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Synthesis of Orotidine by Intramolecular Nucleosidation

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An intramolecular nucleosidation approach provides easy access to orotidine in high yields. Notably, orotate itself is used as a leaving group at the anomeric position. This method has the potential for facile access to derivatives of orotidine of therapeutic interest, with implications for prebiotic formation of nucleosides.

Orotidine (as the 5'-monophosphate) plays a crucial role in contemporary biology. Extant de novo biosynthetic pathway uses orotidine 5'-monophosphate to synthesize the canonical pyrimidine nucleotides in RNA and DNA. In this context, orotide is the only nucleotide that is synthesized through a ‘direct intermolecular nucleosidation’ step, with an attack of the fully-preformed nucleobase (orotic acid) on the activated 5-phosphoribosyl-diphosphate as opposed to the purine nucleotides whose heterocyclic rings are constructed stepwise on the sugar. However, for the organic synthesis of canonical nucleosides the situation is quite different: while the purine and the pyrimidine nucleosides are easily synthesized by ‘direct intermolecular nucleosidation’ of the sugar derivative with the nucleobase, synthesis of orotidine by ‘direct intermolecular nucleosidation’ with orotic acid, has been the most inefficient of all the canonical nucleosides. Herein, we report on an alternative ‘intramolecular’ route that overcomes this ‘nucleosidation hurdle’ and provides a straightforward synthesis of orotidine and opens the door for easy access to its derivatives for medicinal and biological applications. Importantly, this approach also has implications for solving the ‘nucleosidation problem’ in the context of prebiotic chemistry.

The synthesis of nucleosides is well established based on a ‘direct nucleosidation’ approach following the Vorbrüggen-Hilbert-Johnson (VHJ) method. However, application of the VHJ methodology to the synthesis of orotidine has been inefficient and not successful. The yields of the desired N(1)-nucleoside are low, with the undesired N(3)-regioisomer dominating. Therefore, alternative approaches to synthesize orotidine derivatives have been developed, and almost all of them start from protected uridine derivatives. However, access to orotidine and its derivatives have not been straightforward and have constrained its wide application. Easy access to orotidine and its derivatives would be extremely useful for therapeutic investigations.

As part of an investigation of orotidine containing oligonucleotides, we needed to have an easy access to large quantities of orotidine I. Given the prohibitive cost of obtaining orotidine, we re-examined the difficulties associated with the direct nucleosidation approach for orotidine; the presence of the C(6)-carboxylic acid group on orotic acid has been singled out as a likely culprit for the problems associated with the intermolecular nucleosidation approach. We considered whether the C(6)-carboxylic acid could be taken advantage of, by making suitable orotic acid derivatives of ribose, which now may be in a position to undergo intramolecular nucleosidation and overcome the troubles faced in the direct, intermolecular VHJ nucleosidation methodology (scheme1).

This intramolecular approach involves three key steps: (1) The formation of an ester bond: between the 5-OH group of a suitably protected ribofuranosyl derivative with an activated C(6)COOH group of orotic acid. (2) Intramolecular nucleosidation: ester derivative B is now poised to react intramolecularly to deliver the orotic acid moiety via the correct
regiochemistry (N(1)-position) to the anomeric center to give C, since reaction at the N(3)-position is sterically not possible. Additionally, configuration at the 4'-position should ensure the formation of the desired β-anomer.\(^\text{10}\) (3) Hydrolytic ring opening: attack at the lactone carbonyl (C(6)-carboxyl and 5'-O-position) of C with various nucleophiles should afford the orotidine derivatives (Scheme 1).

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\text{Scheme 2 Attempted intramolecular nucleosidation using 1',2',3'-tri-O-benzyl-5'-orotyl-D-ribofuranose (3). Conditions: (a) EEDQ, N-methylmorpholine, DMF, 50 °C (63% based on the recovery of unreacted starting material). EEDQ = 1-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline.}
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The synthesis began with orotic acid to afford 5'-O-orotyl-D-ribofuranose (5a) in 63% yield. However, attempts to produce the desired lactone by intramolecular nucleosidation from 2 under various conditions were unsuccessful (Scheme 2). The “floppiness” (north-south conformations) in the furanose ring of 3 coupled with the necessity of a higher energy “cis-ester” conformation to orient the nucleobase could be thwarting the intramolecular nucleosidation. If the ribofuranose skeleton can be ‘rigidified’ by 2',3'-cyclic ketal, it could facilitate the conformation necessary for the intramolecular nucleosidation.

We prepared three derivatives of 1-acyl-D-ribofuranose, 4a, 4b and 4c (scheme 3)\(^\text{11,12}\) in order (1) to probe the propensity of the leaving group for intramolecular nucleosidation and (2) to check the interference from a competing transacylation (from the 1- to the 5- position) during dehydration of the 5-O-silyl group, a process that would block the 5-O-position and prevent the formation of required 5’-O-orotate ester bond (such as B in scheme 1).\(^\text{13,14,15}\) The desired ribosides 5b and 5c were formed using Et3N·3HF (with 14% of benzoyl and 0% acetyl migration by-products) respectively; 5a was synthesized using TBAF with no by-product. The exact position of acyl groups in compounds 5a–5c was confirmed by HMBC NMR spectroscopy (Fig. S6, S11 and S16, supplemental information). The desired orotate ester derivatives, 6a–6c were synthesized in high yields. The position of the orotate ester bond in 6a, 6b and 6c was confirmed by the correlation between H-C(5') sugar proton and the C(6) carbonyl carbon of the orotate group by HMBC NMR spectroscopy (Fig. S21, S27 and S31, supplemental information). We further streamlined the synthetic process: starting from D-ribose in five-steps without any purification of intermediates, ribofuranoside 6c was produced in 63% of overall yield (Scheme 3). Additionally we shortened the synthetic route, by preparing the ketal 8 directly from ribose,\(^\text{11}\) which we could selectively esterify with orotic acid at the 5'-O-position to afford 9 followed by acetylation at the anomeric position to produce 6e in just 3 steps starting from D-ribose. Intramolecular nucleosidation of esters 6a–6c under VHI conditions led to successful formation of 5'-O-orotate 7, whose structure was confirmed by x-ray (Scheme 3).\(^\text{16}\) The acetyl derivative 6e turned out to be the most efficient substrate (76%) for intramolecular nucleosidation, followed by the benzoyl derivative 6b (51%) and the pivaloyl derivative 6d (29%).\(^\text{15}\) Methanolysis of 7 and subsequent removal of the isopropylidene group yielded orotidine methyl ester 10 in 73% over two-steps. Thus, methyl orotidine 10 was synthesized in overall 35% yield starting from ribose in six steps. Hydrolysis of 10 afforded orotidine 1;\(^\text{11}\) this material was found to be identical to authentic β-orotidine in all respects (Fig. S43, S44 and S64, supplemental information).

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\text{Scheme 3 Synthesis of Orotidine. (a) TBAF, THF, RT (92% for 5a); Et,N 3HF, THF, 0 °C → RT (73% for 5b and 97% for 5c). (b) orotic acid, CDI, pyridinium chloride, DMF (90% for 5a, 94% for 6b and 98% for 6c, 68% for 9). (c) BSA, CH3CN, RT, 1h followed by TMSOTf (29% from 6a, 51% from 6b, 76% from 6c). (d) 0.2 eq NaOMe, MeOH, RT (91%). (e) aq. 60% TFA, 0 °C → RT (80%). (f) NaOH, CH3CN/H2O (1:1, v/v) followed by IR-120(H+) (quantitative). (g) H2SO4, acetone, RT (97%). (h) Ac2O, DMAP, pyridine, RT (64%), CDI = 1,1' carbonyldimidazole.}
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In order to investigate whether direct intermolecular glycosylation can be forced at the N(1) position of orotate, N(3)-benzoyl protected methyl orotate 11\(^\text{8}\) was prepared and...
reacted with 1-O-acetyl-2,3,5-tri-O-benzoyl β-D-ribofuranose (Scheme 4). The $^1$H NMR of isolated product (12, 72%) indicated the loss of the N(3)-benzoyl group. In order to determine the position of nucleosidation, product 12 was converted to the free nucleoside 13 and compared with synthesized orotidine methyl ester 10 (Fig. S57, supplemental information). Comparisons were also made with orotidine 1, and product 14 that was obtained by hydrolysis of 13. The dissimilarity of the spectral data (Fig. S61 and S64 supplemental information) indicated that the nucleosidation product 12 from this direct glycosylation process is the N(3)-β-riboside (perbenzoylated isoorotidine methyl ester). This was confirmed by NMR (NOESY) and by comparison with data for the authentic N(3)-β-isoorotidine compound available from the literature. The failure of 11 to give the desired N(1)-riboside, indicates the lability of the N(3)-benzoyl group of 11 under the reaction conditions to give the unprotected methyl(orotate) 6, which then reacts to afford the N(3)-isoorotidine derivative 12.

Finally, we considered the possibility that the nucleobase (orotate) itself could be used as a leaving group at the anomeric position of ribofuranose 8 (Scheme 5). This would exploit the uniqueness of orotate among the canonical nucleobases (i.e. having a 6-carboxyl group) and convert its perceived drawback (steric hindrance offered for the intermolecular nucleosidation) into an asset (ester bond formation at the anomeric position) that could be activated towards intramolecular nucleosidation. Also, this approach has the potential, when translated under prebiotic conditions, to overcome the longstanding “pyrimidine nucleosidation problem” when starting from orotate and ribose (instead of uracil).

We have demonstrated a short and concise route to orotidine in high yields, one that is compatible with conventional synthetic methodologies. This approach is general and not limited to the ribofuranosyl skeleton but can be extended to other sugars as well. Moreover, the bicyclo ester intermediate 7 (and its corresponding sugar variations) can be considered a central target from which diversification to many orotic acid-containing derivatives (Scheme 6) is possible. This approach has the potential to provide quick access to a diverse library of compounds that may be useful in applications targeting the de novo pyrimidine biosynthetic pathway and for developing broad-spectrum antiviral, anticancer and antimalarial therapeutics.

Notes and references
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† This is highlighted by the fact that orotidine is extremely expensive: 5 mg of Orotidine is $330.50 (Sigma-Aldrich) and US $525 (Carbosynth).

‡ In an effort to increase the nucleosidation yield, other reaction conditions e.g. solvent (1,2-dichloroethane, 1,4-dioxane and ethylene glycol dimethyl ether), Lewis acid (SnCl$_4$, TMSI and BF$_3$·OEt$_2$) and higher temperature (80 °C) were also explored.

¶ In the case of 6b the product distribution was found to be concentration dependent: the optimal concentration was found to be 10 mM of 6b. At 20 mM concentration, 6% of a dimer of 6b (formed by intermolecular nucleosidation) along with 20% of expected product 7 was observed.

© Alternatively, treatment of 7 with aq. NaOH followed by aq. HCl yielded orotidine 1 in 35% yield. However, reaction of 7 with only aq. HCl at 50 °C did not produce orotidine.

Scheme 6 The bicyclo-ester approach has the potential to generate structurally (and functionally) diverse library of orotate derivatives.

Conclusions
The position of the benzoyl group at the N(3)-position in 11 was confirmed by UV spectral-comparison with the corresponding N(1)-benzoyl and N(1), N(3)-dibenzyol derivatives (see Fig. S1 in supplementary information).

When 11 alone was subjected to the reaction conditions (acetonitrile, TMSOTf) the N(3)-benzoyl group of 11 was lost (as confirmed by TLC analysis).

This approach can be extended for other carboxyl-containing pyrimidines and purines. Work in this direction is currently underway in our laboratory.

Electronic Supplementary Information (ESI) available: Experimental details and spectral data for compounds are provided in the supplementary information. See DOI: 10.1039/e000000x/


13 However, the acetyl migration can be suppressed by addition of CAN: O.P. Chevallier, M.E. Migaud, *Beil. J. Org. Chem.* 2006, 2.


16 Crystallographic data for lactone 7 has been deposited with the Cambridge Crystallographic Data Center with CCDC No. 1034691. This lactone has been prepared by a different method: M. P. Groziak, R. Lin, *Tetrahedron* 2000, 56, 9885-9893.

17 The reaction of 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose with methyl orotate (N(3) position was not protected) affords only the isoosorotidine (N(3)-ribose) derivative in 67% yield; see I. A. Mikhailopulou, E.N. Kalinichenko, A.A. Akhrem, *J. Carbohydr. Nucleosid. Nucleotid.* 1981, 8, 227-260.

