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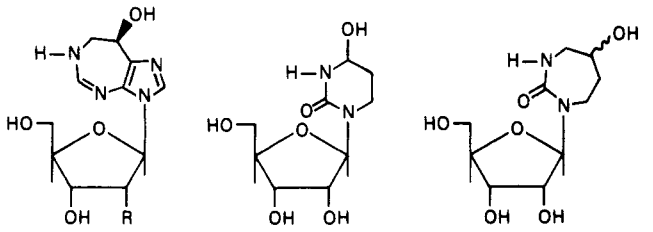
Communications to the Editor

Synthesis of 1,3-Diazepin-2-one Nucleosides as Transition-State Inhibitors of Cytidine Deaminase

Sir:

Potent inhibition of adenosine deaminase by coformycin (**1a**) [or 2′-deoxycoformycin (**1b**)] and of cytidine deaminase by tetrahydrouridine (THU, **2**) is believed to result from the similarity between these stable compounds and the respective tetrahedral substrate intermediates formed in the course of the enzymatic deamination reaction.[1,2] These compounds are probably the best examples of the transition-state analogue concept.[3,4] While the K_m values of these two enzymes for their respective substrates differ only by about an order of magnitude depending on the enzyme source,[5,6] coformycin and its 2′-deoxy analogue are far more potent in inhibiting adenosine deaminase than THU is in inhibiting cytidine deaminase. The purine deaminase inhibitors ($K_i = 10^{-11}$ to 10^{-12} M) are roughly 10^4 to 10^5 times more tightly bound to their enzyme than THU is to cytidine deaminase ($K_i = \sim 10^{-7}$ M).



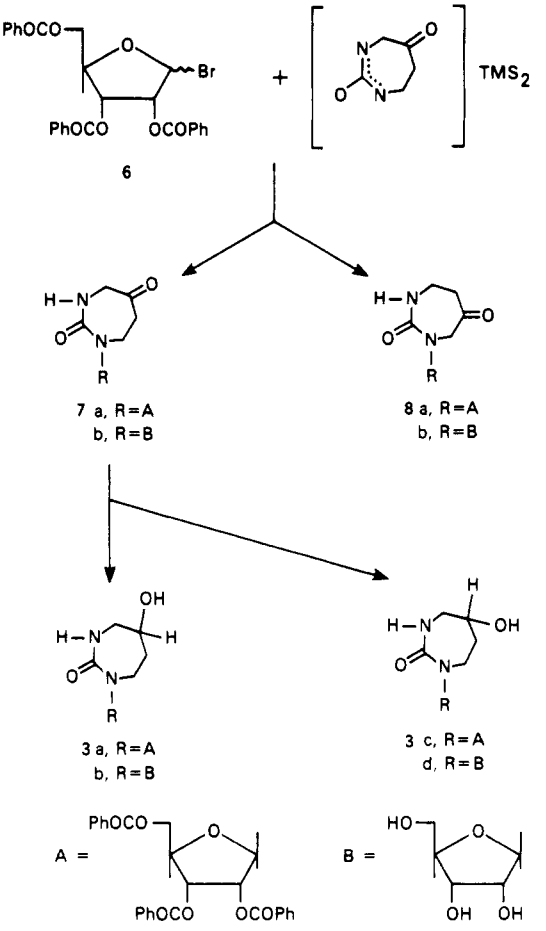
1 a, R=OH
b, R=H

2

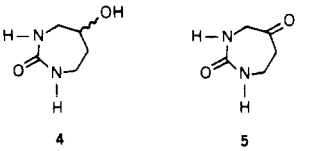
3 b,d

Two unique features appear in the structure of the aglycons of the potent purine deaminase inhibitors: an expanded seven-membered ring and a hydroxyl group located two carbons away from a neighboring nitrogen atom in this ring. It was, therefore, of interest to determine if similar changes in the pyrimidine nucleus would result in compounds with affinities for cytidine deaminase approaching the values observed for the coformycins with adenosine deaminase. In this investigation we describe the synthesis and preliminary enzyme inhibition activity of the new, seven-member, pyrimidine-like nucleosides **3b** and **3d**.

Scheme I



The plan to prepare nucleosides **3b,d** involved first the synthesis of the seven-membered ring heterocycle and second its condensation with an appropriate sugar derivative. Two methods were devised for the synthesis of heterocycle **4**, and both routes successfully afforded the desired compound.[7] However, experimental considera-



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(1) Cha, S.; Agarwal, R. P.; Parks, R. E. *Biochem. Pharmacol.* **1975**, *24*, 2187.
(2) Wentworth, D. F.; Wolfenden, R. *Biochemistry* **1975**, *14*, 5099.
(3) Wolfenden, R. *Acc. Chem. Res.* **1972**, *5*, 10.
(4) Wolfenden, R. "Transition States of Biochemical Processes"; Gandour, R. D.; Schowen, R. L., Eds.; Plenum Press: New York and London, 1978; pp 555–578.
(5) Wentworth, D. F.; Wolfenden, R. *Methods Enzymol.* **1978**, *51*, 401–408.
(6) Agarwal, R. P.; Parks, R. E. *Methods Enzymol.* **1978**, *51*, 502–508.
(7) Marquez, V. E.; Liu, P. S.; Kelley, J. A.; Driscoll, J. S. *J. Org. Chem.* **1980**, *45*, 485.

tions caused us to prepare first the nucleoside of the precursor heterocycle **5**. Compound **5** was smoothly silylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) at room temperature and the Me_3Si derivative was then reacted in refluxing benzene with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**6**) in the presence of mercury catalysts ($HgO/HgBr_2$) to afford the corresponding protected nucleosides (Scheme I).[8] Two compounds were isolated which correspond to the nucleosides formed at N^1 and N^3 (**7a** and **8a**). Only β isomers were observed. This fact is probably due to the participation of the 2'-benzoyl function during the condensation reaction.[9] Compounds **7a** and **8a** were readily separated by column chromatography on silica gel using ethyl acetate–hexane (3:1). The low R_f isomer corresponds to the N^3-nucleoside **8a** [22%; syrup; NMR δ 2.80 (m, 2, $HNCH_2CH_2COCH_2N$), 3.35 (m, 2, $HNCH_2CH_2COCH_2N$), 3.95 (s, 2, $HNCH_2CH_2COCH_2N$), 4.60 (br s, 3, H-4', H-5', H-5'a), 5.50 (m, 2, H-2', H-3'), 5.80 (br s, 1, NH), 6.10 (d, 1, H-1', $J_{1',2'}$ = 7 Hz), 7.40 (m, 10, aryl), 7.90 (m, 5, aryl); M^+· 572]. The high R_f isomer corresponds to the N^1-nucleoside **7a** [27%; syrup; NMR δ 2.75 (m, 2, $HNCH_2COCH_2CH_2N$), 3.65 (m, 4, $HNCH_2COCH_2CH_2CH_2N$), 4.70 (m, 3, H-4', H-5', H-5'a), 5.80 (m, 2, H-2', H-3'), 6.20 (m, 1, H-1'), 6.35 (t, 1, NH, J = 4 Hz), 7.40 (m, 10, aryl), 7.90 (m, 5, aryl); M^+· 572]. These assignments were based on the differences observed for the signal corresponding to the isolated methylene group of the aglycons. In compound **8a**, this signal was a singlet at δ 3.95, whereas in **7a** the equivalent signal appeared more complex due to NH coupling (D_2O exchange) and the presence of a second methylene absorption.[10] We were able to develop conditions to generate the N^1 isomer (**7a**) exclusively by controlling the reflux time of the persilylated heterocycle **5** with the mercury catalysts prior to the addition of **6**. The yield of pure **7a** ranged from 45 to 55%.

Sodium borohydride reduction of **7a** proceeded quantitatively, affording the two expected diastereomers **3a** and **3c** in almost equal amounts. These compounds proved to be separable by preparative thin-layer chromatography. Although the absolute configurations at C-5 of the aglycons are not yet known, all spectral and analytical data are in agreement with these structures.

Deprotection of **3a** and **3c** with methanolic ammonia afforded **3b** and **3d** in ca. 80% yield. The final products were recrystallized ($MeOH–CHCl_3–Et_2O$) to afford crystalline **3b** [mp 110–112 °C; $[\alpha]^{25}_D$ −115° (*c* 0.1, H_2O); NMR δ 1.20–2.20 (m, 2, $HNCH_2CHOHCH_2CH_2N$), 3.15 (m, 4, $HNCH_2CHOHCH_2CH_2N$), 3.35–3.75 (m, 3, H-2', H-3', H-4'), 3.85 (m, 1, $HNCH_2CHOHCH_2CH_2N$), 4.10 (m, 2, H-5', H-5'a), 5.40 (m, 1, H-1'); M^+· 622 ($(Me_3Si)_5$ derivative)] and crystalline **3d** [mp 162–164 °C; $[\alpha]^{25}_D$ −183° (*c* 0.1, H_2O); NMR δ 1.90 (m, 2, $HNCH_2CHOHCH_2CH_2N$), 3.60 (m, 4, $HNCH_2CHOHCH_2CH_2N$), 3.75 (m, 2, H-2', H-3'), 4.00 (m, 2, $HNCH_2CHOHCH_2CH_2N$, H-4'), 4.15 (m, 2, H-5', H-5'a), 5.40 (d, 1, H-1', $J_{1',2'}$ = 7 Hz); M^+· 622 ($(Me_3Si)_5$ derivative)].[11] The GC retention times and mass spectral fragmentation pattern of the persilylated diastereomers (**3b** and **3d**) are identical, giving single GC peaks with the expected molecular ion.

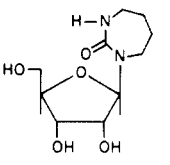
Through an identical experimental approach, nucleoside **9** was obtained using tetramethyleneurea in the condensation reaction. Similarly, deblocking of **7a** and **8a** afforded **7b** and **8b**, respectively.[11]

All deprotected nucleosides were tested as cytidine deaminase (EC 3.5.4.5) inhibitors against the mouse kidney and human liver enzymes[12] (Table I). For this preliminary communication, all K_i values were calculated assuming competitive inhibition.[13,14] As can be seen in Table I, the difference between these two enzyme systems is not great.

Table I. Cytidine Deaminase Inhibition

no.	mp, °C	K_i, M	
		mouse kidney	human liver
2		2.2×10^{-7}	1×10^{-7}
10		4×10^{-6}	
7b		2×10^{-5}	9×10^{-6}
9		3×10^{-7}	4×10^{-7}
3d	162–164	4×10^{-7}	9×10^{-7}
3b	110–112	2×10^{-8}	4×10^{-8}

Based on mechanistic considerations it is quite possible that cytidine deaminase catalyzes the displacement of ammonia by water through an addition–elimination mechanism analogous to that proposed for adenosine deaminase.[15,16] The fact that one of the diastereomers of **3**, which we designed as a coformycin analogue in the pyrimidine series, is the most potent known inhibitor of cytidine deaminase (ten times more potent than THU) tends to support the idea of an intrinsic mechanistic similarity between these two important deaminases. An activity comparison between **9** and a compound (**10**) prepared previously by us[17] reflects a potency increase of more than an order of magnitude in going from a six- to a seven-membered ring aglycon. The correct stereochemistry of the hydroxyl group as in **3b** is further responsible for another order of magnitude increase in binding. The precursor keto compound **7b**, as expected, behaved as a poor inhibitor of the enzyme. This can be rationalized in terms of the lack of a tetrahedral carbon at C-5 in the aglycon of this compound.



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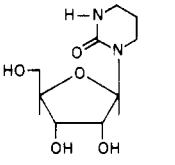

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(8) To our knowledge, this is the first condensation of a fully saturated urea with a sugar derivative. The procedure is efficient and of general applicability to other saturated heterocycles. The persilylated heterocyclic urea, possibly as bis-[*N*-(Me_3Si)] derivative, does not condense with the halo sugar unless the mercury catalysts are present. This would indicate that the Hg^{2+} helps shift the equilibrium toward the imino *O*-(Me_3Si) forms, which can give rise to condensation products at N^1 and N^3. This new reaction is currently under detailed investigation.

(9) Watanabe, K. A.; Hollenberg, D. H.; Fox, J. J. *J. Carbohydr., Nucleosides, Nucleosides* **1974**, *1*, 1.

(10) NMR data for the protected nucleosides were obtained in $CDCl_3$ solutions (~5%) with tetramethylsilane as internal standard. The spectra were recorded with either a Varian T-60 or Varian HA-100D instrument.

(11) NMR data for the free nucleosides were obtained in D_2O solutions (~5%) with deuterated sodium 3-(trimethylsilyl)-propionate as an internal standard. All final target compounds described in the text gave satisfactory elemental analyses.

(12) The experimental details of the enzyme assay for cytidine deaminase are identical with those reported in ref 17.

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With respect to the enzyme's specificity and the anomeric configuration of the glycosidic bond, the enzymatic assay proves to be a useful tool for assigning the β configuration to all the inhibitors prepared. In addition to the chemical and spectral evidence, the selectivity of cytidine deaminase for the β anomers constitutes additional proof for the β configuration assigned to these nucleosides. In our hands, only β-cytidine, the natural substrate, is deaminated. α-Cytidine, with the opposite configuration, was neither a substrate nor an inhibitor. This indicates that only nucleosides with the β configuration fit the enzyme's receptor site.

The combination of these inhibitors with cytidine analogues possessing antitumor properties (e.g., cytosine arabinoside) and the study of the biological consequences of total depletion of cytidine deaminase activity constitute fruitful areas for additional antitumor drug research.

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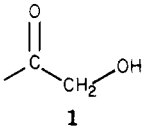
Prostaglandins and Congeners. 25.[1] Inhibition of Gastric Acid Secretion. Replacement of the Carboxylate Moiety of a Prostaglandin with a Hydroxymethylketo Functional Group

Sir:

In testing various prostaglandin congeners for their ability to inhibit histamine-induced gastric acid secretion in the dog, we observed, as have others,[2] that several 15-deoxy-16-hydroxy-16-methyl analogues were orally active at doses which did not produce the gastrointestinal side effects characteristic of the natural prostaglandins. These β (C_{13}–C_{20}) chain modifications at C-15 and C-16 apparently render these analogues more resistant to metabolic degradation by prostaglandin 15-dehydrogenase[3] with retention of biological activity. Insofar as fatty acid β oxidation is also one of the important deactivation routes for the prostaglandins,[3] we have further sought to enhance duration and potency through chemical modification of the α chain, specifically by substitution of a functional group which would mimic the carboxylate moiety with respect to receptor interaction while conferring greater metabolic stability.

A terminal hydroxymethylketo functional group (1),



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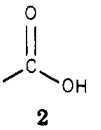

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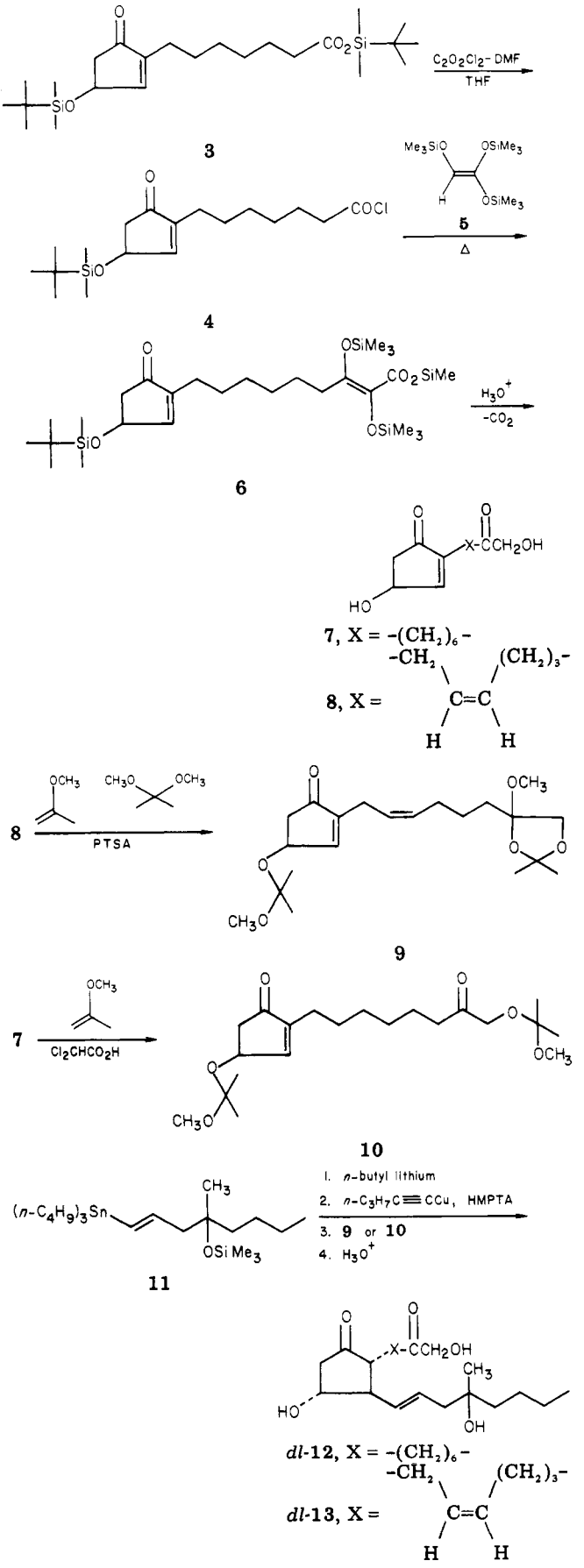
which differs from a carboxylate group (2) in having a methylene group interposed between the carbonyl and hydroxy functions, may have the potential for forming hydrogen bonds in a manner similar to that of a carbox-

Scheme I



(1) For Paper 24 in this series, see C. V. Grudzinskas, J. S. Skotnicki, S.-M. L. Chen, M. B. Floyd, W. A. Hallett, R. E. Schaub, G. J. Siuta, A. Wissner, M. J. Weiss, and F. Dessy, *ACS Symp. Ser.*, **no. 118**, 301 (1980).
(2) P. W. Collins, E. Z. Dajani, D. R. Driskill, M. S. Bruhn, C. J. Jung, and R. Pappo, *J. Med. Chem.*, **20**, 1152 (1977).
(3) B. Samuelsson, E. Granstrom, K. Green, and M. Homberg, *Ann. N.Y. Acad. Sci.*, **180**, 138 (1971).

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