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# Synthesis of 5-(β-D-Ribofuranosyl)-Pyridin-2-one: A "Deletion-Modified" Analogue of Uridine

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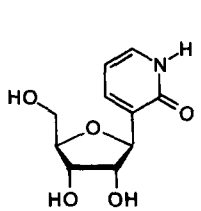
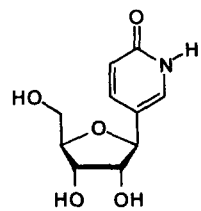
**Abstract:** Pyridin-2-one *C*-nucleoside **2** was prepared using several different approaches. The most efficient pathway utilized the condensation of 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (**4**) and 2-(benzyloxy)-5-bromopyridine (**5**), followed by deoxygenation with $Et_3SiH/BF_3{\bullet}Et_2O$ and removal of the benzyl protecting groups.


A number of base modified nucleosides have been incorporated into hammerhead ribozymes to study their effects on ribozyme activity.[1-5] Nucleoside analogs that are only minimally altered with respect to the corresponding natural nucleosides can be valuable tools in structure-activity studies at a monomer level and when incorporated into DNA or RNA. Recently we found that substitution of the nonconserved U7 residue in the catalytic core of a hammerhead ribozyme by a number of base modified nucleosides has a profound effect on catalytic activity.[2] We have reported[6] the synthesis of a "deletion-modified" uridine analog **1** that lacks an $O^4$ carbonyl group. Herein we report the synthesis of its ismeric analog **2** that lacks an $O^2$ carbonyl. To the best of our knowledge the preparation of this compound has not been reported.[7] These analogs of pyrimidine nucleosides with novel H-bonding patterns could serve as valuable tools for identification of essential intramolecular hydrogen bonding interactions.[8]




**1**



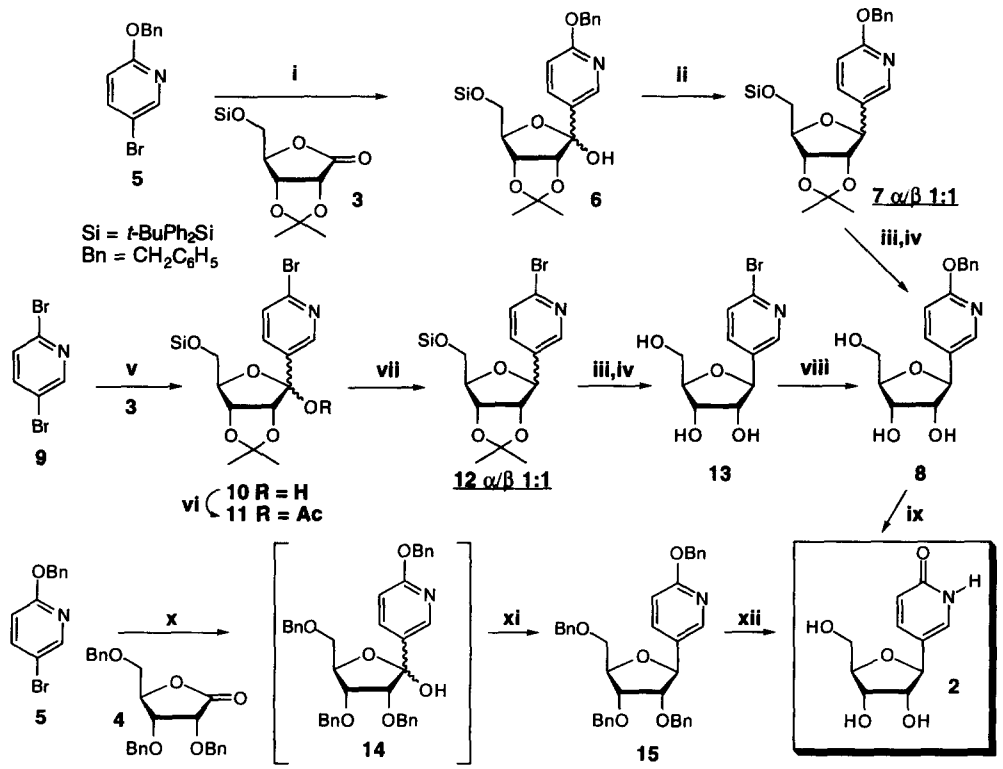

**2**

In our first attempt to synthesize **2** (Scheme 1) we converted 2-(benzyloxy)-5-bromopyridine (**5**) (synthesized from commercially available 2,5-dibromopyridine using NaH/BnOH/DMF)[9] to the 5-lithio derivative with LDA at -78 °C. Addition of 5-*O*-*t*-butyldiphenylsilyl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone (**3**)[10] to this intermediate yielded a mixture of α and β lactols **6** in a 1:8 ratio (47% yield).[11] The assignments of the anomeric configurations were based on Δδ values for the isopropylidene Me groups in the $^1H$ NMR spectrum.[12] It is known from the previous work[6,13,14] that reductive 1'-deacetoxylation is an efficient approach to 1'-deoxygenation in these types of compounds. Unfortunately, acetylation of **6** with $Ac_2O$/DMAP/TEA in acetonitrile[6] failed; the 1'-*O*-acetyl derivative was isolated in only 3% yield, the major product did not contain acetyl group by $^1H$ NMR. Reduction of **6** with triethylsilane

($Et_3SiH$)/$BF_3$•$Et_2O$ in acetonitrile at rt was slow, yielding an 1:1 α/β mixture of *C*-nucleosides **7** in 21% yield. Nucleosides **7α** and **7β** were easily separated by flash column chromatography using a 5-10% gradient of methanol in dichloromethane for elution. The desired anomer **7β** was treated with TBAF to remove the 5'-silyl ether protection followed by the cleavage of the isopropylidene group with acid to yield **8** in good yield.

Parham and Piccirilli[15] described an unexpected and highly selective halogen-lithium exchange between 2,5-dibromopyridine (**9**) and *n*-butyllithium at very low temperature (-100 °C), where only the 5-bromo substituent was exchanged. This finding encouraged us to synthesize **2** by an alternate approach starting from **9** and D-ribono-1,4-lactone (**3**). Lithiation of **9** at -100 °C in THF and condensation of the resulting lithiated pyridine with **3** at -100 °C afforded the expected hemiacetal **10** (44% yield, α/β 1:12)[11] along with two by-products in 6% and 17% yield, respectively.

## Scheme 1




**Reagents and Conditions**: **i)** LDA/-78 °C/THF, **ii)** $Et_3SiH/BF_3$•$Et_2O/CH_3CN$, 0 °C→ rt, 2.5 h, **iii)** 1 M TBAF/THF, 45 min, **iv)** 80% aq. $CH_3COOH$, reflux, 1.5 h, **v)** *n*-BuLi/-100 °C/THF, **vi)** $Ac_2O$/DMAP/TEA/$CH_3CN$, 1 h, **vii)** $Et_3SiH/BF_3$•$Et_2O/CH_2Cl_2$, 0 °C→ rt, 20 min, **viii)** KH/BnOH/DMF, 140 °C, 5 min or 0.1 M KOH in BnOH, 140 °C, 24 h, **ix)** $H_2$/Pd-C, 45 psi, 8 h, **x)** *n*-BuLi/THF/-78 °C, 2 h, -20 °C, 2 h, **xi)** $Et_3SiH/BF_3$•$Et_2O/CH_2Cl_2$, -78 °C, 2 h, -78 °C→ 0 °C, 4 h, **xii)** TMSI/$CH_3CN$, rt, 3h.

All three products exhibited sugar proton NMR signals consistent with the hemiacetal structure. No attempts were made to elucidate the site of attachment of the sugar to the pyridine ring for the two byproducts. This result confirms the reported[15] selectivity in the lithiation of **9**, but the formation of other products suggests that competing halogen-lithium exchange also occurred. Furthemore, the reaction of the 2,5-dibromopyridine **9** with **3** suffered from poor reproducibility in our hands. Deoxygenation of hemiacetal **10** using the same procedure as for the reduction of **6** yielded, as in the case of **6**, a 1:1 mixture of α/β nucleosides in a low yield. On the other hand, acetylation of the C-1' OH group in **10** proceeded in quantitative yield to give **11** (only the β-anomer **11** was obtained). This result demonstrates that acetylation of the 1'-OH group is strongly dependent on even small changes in the aglycon structure; while 2-*O*-Bn analog **6** gave the desired acetylated derivative in only 3% yield, under the same reaction conditions 2-bromo derivative **10** was acetylated in quantitative yield. Reduction of **11** using $Et_3SiH/BF_3{\bullet}Et_2O/CH_2Cl_2$ at 0 °C→ rt proceeded in 82% yield but again without selectivity (12α/12β 1:1). The anomeric assignments were based on both Δδ of the methyl groups and on the well known upfield shift of the 1'-H signal for the β-anomer compared to the α-anomer.[16,17] Intermediate 12β was deprotected to free nucleoside **13** in two steps (1 M TBAF, followed by refluxing 80% acetic acid).[18] It is worth noting that nucleoside **13** was highly resistant to displacement of the 2-bromo substituent with either methoxide or benzylate anions; reaction with KH/BnOH/DMF at 140 °C or 0.1M KOH in BnOH at 140 °C afforded **8** in 23% and 20% yield, respectively, identical to the compound synthesized from 2-(benzyloxy)-5-bromopyridine (**5**). This comparison proved unequivocally that the 5-pyridyl regioisomer was the main product obtained from 2,5-dibromopyridine (**9**). Catalytic hydrogenation[19] of **8** afforded free nucleoside **2** in 70% yield.

Because both synthetic pathways described above suffered from the lack of α/β selectivity and low yields in some reaction steps we decided to use a differently protected ribonolactone, *i.e.* 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (**4**)[20] for the synthesis of **2**. We assumed that the presence of 2,3-dioxolane ring in the intermediate hemiacetals **6**, **10** and **11** may impose conformational rigidity on the furanose ring resulting in the observed poor β selectivity during reduction. The benzyl protection for hydroxyl groups was chosen because of its compatibility with organometallic reagents and reduction conditions.[21] Condensation of 2-(benzyloxy)pyridine (**5**) with **4** followed by $Et_3SiH$ reduction of the crude lactol afforded exclusively β-anomer **15** in 61% overall yield (m.p. 56-57 °C from abs. ethanol).[22] This result demonstrated the importance of ribonolactone protecting groups in directing the stereochemical outcome of this two step reaction. Removal of benzyl groups from **15** was effected with iodotrimethylsilane (TMSI)/$CH_3CN$ yielding **2** in 53% yield [m.p. 173-174 °C, from abs. ethanol, UV (MeOH) $\lambda_{max}$ 305 nm].[23] Interestingly, $BBr_3/CH_2Cl_2$ removed only the sugar benzyl groups from **15** while the 2-*O*-benzyl group remained intact. Catalytic hydrogenation[19] of perbenzylated compound **15** yielded, contrary to the analogous smooth debenzylation of **8**, a complex mixture of products.

In conclusion, an efficient three step procedure for the synthesis of a "deletion-modified" uridine analog **2** starting from 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (**4**) and 2-(benzyloxy)-5-bromopyridine (**5**) is described. Work is in progress in our laboratory on the incorporation of these nucleosides into ribozymes.

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**References & Notes:**


1. For a review of the modified nucleosides utilized in hammerhead ribozyme studies see: McKay, D.B. *RNA* **1996**, *2*, 395-403.
2. Burgin, A.B, Jr.; Gonzalez, C.; Matulic-Adamic, J.; Karpeisky, A.M.; Usman, N.; McSwiggen, J.A.; Beigelman, L. *Biochemistry* **1996**, *35*, 14091-14097.
3. Murray, J.B.; Adams, C.J.; Arnold, J.R.P.; Stockley, P.G. *Biochem. J.* **1995**, *311*, 487-494.
4. Tuschl, T.; Ng, M.M.P.; Pieken, W.; Benseler, F.; Eckstein, F. *Biochemistry* **1993**, *32*, 11658-11668.
5. Bevers, S.; Xiang, G.; McLaughlin, L.W. *Biochemistry* **1996**, *35*, 6483-6490 and references cited therein.
6. Matulic-Adamic, J.; Beigelman, L. *Tetrahedron Lett.* **1997**, in press.
7. Recently, the preparation of 2-amino analog of **2** was reported: Hildbrand, S.; Leumann, C. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1968-1970.
8. Starzewski, P.; Tamm, C. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 36-57.
9. Sieburth, S. McN.; Hiel, G.; Lin, C.-H.; Kuan, D.P. *J. Org. Chem.* **1994**, *59*, 80-87.
10. Drew, M.G.B.; Mann, J.; Thomas, A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 2279-2285.
11. It should be noted that for hemiacetals **6, 10** and **11**, the prefix α refers to the position of the glycosidic OH group relative to the configuration at the reference C-atom (C4' in **2**; *i.e.* the pyridyl moiety is in the β-position).
12. Tapiero, C.; Imbach, J.-L. In *Nucleic Acid Chemistry*, Part 1, pp.1055-1059, Townsend, L.B. and Tipson, R.S., Eds.; J. Wiley & Sons, Inc.: New York, 1978. Ohrui, H.; Jones, G.H.; Moffatt, J.G.; Maddox, M.L.; Christensen, A.T.; Byram, S.K. *J. Amer. Chem. Soc.* **1975**, *97*, 4602-4613.
13. Dondoni, A.; Scherrmann, M.-C. *J. Org. Chem.* **1994**, *59*, 6404-6412.
14. Gudmundsson, K.S.; Drach, J.D.; Townsend, L.B. *Tetrahedron Lett.* **1996**, *37*, 2365-2368.
15. Parham, W.E.; Piccirilli, R.M. *J. Org. Chem.* **1977**, *42*, 257-260.
16. Tam, S.,Y.-K.; Klein, R.S.; de las Heras, F.G.; Fox, J.J. *J. Org. Chem.* **1979**, *44*, 4854-4862.
17. Sokolova, T.N.; Yartseva, I.V.; Preobrazhenskaya, M.N. *Carbohydr. Res.* **1981**, *93*, 19-34.
18. $^1$H NMR ($D_2O$) data for **13**: δ 8.26 (d, $J_{6,4}$=2.6, 1H, H6), 7.69 (dd, $J_{4,3}$=8.3, $J_{4,6}$=2.6, 1H, H4), 7.55 (d, $J_{3,4}$=8.3, 1H, H3), 4.70 (d, $J_{1',2'}$=7.2, 1H, H1'), 4.06 (dd, $J_{3',2'}$=5.5, $J_{3',4'}$=3.8, 1H, H3'), 3.99 (m, 1H, H4'), 3.93 (dd, $J_{2',1'}$=7.2, $J_{2',3'}$=5.5, 1H, H2'), 3.73 (dd, $J_{5',4'}$=3.6, $J_{5',5''}$=12.4, 1H, H5'), 3.65 (dd, $J_{5'',4'}$=5.2, $J_{5'',5'}$=12.4, 1H, H5'').
19. Hsieh, H.-P.; McLaughlin, L.W. *J. Org. Chem.* **1995**, *60*, 5356-5359.
20. Timpe, W.; Dax, K.; Wolf, N.; Weidman, H. *Carbohydr. Res.* **1975**, *39*, 53-60.
21. Kraus, G.A.; Molina, M.T. *J. Org. Chem.* **1988**, *53*, 752-753.
22. $^1$H NMR ($CDCl_3$) data for **14**: δ 8.19 (d, $J_{6,4}$=2.0, 1H, H6), 7.57 (dd, $J_{4,3}$=8.4, $J_{4,6}$=2.0, 1H, H4), 7.46-7.15 (m, 20H, phenyl), 6.69 (d, $J_{3,4}$=8.4, 1H, H3), 5.38 (s, 2H, 2-*OCH2*), 4.95 (d, $J_{1',2'}$=7.3, 1H, H1'), 4.32 (m, 1H, H4'), 4.01 (dd, $J_{3',2'}$=5.2, $J_{3',4'}$=3.6, 1H, H3'), 3.79 (dd, $J_{2',1'}$=7.3, $J_{2',3'}$=5.2, 1H, H2'), 3.62 (dd, $J_{5',4'}$=4.2, $J_{5',5''}$=10.5, 1H, H5'), 3.57 (dd, $J_{5'',4'}$=3.6, $J_{5'',5'}$=10.5, 1H, H5'').
23. $^1$H NMR ($CD_3OD$) data for **2**: δ 7.73 (dd, $J_{4,6}$=2.4, $J_{4,3}$=9.6, 1H, H4), 7.48 (d, $J_{6,4}$=2.4, 1H, H6), 6.55 (d, $J_{3,4}$=9.6, 1H, H3), 4.50 (d, $J_{1',2'}$=7.2, 1H, H1'), 4.05 (dd, $J_{3',2'}$=5.7, $J_{3',4'}$=3.4, 1H, H3'), 3.93 (m, 1H, H4'), 3.84 (dd, $J_{2',1'}$=7.2, $J_{2',3'}$=5.7, 1H, H2'), 3.74 (dd, $J_{5',4'}$=3.6, $J_{5',5''}$=11.9, 1H, H5'), 3.67 (dd, $J_{5'',4'}$=4.6, $J_{5'',5'}$=11.9, 1H, H5'').


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