1-(β-D-Ribofuranosyl)imidazo[4,5-*d*]pyridazin-4(5*H*)-one: A New Analogue of Inosine (1)

R. Paul Gagnier, Michael J. Halat and Brian A. Otter*

Laboratory of Organic Chemistry, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

Received January 6, 1982

Imidazo[4,5-*d*]pyridazine-4(5*H*)-one, which normally forms the *N*-6 nucleoside, can be induced to form the N-1 and N-3 nucleosides when a benzyloxymethyl substitutent is incorporated at *N*-5. The *N*-5 blocking group can be removed under mild conditions with boron trichloride.

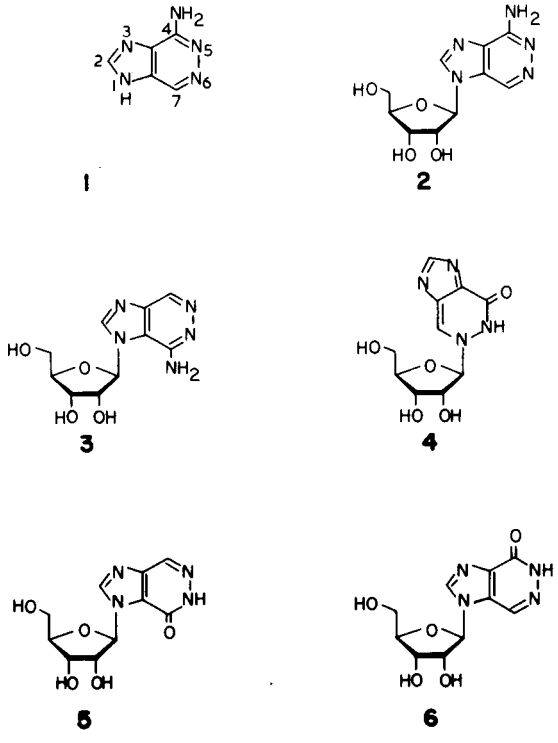
J. Heterocyclic Chem., **19**, 221 (1982).

The imidazo[4,5-*d*]pyridazines (2) attracted early attention as potential purine antimetabolites and certain 4-substituted derivatives do, in fact, function as substrates or inhibitors of purine-requiring enzymes (3). In view of these findings and the report (4) that the adenine analogue **1** (5) is a cytotoxic compound, it is surprising that imidazo-[4,5-*d*]pyridazine nucleosides have not received more attention as candidates for antitumor and antiviral evaluation. Only a few such nucleosides are known (6), and only one of these, compound **2**, can be regarded as a close structural analogue of a natural 6-substituted purine nucleoside. However, the structures assigned (6a) to **2** and its isomer **3** were not established rigorously and they must be regarded as tentative for the reasons outlined below (7). In the area of 4-oxo nucleosides, Cook and coworkers (6b) have prepared the *N*-6 isomer **4** *via* a condensation reaction, and the *N*-3 isomer **5** *via* a multistep procedure. In this paper we present a preliminary report on the synthesis of the *N*-1 nucleoside **6**, namely the imidazo[4,5-*d*]-pyridazine analogue of inosine. This work also demonstrates that proton spin-lattice times (T_1) can be used to assign the structures of isomeric nucleosides in this series.

Since Cook and coworkers (6b) have already shown that the bis-TMS derivative of **9** (Figure 2) cannot be converted into the *N*-1 nucleoside *via* the Vorbrüggen procedure, we have adopted the strategy of using a removable *N*-substituent to influence the site of glycosylation (8). The standard synthesis (3c,9) of imidazo[4,5-*d*]pyridazin-4(5*H*)-ones from pyridazines allows for the convenient introduction of substituents at *N*-5, and we have therefore prepared the 5-benzyloxymethyl derivatives **7** (Figure 2) (10). Our working hypothesis was that if **7** silylates on oxygen to give **8**, then the sites of glycosylation might be limited by mechanistic considerations to *N*-1 and *N*-3.

Condensation of silylated 7 (1 equivalent, possibly **8**) with 1-*O*-acetyltri-*O*-benzoylribofuranose (0.95 equivalent) in 1,2-dichloroethane containing stannic chloride (0.7 equivalent) does indeed afford a mixture of the *N*-1 and *N*-3 nucleosides **10** and **11**. Moreover, the relative yield of each product depends critically on the time span of the reaction. The hplc analysis indicates that **10** is formed first, but that it is converted partially into **11** when the reaction mixture is stored at room temperature for several days. The yields of **10** and **11** isolated by preparative tlc from a 3-day reaction mixture were 33% and 18%, respectively. In contrast, a 1 hour reaction afforded **11** in only 13% yield, whereas **10** was isolated in 56% yield.

Figure 1

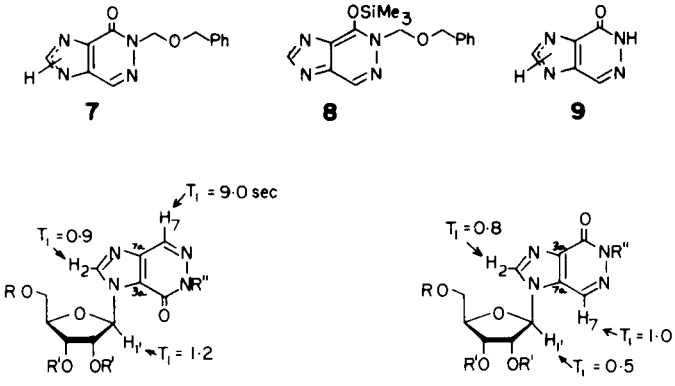


Removal of the benzoyl blocking groups from **10** and **11** and isopropylidenation of the resulting products afforded **12** and **13**, each of which shows $\Delta\delta$ value for the isopropylidene methyl resonances of 23 Hz, clearly indicative of the expected β-anomeric configurations (11a). The appearance of the H-4′ resonances of **12** and **13** as multiplets, rather than pseudotriplets, is also consistent with the β-configuration (11b).

Removal of the *N*-5 benzyloxymethyl groups was accomplished by treating **10** and **11** with boron trichloride in

dichloromethane at -78°. Treatment of the resulting products **14** and **15** with methanolic ammonia then affords the deblocked nucleosides **5** and **6**, respectively in overall yields (from **10** and **11**) of 50-60%. The structures assigned to **5** and **6**, and their precursors, rest on the following evidence.

Figure 2



10 - - - R = R' = COPh, R" = CH_2OCH_2Ph - - - **11**

12 - - R = H, R' = 2',3'-isopropylidene, R" = CH_2OCH_2Ph - - **13**

14 - - - - R = R' = COPh, R" = H - - - - **15**

5 - - - - R = R' = R" = H - - - - **6**

The starting base **7** contains three potential *N*-glycosylation sites and two of the possible products (**4** are **5**) are known compounds (6b). The physical properties (uv, ^{1}H-nmr, ^{13}C-nmr) of **5** and **6** isolated in the present study do not correspond to those reported (6b) for the *N*-6 nucleoside **4** (12). On the other hand, the spectral properties of **5** are in good agreement with those published (6b) for the *N*-3 isomer. Nucleoside **6**, by elimination, is therefore the desired *N*-1 isomer. In addition to this negative evidence, the structures of **5** and **6** were established without reference to previous assignment by using the following nmr techniques.

i) ^{13}C-^{1}H Coupling Constants.

The C-2 resonances (13) of both **5** and **6** appear as double doublets with $^1J_{C\text{-}2,\ H\text{-}2}$ = 215 Hz and $^3J_{C\text{-}2,\ H\text{-}1'}$ = 5Hz. The coupling to H-1′ establishes (14) that both compounds are ribosylated on an imidazole nitrogen, thereby removing the *N*-6 isomer **4** from consideration.

ii) Proton Spin-Lattice Relaxation Times (T_1).

The resonance sequence for the base protons of **5** was shown to be H-2 (δ 8.78) — H-7 (δ 8.43) by selective decoupling of the C-2 and C-7 resonances. This reverses the proton assignments made by Cook, *et al.*, (6b). The H-2 and H-7 resonances of **6** are quite close together (δ 8.66, 8.52), so they were assigned by analogy with **5** rather than by selective decoupling. The T_1 values (seconds) for **5** and **6** (inversion-recovery method, degassed 0.025 *M* solutions in DMSO-d_6 at 22°) are shown on the structures in Figure 2. The large difference in the T_1 values for **5** (T_1 H-7/T_1 H-2 = 10) indicates that only H-2 is being relaxed by dipole-dipole interactions with the sugar ring protons. The long relaxation time of H-7 shows that it is remote from other protons, which is consistent with the *N*-3 nucleoside structure. Conversely, the *N*-1 structure is indicated for **6** because *both* base protons have short relaxation times (T_1 H-7/T_1 H-2 = 1.25) — in other words, both protons are close to the protons of the sugar ring. The reduced T_1 value of H-1′ in **6** (relative to **5**) suggests an H-1′ — H-7 interaction. We are not aware of other examples of the use of T_1 values for assigning glycosylation sites.

iii) Anomeric Protons Chemical Shifts.

The H-1′ proton of **5** (δ 6.38) is deshielded relative to the H-1′ proton of **6** (δ 5.90), a difference (0.48 ppm) that can be attributed to the proximity of the anisotropic C-4 carbonyl group to H-1′ in **5**. Similar differences have been noted (15) for the △δ H-1′ values of the 7- and 9-ribosyl derivatives of hypoxanthine, guanine, and 3-deazaguanine.

iv) ^{13}C Chemical Shifts.

The assignment of glycosylation sites by ^{13}C-nmr relies on analysis of the carbon chemical shift differences between the *N*-nucleoside and the parent heterocycle (16). In one variation of this technique, which is useful for bicyclic systems, *N*-substitution causes upfield shifts of α bridgehead carbons and downfield shifts of β bridgehead carbons (14,16b). For **5**, the observed upfield shift of 4 ppm for C-3a (α) and the downfield shift of 3.1 ppm for C-7a (β), relative to the corresponding carbons in **9**, is consistent with the *N*-3 nucleoside structure. For **6**, the upfield shift of C-7a (α, 2.4 ppm) and the downfield shift of C-3a (β, 2 ppm) indicates the *N*-1 nucleoside structure.

The conversions of **5** and **6** into the adenosine analogues are currently under investigation and are expected to clarify the structures assigned (6a) to nucleosides **2** and **3**.

REFERENCES AND NOTES

(1) This investigation was supported by funds from the American Cancer Society (CH-169) and from the National Cancer Institute (CA 08748).

(2) For a review, see M. Tisler and B. Stanovnik, "The Chemistry of Heterocyclic Compounds", Vol. 27, R. N. Castle, ed., John Wiley and Sons, Inc., New York, N.Y., 1972. p 801.

(3a) R. C. Hartenstein and I. Fridovich, *J. Biol. Chem.*, **242**, 740 (1967); (b) R. E. A. Gadd and J. F. Henderson, *Can. J. Biochem.*, **48**, 295 (1970); (c) S-F. Chen and R. P. Panzica, *J. Org. Chem.*, **46**, 2467 (1981).

(4) L. L. Bennett, Jr., and D. Smithers, *Biochem. Pharmacol.*, **13**, 1331 (1964).

(5a) R. N. Castle and W. S. Seese, *J. Org. Chem.*, **23**, 1534 (1958); (b) J. A. Carbon, *J. Am. Chem. Soc.*, **80**, 6083 (1958).

(6a) J. A. Carbon, *J. Org. Chem.*, **25**, 579 (1960); (b) P. D. Cook, P. Dea and R. K. Robins, *J. Heterocyclic Chem.*, **15**, 1 (1978); (c) Certain 4,7-dioxo nucleosides described in reference 6b have also been prepared by an alternative route. See C. Tapiero, J-L. Imbach, R. P. Panzica and L. B. Townsend, *J. Carbohydr., Nucleosides, Nucleotides*, **3**, 191 (1976).

(7) A. re-examination of Carbon's uv data (6a), made with the benefit of hindsight, suggests that structure **3** [designated (6a) as 7-amino-1-ribofuranosyl[4,5-*d*]pyridazine] is in fact the *N*-6 isomer. Carbon himself recognized this possibility and Chen and Panzica (3c) have recently stated the same conclusion. The structure assigned to **2** rests solely on a uv comparison with the 1- and 3-methyl bases. However, the model compounds were prepared by an ambiguous procedure, which raises the possibility that **2** could be the *N*-3 isomer.

(8) This strategy has been used for a variety of heterocyclic systems. For examples in the purine area that use allyl, propenyl and pivaloyloxymethyl groups, see J. A. Montgomery and J. H. Thomas, *J. Org. Chem.*, **30**, 3235 (1968); *idem, ibid.*, **34**, 2646 (1969); N, J. Leonard and M. Rasmussen, *ibid.*, **33**, 2488 (1968).

(9) S. F. Martin and R. N. Castle, *J. Heterocyclic Chem.*, **6**, 93 (1969).

(10) All new compounds gave satisfactory elemental analyses.

(11a) B. Rayner, C. Tapiero and J-L. Imbach, *Carbohydr. Res.*, **47**, 195 (1976); (b) M. MacCoss, M. J. Robins, B. Rayner and J-L. Imbach, *ibid.*, **59**, 575 (1977).

(12) The dissimilarity of the uv spectra of *N*-5 alkyl imidazo[4,5-*d*]-pyridazin-4(5*H*)-ones (*e.g.*, **7**, λ max water 255, 265 nm) and nucleoside **4** (λ max water 302 nm) can be regarded as additional evidence for the *N*-6 structure of **4**, which was assigned originally by ^{13}C-nmr (6b).

(13) For each isomer, C-2 (δ 143.9 in **5**, 142.4 in **6**) appears downfield from C-7 (δ 133.7 in **5**, 127.3 in **6**), and $^{1}J_{C\text{-}2,\ H\text{-}2}$ (both 215 Hz) is larger than $^{1}J_{C\text{-}7,\ H\text{-}7}$ (both 187 Hz). Coupling constants estimated by Malinowski's additivity rules are 211 Hz and 180 Hz, respectively. See E. R. Malinowski, L. Z. Pollara and J. P. Larman, *J. Am. Chem. Soc.*, **84**, 2649 (1962), and K. Tori and T. Nakagawa, *J. Phys. Chem.*, **68**, 3163 (1964).

(14) B. L. Cline, P. E. Fagerness, R. P. Panzica and L. B. Townsend, *J. Chem. Soc., Perkin Trans. II*, 1586 (1980).

(15) P. D. Cook, R. J. Rousseau, A. M. Mian, P. Dea, R. B. Meyer, Jr. and R. K. Robins *J. Am. Chem Soc.*, **98**, 1492 (1976), and references therein.

(16a) P. Dea and R. K. Robins, "Chemistry and Biology of Nucleosides and Nucleotides", R. E. Harmon, R. K Robins and L. B. Townsend, eds., Academic Press, New York, N.Y., 1978, p. 301; (b) M-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica and L. B. Townsend, *J. Am. Chem. Soc.*, **97**, 4627 (1975).