried (Na_2SO_4). The residue obtained after removal of the solvent under vacuum was column chromatographed (silica gel, ethyl acetate–hexanes, 3:7) to give 0.118 g (18%) of **24** as a solid product which was in all respects identical with that obtained previously under method A.

4-(Hydroxymethyl)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (10). A solution of compound **24** (0.515 g, 0.76 mmol) in 3 mL of chloroform was treated with 100 mL of saturated methanolic ammonia and kept at 4 °C for 24 h in a pressure bottle. After removal of the solvents under reduced pressure, the residue was dissolved in water (15 mL) and extracted six times with 10-mL portions of chloroform. The aqueous solution was lyophilized to give 0.184 g (94%) of compound **10**, which was further purified using C-18 reversed-phase chromatography with water as eluant. Collection of the fractions was monitored at 254 nm and the flow rate adjusted to 2 mL/min. The peak containing the desired compound eluted after 38 min, and the combined fractions were lyophilized to give a white fluffy powder: $[\alpha]^{24}_D$ +94.2° (*c* 0.128, H_2O); NMR (D_2O) δ 3.90 (dd, 2 H), 4.20 (m, 2 H), 4.35 (m, 1 H), 4.60 (s, 2 H), 5.92 (s, 1 H), 6.90 (d, 1 H, J = 7 Hz), 8.50 (d, 1 H, J = 7 Hz); FAB mass spectrum, m/z (relative intensity) 259 (MH^+, 59), 127 (100, base + 2 H). Anal. ($C_{10}H_{14}N_2O_6 \cdot 1.25H_2O$) C, H, N.

Sodium Borohydride Reduction of 24. Compound **24** (1.24 g, 1.83 mmol) dissolved in dry THF (30 mL) was reacted with a solution of 0.076 g (2 mmol) of $NaBH_4$ in 15 mL of methanol at 0 °C. After 1 h, the solvents were removed at reduced pressure and the residue dissolved in chloroform. The chloroform solution was washed with water and brine and dried (Na_2SO_4) before being reduced to dryness. The crude products were separated by preparative TLC (silica gel, 2000 μm, ethyl acetate–hexane, 1:1) to give 0.469 g of a fast-moving (R_f 0.43) compound and 0.468 g of slow-moving (R_f 0.30) material (overall yield 76%).

The fast-moving compound, mp 59–61 °C (ether–hexane), was identified as 3,6-dihydro-4-[(benzoyloxy)methyl]-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**26**): NMR ($CDCl_3$) δ 4.10 (m, 2 H), 4.60–5.00 (m, 6 H), 5.90 (m, 2 H), 6.50 (s, 1 H, D_2O exchanged), 6.60 (d, 1 H, J = 6 Hz), 7.50 (m, 12 H), 8.20 (m, 8 H). Anal. ($C_{38}H_{32}O_{10}N_2 \cdot 0.5H_2O$) C, H, N.

The slow-moving material, mp 60–63 °C (ether–hexane), was identified as 3,4-dihydro-4-[(benzoyloxy)methyl]-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**25**): NMR ($CDCl_3$) δ 4.20 (m, 1 H), 4.40 (m, 2 H), 4.60 (m, 2 H), 4.80 (m, 2 H), 5.30 (d, 1 H, D_2O exchanged), 5.70 (m, 1 H), 5.85 (m, 1 H), 6.40 (m, 2 H), 7.50 (m, 12 H), 8.10 (m, 8 H). Anal. ($C_{38}H_{32}O_{10}N_2$) C, H, N.

3,4,5,6-Tetrahydro-4-(dihydroxymethyl)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (28). A solution of **26** (0.40 g, 0.58 mmol) in 3 mL of chloroform was treated with saturated methanolic ammonia (60 mL) and kept in a pressure bottle at 4 °C for 20 h. After removing the excess of ammonia and solvent, the residue was dissolved in 15 mL of water and extracted several times with ethyl acetate. The aqueous layer was lyophilized to give 0.122 g (76%) of crude material, which was purified by C-18 reversed-phase flash chromatography, as described for compound **10** (elution time 25 min), to produce **28** as a lyophilized powder: $[\alpha]^{25}_D$ −42.5° (*c* 0.130, H_2O); NMR (D_2O) δ 2.00 (m, 2 H), 3.30–3.65 (m, 6 H), 3.95 (m, 1 H), 4.10 (m, 1 H), 4.30 (m, 1 H), 5.95 (d, 1 H, J = 6 Hz); FAB mass spectrum, m/z (relative intensity) 279 (MH^+, 100), 261 (MH − H_2O, 26), 147 (b + 2 H, 21), 129 (b + 2 H − H_2O, 12). Anal. ($C_{10}H_{18}N_2O_7$) C, H, N.

3,4-Dihydro-4-(hydroxymethyl)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (7). In a similar fashion as in the preceding experiment, compound **25** (0.345 g, 0.51 mmol) was deblocked to afford 0.096 g (72%) of crude **7**. Purification by C-18 reversed-phase flash chromatography as performed above (elution time 26 min) produced **7** as a lyophilized powder: $[\alpha]_D^{25}$ −46.2° (*c* 0.104, H_2O); NMR (D_2O) δ 3.50 (m, 2 H), 3.70 (m, 2 H), 4.00 (m, 1 H), 4.20 (m, 3 H), 5.00 (m, 1 H), 5.70 (t, 1 H, J = 7 Hz), 6.40 (t, 1 H, J = 8.5 Hz); FAB mass spectrum, m/z (relative intensity) 261 (MH^+, 100), 129 (b + 2 H, 31). Anal. ($C_{10}H_{16}N_2O_6 \cdot 0.75H_2O$) C, H, N.

Sodium Borohydride Reduction of 29. Compound **29**[4,32] (1.08 g, 2 mmol) was dissolved in dry THF (200 mL) and reacted with a solution of 0.080 g (2.1 mmol) of $NaBH_4$ in 10 mL of methanol at 0 °C. After 1 h, the solvents were removed and the residue dissolved in ethyl acetate. Following extractions with water and brine, the organic layer was dried (Na_2SO_4) and separated by preparative HPLC using a Waters LC-500 instrument with silica cartridges and eluting with ethyl acetate–hexane (3:2). Two peaks were clearly resolved and collected to give, respectively, 0.27 g (25%) of **30** and 0.69 g (64%) of **31** as foams.

Compound **30**: NMR ($CDCl_3$) δ 4.00 (m, 2 H), 4.50 (m, 2 H), 4.75 (m, 2 H), 5.75 (m, 2 H), 5.87 (br t, 1 H), 6.50 (d, 1 H, J = 5 Hz), 6.80 (d, 1 H, D_2O exchanged), 7.50 (m, 9 H), 8.00 (dd, 6 H).

Compound **31**: NMR ($CDCl_3$) δ 3.95 (s, 2 H), 4.60 (m, 2 H), 4.75 (m, 2 H), 5.60 (s, 1 H, D_2O exchanged), 5.70 (t, 1 H, J = 6 Hz), 5.81 (dd, 1 H, J = 6 Hz, J' = 3 Hz), 6.18 (d, 1 H, J = 8 Hz), 6.31 (d, 1 H, J = 6 Hz), 7.43 (m, 9 H), 8.10 (dd, 6 H). These compounds were used directly without further purification in the next step.

3,4-Dihydro-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (9). Under the same general conditions for deblocking with saturated methanolic ammonia, compound **31** (0.27 g) was deprotected. After the usual workup and purification by preparative TLC (silica gel, 2000 μm, 20% methanol in CH_2Cl_2) 0.065 g (57%) of pure **9** was isolated. The sample was further lyophilized from an aqueous solution to give **9** as a fluffy powder: NMR (D_2O) δ 3.82 (m, 2 H), 4.06 (m, 2 H), 4.20 (dd, 1 H, J = 6 Hz, J' = 4 Hz), 4.38 (t, 1 H, J = 6 Hz), 5.06 (m, 1 H), 6.00 (d, 1 H, J = 6 Hz), 6.19 (dt, 1 H, J = 8 Hz, J' = 1.5 Hz); FAB mass spectrum, m/z (relative intensity) 231 (MH^+, 100). Anal. ($C_9H_{14}N_2O_5 \cdot 0.5H_2O$), C, H, N.

3,6-Dihydro-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (8). In a similar fashion to that used for **9**, compound **30** (0.69 g) was deblocked and purified by preparative TLC, followed by lyophylization of an aqueous solution to give 0.062 g (21%) of pure **8**: NMR (D_2O) δ 3.78 (m, 2 H), 4.07 (m, 2 H), 4.25 (dd, 1 H, J = 6.5 Hz, J' = 3.5 Hz), 4.31 (t, 1 H, J = 6.5 Hz), 5.21 (m, 1 H), 5.87 (d, 1 H, J = 6.5 Hz), 6.38 (dt, 1 H, J = 8 Hz, J' = 3.5 Hz); FAB mass spectrum, m/z (relative intensity) 231 (MH^+, 100). Anal. ($C_9H_{14}N_2O_5 \cdot 0.5H_2O$) C, H, N.

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