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Synthesis of 3-(β-D-Ribofuranosyl)-2-Fluoropyridine and 3-(β-D-Ribofuranosyl)-Pyridin-2-one

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Abstract: Pyridin-2-one *C*-nucleoside **13** was prepared in 7 steps from 2-fluoropyridine **1** and D-ribono-1,4-lactone **2**. The successful approach to β-ribofuranosides **12** and **13** consisted of the reductive opening of the furanose ring of hemiacetal **3** followed by intramolecular Mitsunobu cyclization. Copyright © 1996 Elsevier Science Ltd

A number of modified nucleosides were incorporated into hammerhead ribozymes to study their effects on the ribozyme activity.[1] Nucleoside analogs that are only minimally altered with respect to the corresponding native nucleosides can be valuable tools in structure-function studies at a monomer level and when incorporated into DNA or RNA.[2,3] Recently, we found that substitution of nonconserved U7 residue in the catalytic core of the hammerhead ribozyme with a variety of base modified nucleosides has a profound effect on the catalytic activity.[4,5] Herein we report the synthesis of a pyrimidine ribonucleoside analogs **12** and **13** that lack an O^4 carbonyl group and can be used for the identification of essential intramolecular hydrogen bonding interactions.

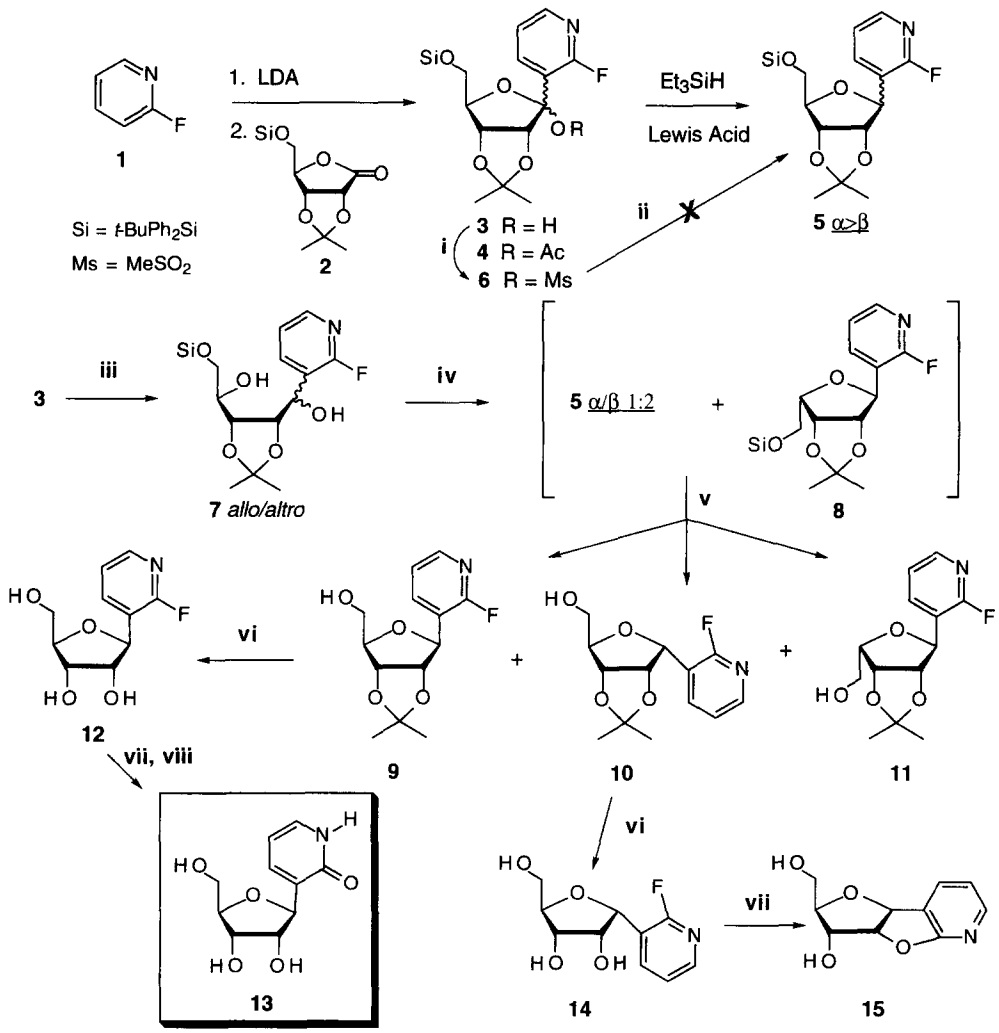
The synthesis of **13** as an anomeric mixture is described from 2,4:3,5-di-*O*-benzylidene-aldehydo-D-ribose and 3-lithio-2-fluoropyridine.[6] This procedure was inefficient and not amenable to large-scale preparations due to the involvement of a ribose dithioacetal during preparation of the key starting material. In addition, 2-fluropyridine derivative **12** could not be isolated by this route because of the solvolytic displacement of the fluorine atom during the acid catalyzed cyclization of an open chain precursor.

We therefore used the easily accessible 5-*O*-*t*-butyldiphenylsilyl-2,3-*O*-isopropylidene-D-ribonolactone (**2**)[7] in the condensation with 3-lithio-2-fluoropyridine[8] (-78 °C, then rt, 18 h, THF) and obtained a 1:5 α/β[9] mixture of lactols **3** in 63% yield (Scheme 1). The assignments of the anomeric configurations were based on Δδ values for the isopropylidene Me groups in ^{1}H NMR spectra.[10] All attempts to dehydroxylate **3** using triethylsilane (Et_3SiH) in the presence of BF_3•Et_2O or TMSOTf in a variety of solvents resulted in poor yields of nucleosides **5**. Futhermore, the desired β-nucleoside was always the minor component in anomeric mixture. In addition to the Imbach's rule[10] anomeric assignments were based on the well known upfield shift of the 1'-H signal for the β-anomer *vs* the α-anomer.[11,12]

It has been demonstrated that a more efficient and/or selective reduction of 1'-hydroxyl group can be achieved through intermediate 1'-*O*-Ac derivative (i.e. **4**).[13,14] We found that alcohol **3** could be acetylated in a quantitative yield using Ac_2O/TEA/DMAP in acetonitrile to give **4** (only one isomer was generated, no attempt was made to determine its configuration). Reductive deacetoxylation of **4** with Et_3SiH/TMSOTf in dichloromethane (0 °C, then rt) proceeded efficiently and afforded **5** (α/β 10:1) in 85% yield. When Et_3SiH was used as solvent the α/β ratio improved to 5:1. These results were sur-

prising because under similar reaction conditions the exclusive β-selectivity in the reduction of pyrazine[15] and imidazo[1,2-a]pyridine lactols[13] was reported.

Scheme 1



Reagents & Conditions: **i)** MsCl/DMAP/TEA/CH_3CN, 0 °C/rt, **ii)** LAH/Et_2O or $LiEt_3BH$/THF, **iii)** $NaBH_4$/MeOH, rt, 1 h, **iv)** Ph_3P/DEAD/THF, reflux, 1 h, **v)** 1M TBAF/THF, **vi)** 80% aq. AcOH, reflux, 1 h, **vii)** KOH/ BnOH, 140 °C, **viii)** TMSI/CH_2Cl_2, rt, 5 h.

To obtain gram quantities of β *C*-nucleoside **5** we investigated several different pathways. Mesylation of **3** afforded 1'-*O*-mesyl derivative **6** in a moderate yield. Treatment of **6** with LAH did not reduce the sulfonate as reported for 2-pyridine *C*-nucleosides.[16,17] The more reactive $LiEt_3BH$[18] also failed.

Interestingly, preparation of a more reactive 1'-*O*-Tf derivative failed due to the opening of the furanose ring under triflylation conditions.

Pankiewicz *et al.*[19] reported the hydrogenolytic opening of the hemiacetal ring of 6-pyridine lactol derivatives to generate mixtures of *allo* and *altro* isomers. Reaction of **3** with $NaBH_4$ proceeded in a quantitative yield to give *ca* 1:1 ratio of *allo/altro* **7**. These epimers were not separated but treated under standard Mitsunobu conditions [diethyl azodicarboxylate (DEAD)/Ph_3P/THF, reflux] to give **5** (α/β ratio 1:2) and 3-(2,3-*O*-isopropylidene-5-*O*-*t*-butyldiphenylsilyl-α-L-*lyxo*-furanosyl)-2-fluoropyridine (**8**), the latter arising from the competitive formation of 4'-oxyphosphonium intermediate. Yokoyama *et al.*[20] speculated that such an intermediate is formed because of the hydrogen bonding between 1'-OH and the hydrogen acceptor on the 2-pyridyl base. It is worth noting that if Mitsunobu cyclization of **7** was conducted at rt more *lyxo* derivative **8** was obtained than at reflux temperature. This may indicate the existence of the hydrogen bond between 1'-OH and 2-fluorine in its close proximity which could be disrupted by increasing the reaction temperature. During preparation of this manuscript an efficient synthesis of imidazole *C*-nucleosides was reported using similar Mitsunobu cyclization as the key step.[21]

Since it was difficult to separate the mixture of **5** and **8**, these compounds were 5'-desilylated with TBAF and then purified using flash chromatography.[22] The β-anomer **9** was obtained in 54% yield in two steps from **7**, while α-anomer **10** and *lyxo* derivative **11** were both obtained in 19% yield. Compound **9** was converted into free 2-fluoro nucleoside **12** (mp 134-135 °C, from THF)[23] by boiling in 80% acetic acid and then converted into 2-(benzyloxy) derivative with BnOK. Catalytic hydrogenolysis of the benzyl group (H_2, Pd-C) simultaneously cleaved the C1'-O4' bond, therefore debenzylation was carried out with trimethylsilyl iodode (TMSI) to afford 3-(β-D-ribofuranosyl)pyridin-2-one (**13**) in 83% yield as a syrup [UV(MeOH) λ_{max} 302 nm, ^1H NMR corresponds to that reported in reference 6]. The α-anomer **10** was deprotected in the same manner as **9** to give **14** (mp 173-174 °C, from CH_2Cl_2).[24] An attempt to displace the fluorine in **14** with benzylate yielded the anhydro derivative **15** in a quantitative yield [mp 195-196 °C, UV(MeOH) λ_{max} 286 nm].[25] This unexpected cyclization can be explained by an intramolecular nucleophilic displacement of fluorine by an adjacent 2'-hydroxyl. The structure of **11** was confirmed by NOE experiments in which mutual enhancement was observed between H4' and H3' (6% NOE) and between H4' and H4 of pyridine base (5% NOE).

In conclusion, we found a useful method for the synthesis of gram quantities of β-anomers of *C*-nucleosides **12** an **13** as starting materials for the preparation of building blocks for oligonucleotide synthesis. These analogs are also interesting as potential antiviral and/or anticancer agents.

Work is in progress in our laboratory on the incorporation of these nucleosides into ribozymes.

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22. In a typical procedure **7** (7 g, 13 mmol) and Ph_3P (5.25 g, 20 mmol) were dissolved in THF and the mixture was heated to reflux. DEAD (3.15 mL, 20 mmol) was than added to the refluxing mixture and heating continued for 1 hour. Solvent was removed *in vacuo* and the residue purified by flash column chromatography using a 9-11% gradient of ethyl acetate in hexanes for elution. 5.6 g, 83% of the mixture of **5** and **8** was obtained after removal of solvents. The above mixture was dissolved in THF (75 mL) and treated with 1 M TBAF in THF (22 mL, 2 eq) for 1 hour. It was then concentrated *in vacuo* and chromatographed on the column of silica gel using a 15-70% gradient of ethyl acetate in hexanes for elution. α-Anomer **10** eluted first (0.56 g, 19%), followed by β-anomer **9** (1.6 g, 54%). *Lyxo* derivative **11** eluted last (0.55 g, 19%).
23. ^{1}H NMR (CD_3OD) data for **12**: δ 8.19 (m, 1H, H6), 8.11 (m, 1H, H4), 7.32 (m, 1H, H5), 4.98 (d, $J_{1',2'}$=5.6, 1H, H1'), 4.05-3.96 (m, 3H, H2',H3',H4'), 3.84 (dd, $J_{5',4'}$=3.0, $J_{5',5''}$=12.0, 1H, H5'), 3.73 (dd, $J_{5'',4'}$=4.6, $J_{5'',5'}$=12.0, 1H, H5").
24. ^{1}H NMR (CD_3OD) data for **14**: δ 8.12-8.06 (m, 2H, H6,H4), 7.31 (m, 1H, H5), 5.26 (d, $J_{1',2'}$=2.8, 1H, H1'), 4.35-4.25 (m, 2H, H2',H3'), 4.04 (m, 1H, H4'), 3.88 (dd, $J_{5',4'}$=2.6, $J_{5',5''}$=11.8, 1H, H5'), 3.68 (dd, $J_{5'',4'}$=4.6, $J_{5'',5'}$=11.8, 1H, H5").
25. ^{1}H NMR ($(CD_3)_2CO$ data for **15**: δ 8.10 (dd, $J_{6,5}$=5.2, $J_{6,4}$=1.6, 1H, H6), 7.80 (dd, $J_{4,5}$=7.3, $J_{4,6}$=1.6, 1H, H4,), 6.95 (dd, $J_{5,6}$=5.2, $J_{5,4}$=7.3, 1H, H5), 5.59 (d, $J_{1',2'}$=6.0, 1H, H1'), 5.36 (d, $J_{OH,3'}$=7.2, 1H, OH3'), 5.02 (dd, $J_{2',1'}$=6.0, $J_{2',3'}$=5.2, 1H, H2'), 4.64 (t, $J_{5',OH}$=5.4, 1H, OH5'), 3.97 (m, 1H, H3'), 3.62 (dq, $J_{5',4'}$=2.2, $J_{5',5''}$=12.2, $J_{5',OH}$=5.4, 1H, H5'), 3.39 (m, 1H, H5"), 3.22 (m, 1H, H4').

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