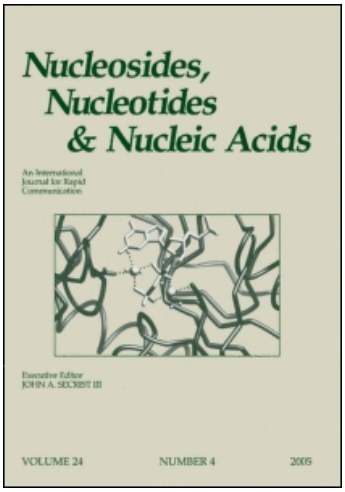

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Synthesis of 2'-Deuterio and 3'-Deuterio Cytidine 5'-Diphosphate

Jessica Sandbrink[a]; Roger Strömberg[ab]
[a] Division of Organic and Bioorganic Chemistry, MBB Scheele Laboratory, Karolinska Institute, Stockholm, Sweden [b] MBB-Division of Organic and Bioorganic Chemistry, Karolinska Institutet, Stockholm, Sweden

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Synthesis of 2′-Deuterio and 3′-Deuterio Cytidine 5′-Diphosphate

Jessica Sandbrink and Roger Strömberg*

Division of Organic and Bioorganic Chemistry, MBB Scheele Laboratory, Karolinska Institute, Stockholm, Sweden

ABSTRACT

2′-^{2}H- and 3′-^{2}H-CDP were synthesized from 5′-MMT-3′-O-TBDMS and 2′,5′-O-diTBDMS cytidine derivatives, respectively, by oxidation followed by acidic removal of 5′-protection, reduction with [$NaBD(OAc)_3$] and finally displacement of a tosyl group by pyrophosphate.

Key Words: CDP; Deuteriumlabelled; Stereoselectivity; Nucleotides.

Monodeuterated nucleoside diphosphates can be valuable tools in mechanistic studies on the enzyme ribonucleotide reductase[1] (e.g., through EPR analysis where the deuteron gives a unique coupling pattern). Thus, we considered it most significant to devise syntheses of the deuterated cytidine 5′-diphosphates **4** and **8**.

Ketonucleosides of adenosine have earlier been employed to synthesize deuterium labelled analogues from the parent ribonucleoside via an oxidation-reduction sequence, using sodium triacetoxyborodeuteride [$NaBD(OAc)_3$][2] which gives excellent stereoselectivity when the 5′-OH is left unprotected during the reduction step. Hence, this methodology was an obvious choice for introduction

*Correspondence: Roger Strömberg, Karolinska Institutet, MBB–Division of Organic and Bioorganic Chemistry, Scheele Vag 2, Stockholm S-17177, Sweden; Fax: +46 8 311 052; E-mail: Roger.Stromberg@mbb.ki.se.

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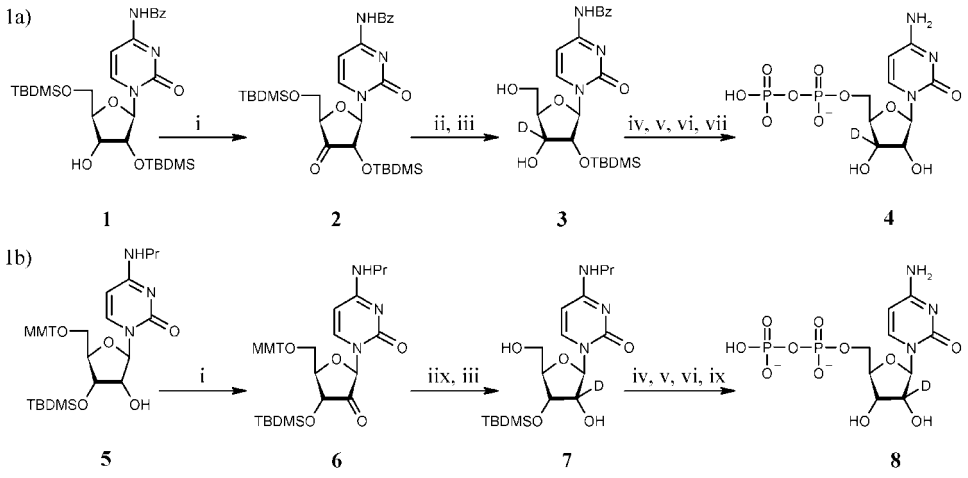
of the deuterium label. The diphosphate moiety was introduced by the known procedure of substituting a tosyl group by pyrophosphate.[3]

The synthesis of the 3′-deuterio cytidine 5′-diphosphate **4** was performed as outlined in Sch. 1a. Oxidation of **1** by CrO_3/acetic anhydride/pyridine gave **2**, 72%. Selective acidic cleavage of the 5′-O-TBDMS by TFA-water 9 : 1[4a] at −15°C prior to reduction afforded **3** in 92% yield (ribo-xylo ratio = 97.5 : 2.5). For incorporation of the diphosphate functionality the 5′-hydroxy group was first converted to the tosylate by treatment with tosyl chloride in MeCN-pyridine (2 : 1). The 2′-O-TBDMS was then cleaved off by means of 0.1 M TBAF in THF. Substitution of the tosyl moiety by tris(tetra-*n*-butylammonium) pyrophosphate followed by ammonia-ethanol (3 : 1) treatment resulted in the desired product **4**, in 25% yield after HPLC purification.

For the synthesis of the 2′-deuterio cytidine 5′-diphosphate **8** a slightly different protection strategy had to be used. This was due to difficulties in removal of the 5′-O-TBDMS without cleaving off the 3′-O-TBDMS to a large extent, when attempting to synthesize the 2′-deuterio isomer by the same route as the 3′-deuterio isomer.

Several different acidic conditions were tested for selective removal of the 5′-O-TBDMS without much success. Various TFA treatments cleaved off both the 5′ and the 3′-silyls while the cleavage attempts with acetic acid[4b] resulted in very slow reactions where several byproducts were formed. The above problem was, however, circumvented by changing the strategy, i.e., to the use of the more acid labile monomethoxytrityl group as protection for the 5′-hydroxyl function. The 2′-deuteriocytidine 5′-diphosphate was then synthesised by the sequence shown in Sch. 1b. Compound **5** was oxidized to ketonucleoside **6**, in 81% yield. Selective removal of the 5′-O-MMT from **6** by p-toluenesulfonic acid in CH_2Cl_2-MeOH and subsequent



Scheme 1. i) CrO_3/acetic anhydride/pyridine, CH_2Cl_2, rt., 2 h; ii) TFA-water 9 : 1–15°C, 0.5 h; iii) $NaBD(OAc)_3$/acetic acid (generated in situ by $NaBD_4$/AcOH), rt. 2 h; iv) TsCl, MeCN-pyridine (2 : 1), rt., 24 h; v) 0.1 M TBAF, THF, rt., 15 min.; vi) 2M $((BuN_4)_3)$ $H_2P_2O_7$, MeCN, rt, 4 days; vii) NH_3-EtOH (3 : 1), rt, 12 h; iix) TsOH 1%, CH_2Cl_2-MeOH 7 : 3 15 min; ix) NH_3-EtOH (3 : 1), rt, 2 h.

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reduction gave the 2′-deuterio isomer **7**, in 90% yield (ribo-arabino ratio = 93.5 : 6.5). Introduction of the diphosphate and removal of the base protection gave the 2′-deuteriocytidine 5′-diphosphate **8** in a yield of 23% after HPLC purification.

Both 2′-deuterio- and 3′-deuteriocytidine 5′-diphosphate could be made successfully and should be useful in future mechanistic studies. In addition it is worth noting that the selective cleavage of 5′-O-TBDMS relative to the secondary O-TBDMS under acidic conditions[4] worked well with the 3′-ketocytidine derivative **2** but the corresponding 2′-ketoderivative is an exception to this selectivity.

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