



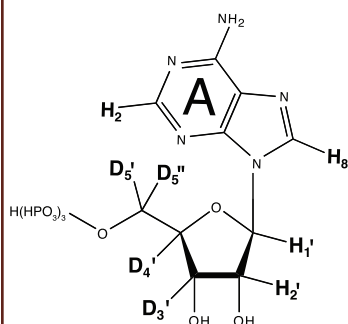
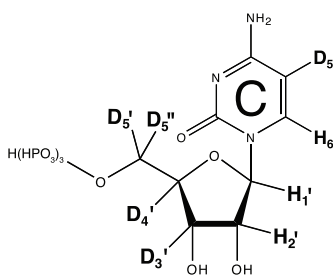
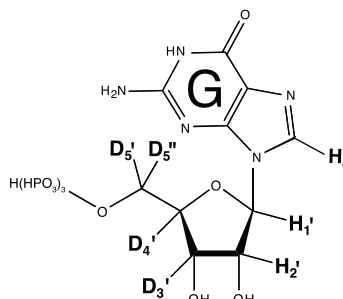
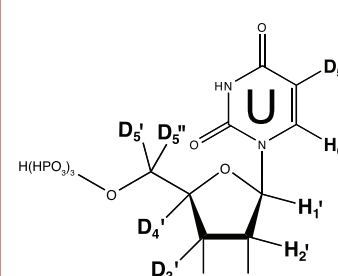
CIL

Cambridge Isotope Laboratories, Inc.
www.isotope.com

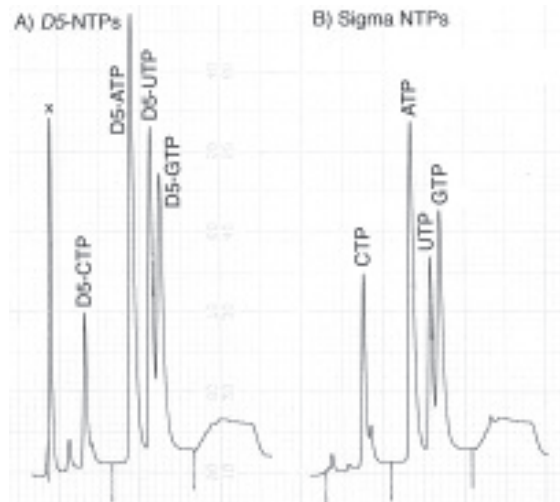
New

RESEARCH PRODUCTS

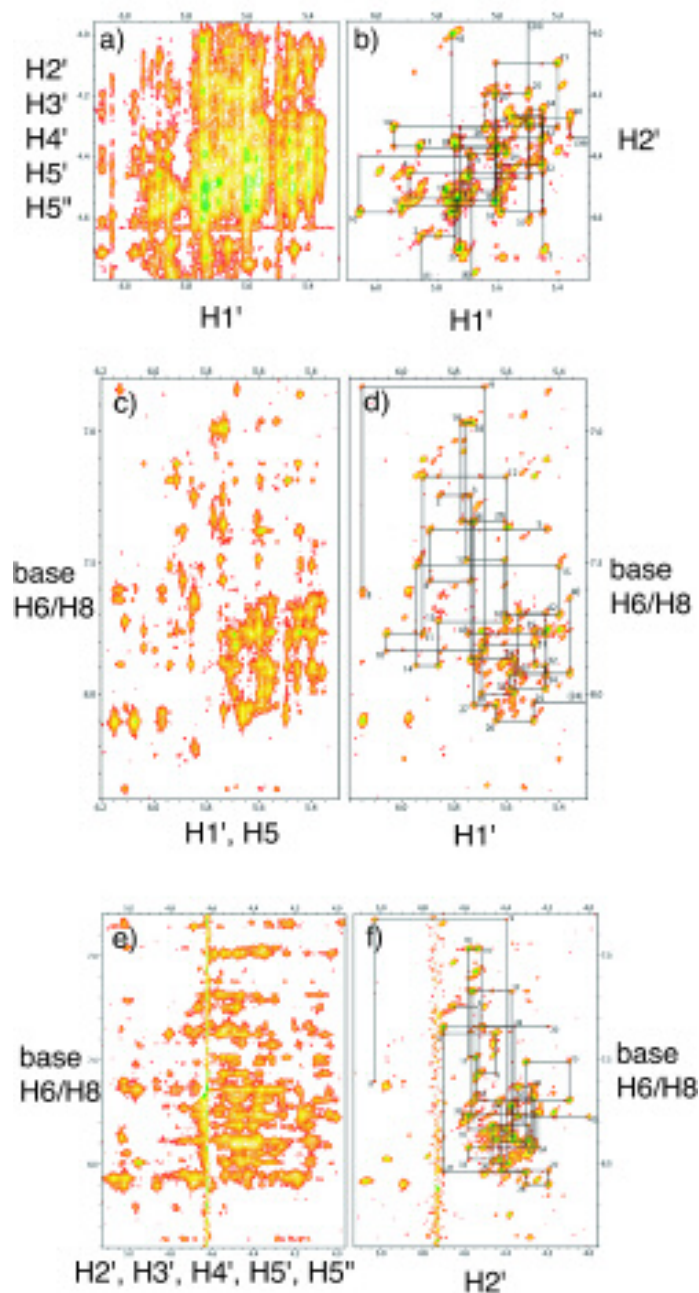
Selectively Deuterated Ribonucleotides

Catalog No. DLM - 7862**\$995/100 mg mixture containing the following ribonucleotide triphosphates:****Adenosine Triphosphate****ribose-3', 4', 5', 5''-d₄, 98%****Cytidine Triphosphate****5-d₁; ribose-3', 4', 5', 5''-d₄, 98%****Guanosine Triphosphate****ribose-3', 4', 5', 5''-d₄, 98%****Uridine Triphosphate****5-d₁; ribose-3', 4', 5', 5''-d₄, 98%**

As recently demonstrated by Dr. Jamie Williamson, of Cassia, LLC, selectively deuterated ribonucleotides can be enzymatically converted into RNA oligomers to produce greatly simplified ¹H NOESY spectra. The spectral overlap for the crowded ribose region is significantly reduced allowing rapid analysis of homonuclear NOESY spectra that greatly facilitates assignments and structure determination of RNAs. CIL is pleased to offer these products, produced by Cassia, LLC, on an exclusive basis. Cassia, LLC was founded by Dr. Lincoln Scott and Dr. Jamie Williamson to produce these materials for the biomolecular NMR community. The D5-labeled nucleotides are supplied as an equimolar mixture of the ammonium salts of ATP, CTP, GTP, and UTP, as shown below. The NTPs are transcription grade and are suitable for RNA synthesis using T7 RNA polymerase.

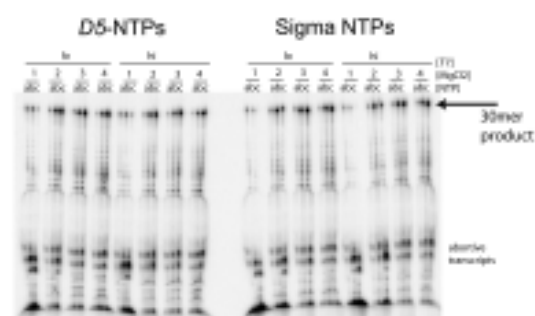


Analytical Ion Exchange HPLC Traces of NTPs. A) D5-NTPs. B) Sigma NTPs. HPLC was carried out on a Vydac anion exchange column using sodium phosphate gradient at pH 2.8. The peak marked with an x is residual chloramphenicol, which has no effect on transcription efficiency.



¹H-¹H-NOESY Spectra of the Tetraloop-Receptor RNA (45 nt dimer; 30 kDa).

Spectra on the left are from unlabeled RNA (a,c,e) and spectra on the right are from D5-labeled RNA (b,d,f). The top two panels (a,b) contain NOEs between the H1' proton and all other ribose protons. The middle two panels (c,d) contain NOEs between the base protons and H1' protons. The bottom two panels (e,f) contain NOEs between the base and other ribose protons. Spectra taken from Davis et al. (2005) were provided courtesy of Prof. Sam Butcher at the University of Wisconsin. The sequential assignment pattern of inter- and intra nucleotide NOEs is shown for the D5-RNA. The advantages of the D5-labeling pattern are evident in these key regions of the spectra.



Polyacrylamide Gel Electrophoresis Analysis of D5-NTP Transcription reactions.

Transcription reactions were carried out under a variety of conditions to determine the optimal transcription conditions for a 30 nucleotide HIV-2 TAR RNA transcript. D5-NTPs were directly compared to unlabeled Sigma NTPs using T7 RNA polymerase and a synthetic DNA template. The reactions were run for 3 hours at 37°C using a matrix of conditions where the concentrations of the reagents were varied: [T7 RNA polymerase] hi=175 units, lo= 25 units. [MgCl₂] 1=8 mM, 2=20 mM, 3=34 mM, 4=48 mM. [NTP] a=4 mM, b=14 mM, c=24 mM. Products were labeled by inclusion of trace ³²P-labeled GTP, and were analyzed on a 20% (29:1) denaturing polyacrylamide gel. The D5-NTPs are equally as active as commercial Sigma NTPs.

Catalog No. DLM - 7862
\$995/100 mg mixture



Cambridge Isotope Laboratories, Inc.
 50 Frontage Road Andover, MA 01810-5413

direct: 978.749.8000
 toll-free: 800.322.1174
 fax: 978.749.2768
 web: www.isotope.com



Produced by Cassia, LLC, exclusively for CIL