

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 substituent is incorporated at *N*-5. The *N*-5 blocking group can be removed under mild conditions with boron trichloride.

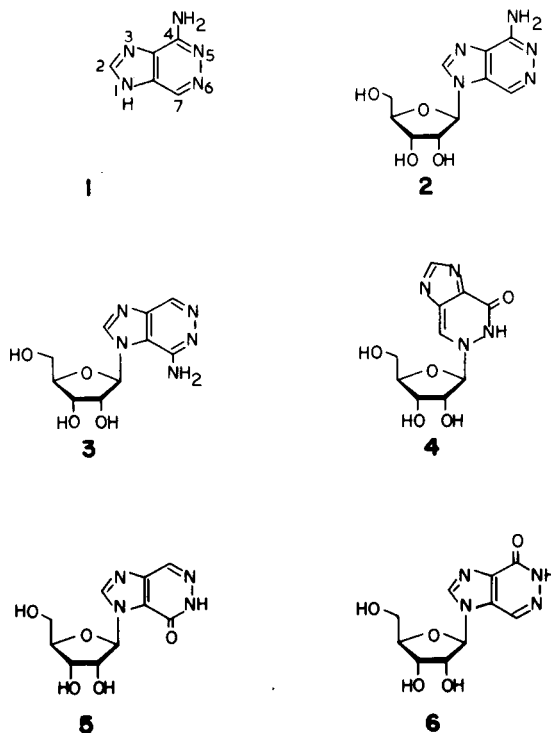
J. Heterocyclic Chem., **19**, 221 (1982).

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

Figure 1



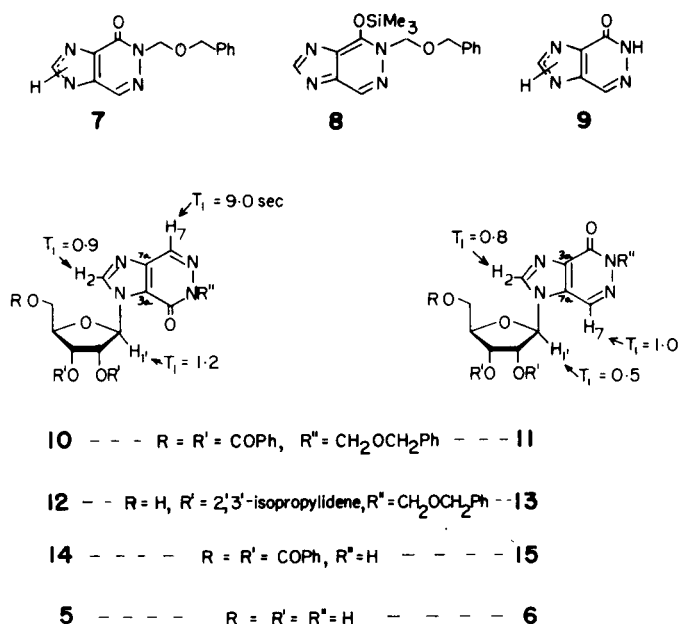
reaction mixture is stored at room temperature for several days. The yields of **10** and **11** isolated by preparative tlc from a 3-day reaction mixture were 33% and 18%, respectively. In contrast, a 1 hour reaction afforded **11** in only 13% yield, whereas **10** was isolated in 56% yield.

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



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, ^1H -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 - ^1H Coupling Constants.

The C-2 resonances (13) of both **5** and **6** appear as double doublets with $^1\text{J}_{\text{C-2, H-2}} = 215$ Hz and $^3\text{J}_{\text{C-2, H-1'}} = 5$ Hz. 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 $\Delta\delta$ 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 pivaloyloxy-methyl 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 $^1\text{J}_{\text{C-2, H-2}}$ (both 215 Hz) is larger than $^1\text{J}_{\text{C-7, H-7}}$ (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).