ON THE ORIGINS OF LIFE: THE PREBIOTIC SYNTHESIS OF CARBOHYDRATES, PRIMITIVE CATALYTIC CYCLES & ENGINEERING THE GENETIC LEXICON

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2010

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To be is to do. -Socrates
To do is to be. -Sartre
Do be do be do. -Sinatra

To my dear parents Herath and Nandani

"I never had problems with my fellow scientists.
Scientists are a friendly, atheistic, hard-working,
beer-drinking lot whose minds are preoccupied with sex,
chess and baseball when they are not preoccupied with science."

-Piscine Molitor Patel in Life of Pi by Yann Martel

ACKNOWLEDGMENTS

I thank my Advisor, Dr. Steven A. Benner, for the trust he has shown me throughout the years. I also thank the members of my committee, Dr. Tom Lyons, Dr. Nigel Richards, Dr. Aaron Aponick and Dr. David Hahn for their support, input, helpful discussions and above all, patience.

I also thank my friends and mentors Photon Rao, Abhijit Roychowdhury, Harch Ooi, Sangita Ghosh, Alonso Ricardo, Matt Carrigan, A. Michael Sismour, Cynthia Hendrickson and Theodore Martinot who helped me through the years. Photon, Abhijit and Theo were instrumental in teaching me the techniques and the art of organic chemistry, while Cynthia taught me everything I needed to know about molecular biology. I am deeply indebted to them all.

I also thank my parents, Herath and Nandani Illangkoon, my aunt and uncle, Hema and Chandra Wijeratne, and finally my close friends John Brennan, Amanda Rowe, Cara Lubner, Kathryn Ranhorn, Jonny Gerstsen, Christos Lampropoulos, Roberto Laos, Nellie Eshleman, Ahu Demir, Sarah Luciano, Tammy Low and Joanna Fried, for their endless support and love. Last but not least I would like to thank Romaine Hughes. Without her, our lives would have simply been chaos.

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By

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May 2010

Chair: Steven A. Benner
Major: Chemistry

Here we report experiments that establish xylulose and threose as stable products from formaldehyde and lower carbohydrates, such as glycolaldehyde and dihydroxyacetone, in the presence of borate. Xylulose is one of two diastereomeric five carbon carbohydrates known as 2-pentuloses, and threose is one of two diastereomeric four carbon tetroses. Arguments are presented that formaldehyde, glycolaldehyde and dihydroxyacetone are all likely to have been available on early Earth and therefore may have supported processes that created carbohydrates that could have been part of the first genetic material. Borate, weathered from igneous rock, is likely to have been available on early Earth. Borate may have also been available, as evaporite minerals, which result from evaporation of superfial bodies of water (e.g. colemanite, ulexite, and kernite); all of these minerals are found today in, for example, Death Valley. 1,2,4,5-Tetrahydroxy-3-pentanone was synthesized and shown to be an intermediate in the formation of 2-pentuloses, including xylulose and ribulose. Rate constants for the enolization of glycolaldehyde, glyceraldehyde, erythrulose, erythrose, and dihydroxyacetone, which are precursors, intermediates, and potential products in this putative prebiotic synthesis of carbohydrates, were estimated by NMR in the presence and absence of borate. Borate had only a modest impact on those rate constants for enolization of linear carbohydrates that presented 1,2-

diol units, but had a dramatic impact on the rate constants for enolization of carbohydrates that could present the 1,2-diol in a cyclic form. In a separate line of work, experiments are directed towards the synthesis of a modified nucleoside heterocycle with an amine linker at the C5 position of cytidine. Molecular biology using the modified cytidine resulted in a successful PCR amplification.

CHAPTER 1
PREBIOTIC CHEMISTRY

Introduction

All life, as we know it, contains genetic material in the form of nucleic acids, which are DNA and RNA. The field that considers the origin of life on Earth, therefore, must explain how nucleic acids arose at some point in the historical past, either on Earth or elsewhere. The field of astrobiology studies the origins, evolution and distribution of life on earth and in the universe.

The study of the origins of life started long ago, though certain discoveries may not have been evident as important at the time they were made. One of the first reports is the polymerization of HCN, reported by Proust in 1806[1, 2]. Further analysis of this polymer showed the presence of adenine, one of four RNA heterocycles. A recent investigation by Minard[3] found the spontaneous polymerization products of HCN which gave a black polyimine chain (Figure 1-1). This shows the formation of polymers from a common prebiotic precursor, and may form adenine.

Soon after Proust's reactions came the eye-opening synthesis of urea by Wöhler[4]. A pioneer of organic chemistry, Wohler was attempting to form ammonium cyanate, and instead formed urea (Figure 1-2). Prior to this event, organic compounds were thought to have been created only by living organisms. This discovery brought about a paradigm shift where once vitalism ruled. Wöhler demonstrated that organic molecules could be formed from 'inorganic' materials. A little known fact, Wöhler also isolated many elements including aluminium, yttrium, beryllium, and titanium.

Strecker's synthesis of alanine from HCN followed in 1850[5, 6]. Alanine was later discovered to be part of natural proteins. At this time, the field began to make real progress

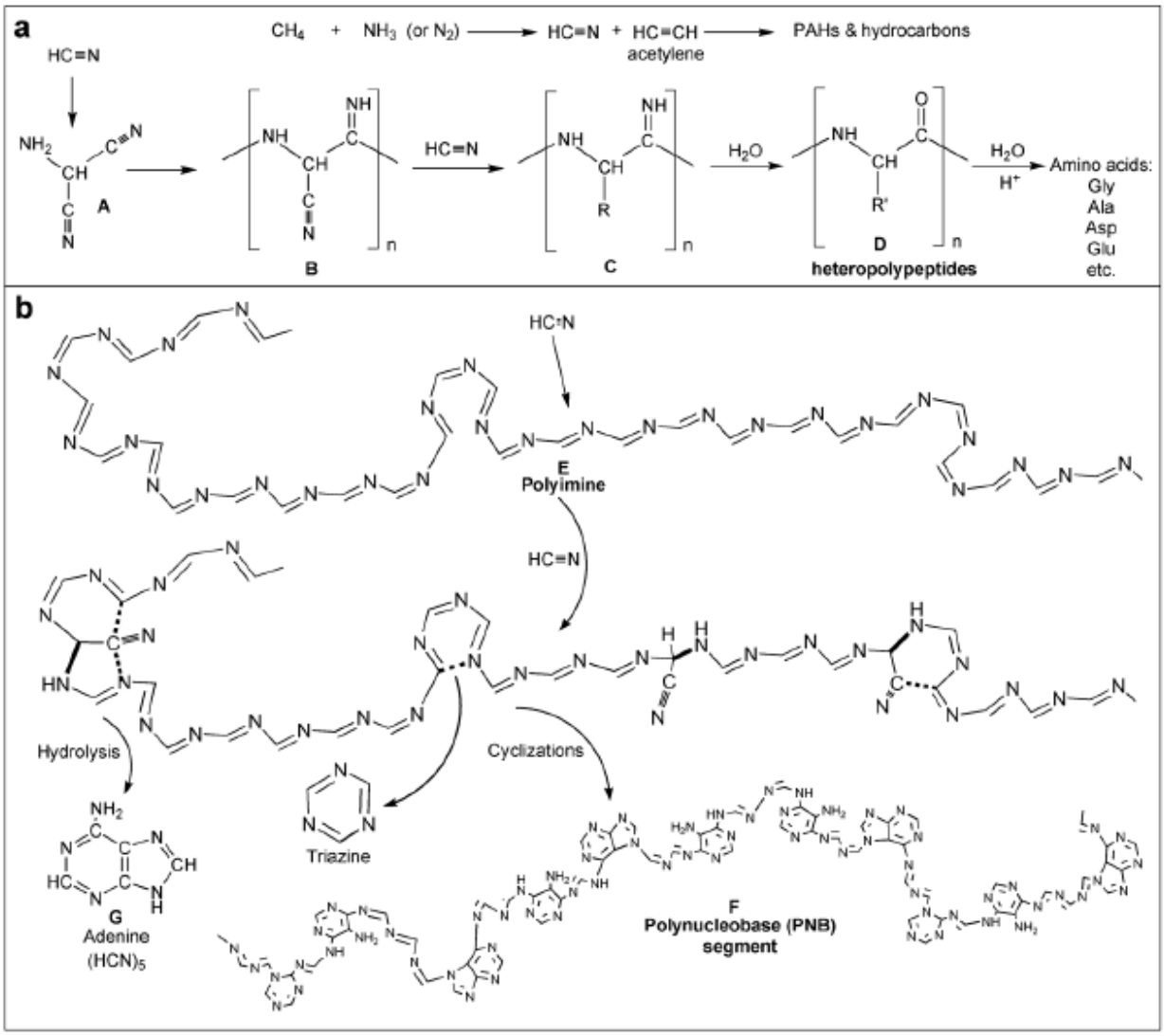


Figure 1-1. Pathways and structures proposed for HCN polymerization. A sample of HCN polymer may possess any or all of these structures including hybrids (multimers).7

Expected Product

$$AgOCN + {}^{+}NH_4\,{}^{-}Cl \xrightarrow{\Delta} {}^{+}NH_4\,{}^{-}OCN + AgCl$$

Reaction Product

$$AgOCN + {}^{+}NH_4\,{}^{-}Cl \xrightarrow{\Delta} H_2N\!-\!\overset{\overset{O}{\|}}{C}\!-\!NH_2$$

Figure 1-2. The Wöhler synthesis of urea.

towards a chemical understanding of life. In 1861, Butlerov reported his now famous formose experiment, where formaldehyde (HCHO) in hot (70 ºC or higher) solutions of calcium hydroxide (at pH 12.5) formed a sweet sugary substance[8]. In an incompletely understood first step, two molecules of HCHO are presumded to join to form a molecule of glycolaldehyde. Then under a proposal by Breslow, this glycolaldehyde then initiates a series of reaction cycles that, over time, fix more HCHO to give higher carbohydrates[9]. This is followed by a long induction period during which HCHO is consumed slowly to generate C4, C5, C6, C7, and some C8 and C9 carbohydrates. Then suddenly, the remaining HCHO is rapidly consumed, and the mixture begins to yellow[10, 11]. A side reaction in this experiment is the disproportionation of HCHO to form methanol and formic acid in a process now known as the Cannizaro reaction (Figure 1-3).

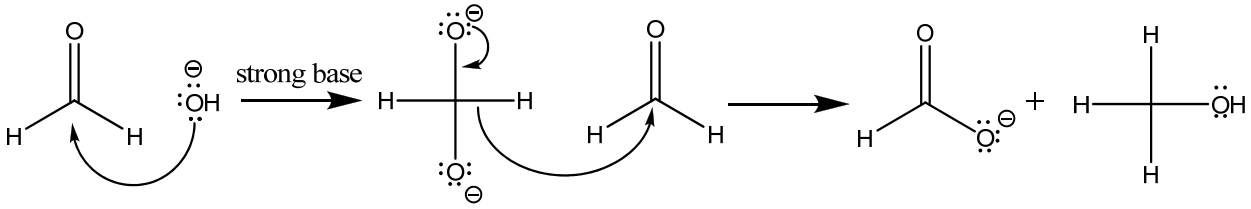


Figure 1-3. The Cannizaro reaction.

In 1913, Löb formed glycine after passing an electric discharge through a mixture of carbon monoxide, ammonia and water vapor[12]. In the same year, Baudisch prepared uracil, a pyrimidine heterocycle found in RNA, by treating urea and malic acid with fuming sulfuric acid in a process less likely to be considered prebiotic on an early Earth but not entirely discounted.

Modern theories on the origins of life came independently from Oparin in 1924 and Haldane in 1929[13]. Although Haldane's work was published before Oparin's Russian manuscript was translated into English, both suggested that a reducing primitive atmosphere coupled with cosmic rays, ultra-violet light and lightning could synthesize a variety of organic compounds. Haldane coined the term "prebiotic soup" in reference to this mixture. This process was shown

experimentally in 1953 with Stanley Miller's famous experiment combining methane, carbon dioxide and electrical discharge simulating lighting. Miller's experiment yielded amino acids, then widely accepted to be key building blocks for life[14]. There still remains a controversy among a small contingent of origins researchers who question the novelty of Miller's experiment, as Löb had conducted similar experiments years before Miller's birth. Also in 1953, Watson and Crick proposed a structure for DNA[15-17] and Frederick Sanger sequenced the amino acids of the protein insulin. Perhaps 1953 should be considered the dawn of the biotechnological era, as scientists made groundbreaking strides towards understanding chemical fundamentals of life, as we know it.

Today, the dominant model for the first form of life holds that the first nucleic acid to arise was RNA (not DNA)[18]. Under this model, an early form of life on Earth used RNA to serve both genetic and catalytic roles (the "RNA world" hypothesis). RNA emerged from an abiotic environment to support the first self-sustaining chemical system capable of Darwinian evolution (the "RNA-first" hypothesis). The central challenge which has long faced this model is the apparent chemical complexity of RNA relative to the organic molecules that were likely to have been present in the primordial soup.

More recently, the RNA first hypothesis has been challenged by some who point out that certain components of RNA are not easily formed under any conditions probable for an early Earth. The belief is that many of the intermediates, including RNA itself, are sufficiently unstable and would not have survived long on an early Earth, including the conditions where they were formed[19]. For example, adenine (a nucleobase within RNA) is formed from hydrogen cyanide polymerization[1] and is well known to be formed abiologically. The molecule hydrolyzes in water to give inosine and the carbohydrate ribose, the "R" in RNA is also unstable.

Current origins of life theory on a larger scale propose that a pool of prebiotically formed, catalytic, self-replicating RNA molecules (known as ribozymes) was subject to a selection pressure where the most 'fit' molecules came to dominate the pool. This genetic material is subject to replication errors, which create slight variations from parent to offspring. Over time, this variation results in a progeny with a higher fitness relative to preceding generations. Given this model, the primary interest in origins of life research is to determine how the sugar and heterocycle components of the first molecule of RNA came to be.

Our hypothesis is that sugars, specifically carbohydrates involved in primitive and modern genetic systems, formed prebiotically on an early earth. Further, we propose that borate played a central role in the reactivity and stability of the carbohydrate products.

Early Earth

The absence of a community-wide agreement concerning a "standard of proof" complicates studies regarding prebiotic relevance. When proposing a solution to this historical problem, a geological hypothesis describing the environment where the proposed prebiotic chemistry occurred is necessary.

Though early models of Earth range widely in their proposed temperature and mineralogy, the Hadean Earth almost certainly had igneous lava flows that carried olivine and tourmaline minerals[20]. In addition, pallasites (meteorites that carry olivines) and chondrites (meteorites that carry organic species; for example, the Murchison meteorite) were almost certainly delivering both to early Earth. Temperature ranges on an early Earth may have varied from 0 ºC to the temperature of molten iron (~1538 ºC)[21]. This provides a vast chemical reaction landscape of possible conditions to be explored.

Prebiotic Organic Compounds

Microwave spectroscopy of interstellar gas clouds confirm the presence of organics including glycolaldehyde, formaldehyde and more recently dihydroxyacetone.[3] Meteorite bombardment would have delivered these molecules to an early Earth. Reactions of glycolaldehyde and formaldehyde on a primitive Earth would provide a source for glyceraldehyde and erythrulose (in an aldol reaction of glycolaldehyde and formaldehyde). Carbonaceous chondrites have been found to contain a variety of organic compounds, including amino acids.[22] It is also well known that atmospheric electrical discharge yields HCHO and H_2O_2, while meteorites deliver abundant (and stable) glycerol[23]. The availability of Fe(II) minerals and H_2O_2 give rise to dihydroxyacetone from glycerol using Fenton chemistry.[24, 25] These factors combined provide a rich pool of chemicals to consider when hypothesizing processes that may have occurred in the primordial soup.

Primordial Soup & The RNA World

The "RNA first" hypothesis is widely held as offering the most likely scenario for the emergence of life as we know it. In 1989, Tom Cech shared the Nobel Prize in chemistry with Sydney Altman for their discovery of the catalytic properties of RNA molecules[26-28]. Catalytic RNA molecules are the key to the success of a RNA world. The RNA world theory presupposes the existence of a pre-biotic soup as the originator of the first 'library of life'. To test this hypothesis, laboratories seek a series of stepwise reactions providing support for the abiotic synthesis of carbohydrates in the prebiotic soup. Of primary interest are sugars implicated in both modern and primitive genetic information systems.

The central argument against the RNA world arising from the primordial soup involves the formation of problematic tar, which is a nearly useless byproduct from highly reactive species. The propensity of organic precursors to life to form tar is an indication of the functionality and

reactivity of these building blocks. Apart from making a sticky mess, the tar prevents in depth product analysis and leads to criticism of the viability of carbohydrates in the primordial soup[19].

Nowhere is this criticism more valid than when directed to the formose process. For example, an analysis performed by Ricardo demonstrated the consumption of formaldehyde to give brown material (Figure 1-4)[10, 11]. Figure 1-4 shows the acceleration of formaldehyde consumption with increased concentrations of HCHO. In this rapid loss of HCHO, the mixture first yellowed, then turned brown, forming mixtures that some suggest precludes the assumption that the formose process was important on early Earth.

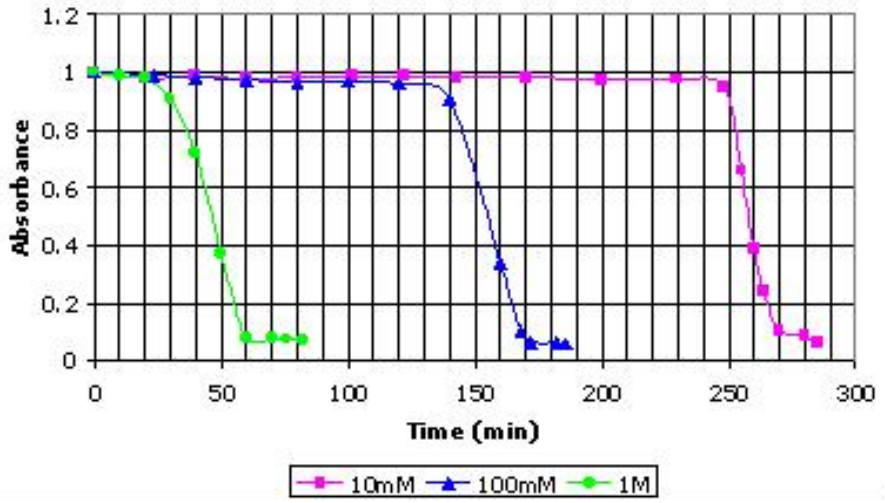


Figure 1-4. Formaldehyde concentration dependence in the progress of the formose reaction.[10,11] Work by Ricardo shows the rapid depletion of formaldehyde at increasing concentrations during the formose reaction.

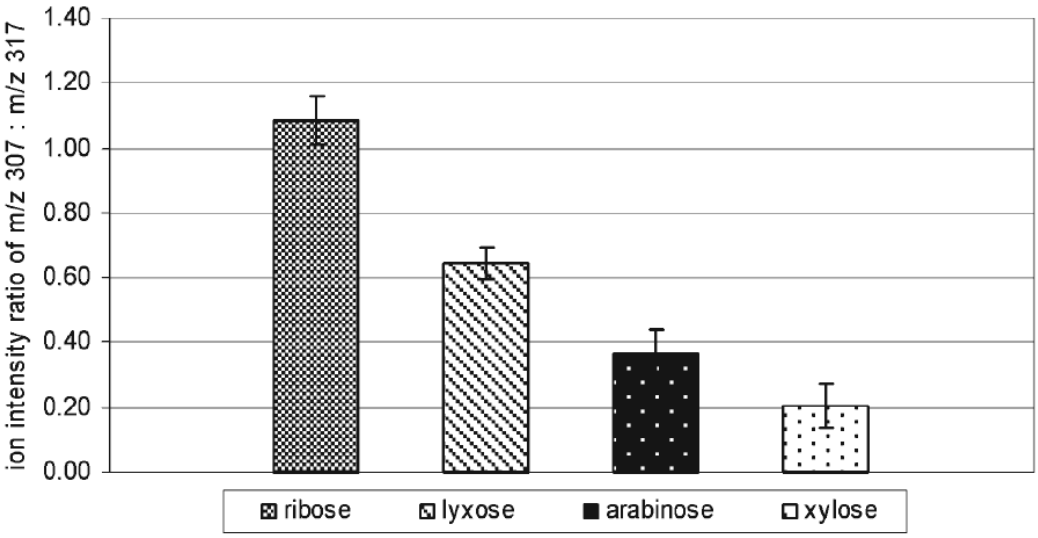


Figure 1-5. The affinity of borate to various carbohydrates, ribose is highest among the aldopentoses[10, 11, 29].

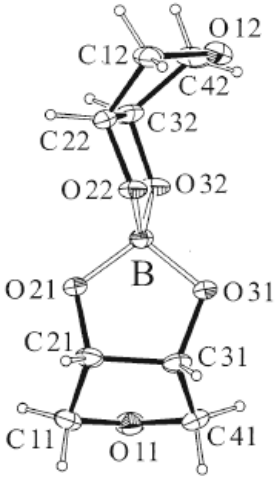


Figure 1-6. Crystal structure of borate complexed with 1,4-anhydroerythritol.[30]

In a paper published in 1995, Stanley Miller states that the instability of ribose precludes it from the primordial soup. However, in 2004, Ricardo et al. postulated that if a species could be found to bind the cis-diols of the formed sugars, this would in reduce their reactivity through the removal of the reacting carbonyl group, thus resulting in a carbohydrate which did not undergo uncontrolled further reaction to form tar[10]. This was achieved through the use of borate. Borate

has the ability to form a complex with cis-diols[30], a motif ubiquitous in sugars (Figures 1-5 & 1-6). Additionally, the affinity of borate complexes varies among pentoses (Figure 1-5)[10, 11, 29].

In their first experiments, Ricardo et al. acquired borate from colemanite, a calcium borate mineral found in the arid desert climates of today. They reported that borate did in fact complex with ribose, arabinose and other pentoses to form a stable complex. Furthermore, this stabilization was effective under conditions where the pentoses were formed from the C3 carbohydrate glyceraldehyde and the C2 glycolaldehyde. This dissertation carries this work further.

CHAPTER 2
THE PREBIOTIC SYNTHESIS OF SUGARS AND PRIMITIVE CATALYTIC CYCLES

When approaching the abiotic synthesis of genetically relevant carbohydrates, the challenge lies within the analysis and structure proof of the formed compounds. For this reason, reactions of one, two and three carbon building blocks in the presence of borate were examined separately.

The reaction of dihydroxyacetone and formaldehyde was studied via the method as outlined in the experimental section (3 carbon unit, dihydroxyacetone + 1 carbon unit, formaldehyde → 4 carbon unit, erythrulose). Next, the reaction between erythrulose and formaldehyde (4 carbon unit, erythrulose + 1 carbon unit, formaldehyde → 5 carbon unit, pentulose) was studied. In order to identify the reaction products, a sample of authentic straight chain pentulose, 1,2,4,5-tetrahydroxy-3-pentulose was synthetically prepared. In its ^{13}C NMR spectrum, major peaks superimposed with those from the prebiotic reaction of erythrulose and formaldehyde, confirming the formation of the straight chain sugar, along with other species (Figure 2-1).

In an effort to look solely at newly formed products, ^{13}C labeled formaldehyde was used in reactions with dihydroxyacetone and erythrulose respectively. These reactions were performed in a standard Carbonate –Boric Acid (CBA) buffer Na_2CO_3 (1.18g, 11.1 mmol, Fisher) and H_3BO_3 (0.172g, 2.78 mmol, Fisher) was dissolved in D_2O (10 mL, Cambridge Isotope Laboratories) resulting in a solution with a measured pH of 10.4 and a final boron concentration of 278 mM. The initial primary consumption of $H^{13}CHO$ to yield $R–^{13}CH_2OH$ products resonating in the 60-62 ppm range was observed. A peak at 72.4 ppm was observed to rise over time (Figure 2-17 & Experimental section A) in these reactions. This peak continued to intensify as a manifold of peaks at 60-62 ppm continued to decrease, signaling a shift in the product ratios.

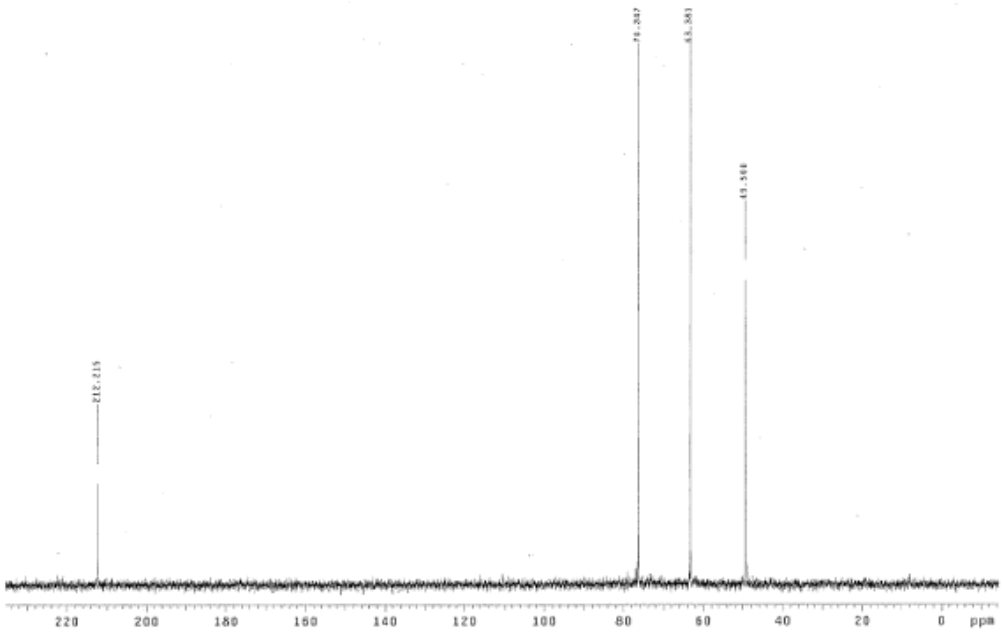


Figure 2-1. ^{13}C Spectrum of synthetic 1,2,4,5-tetrahydroxy-3-pentanone standardized to methanol at 49.5 ppm. Spectrum file: 1,2,3,4-pentan-3-one.pdf

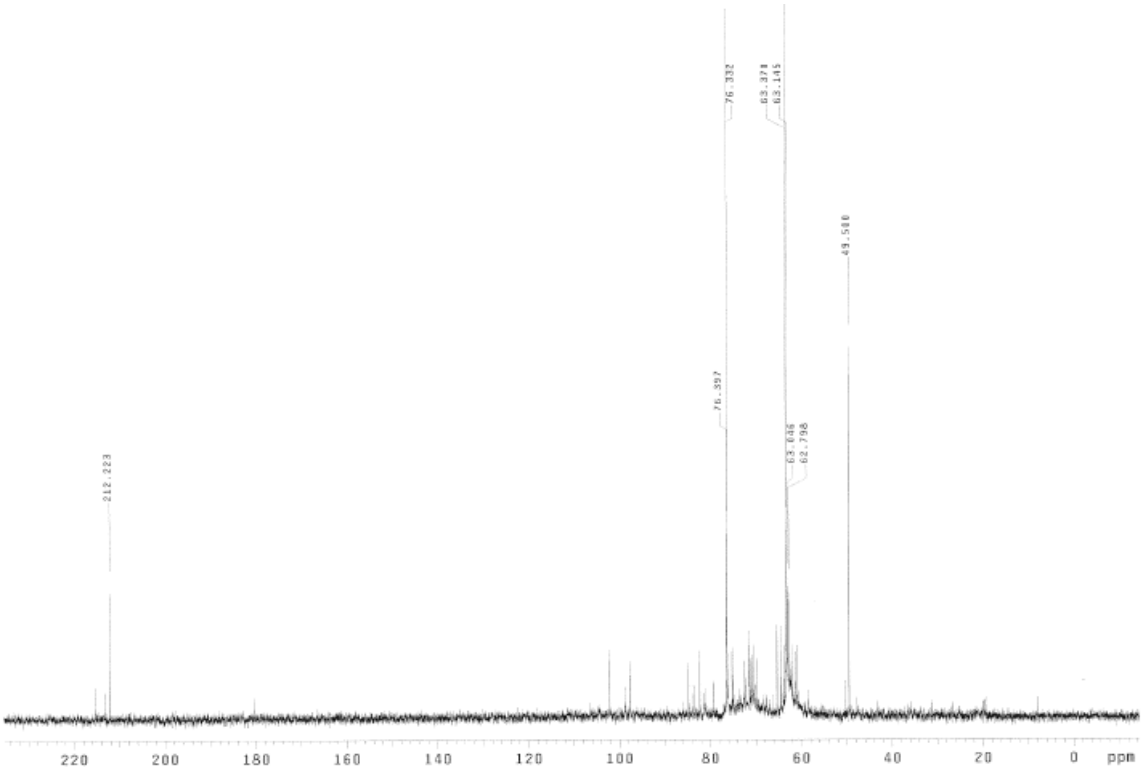


Figure 2-2. Correlation experiment: Addition of pure 1,2,4,5-tetrahydroxy-3-pentanone to the mixture holding the reaction of erythrulose and formaldehyde. This spectrum is standardized to methanol at 49.5 ppm. Spectrum file: pentulose_mixed_with_authentic_20060715.pdf

A single sharp signal emerging at 72.4 ppm is a peak most likely characterized by a single product. Furthermore, NMR superimposition experiments that this peak came from C4 of xylulose. The resonance at 72.47 ppm had a barely split doublet structure (72.47 and 72.42) that was not clearly significant above the noise, but was reproducible. One possibility is that a single borate coordinates two chiral diols in a complex, creating diasteromers. Alternatively, this doubling could be assigned as a cyclic form with two anomers. The addition of an authentic sample of ribulose to this material did not result in any superimpositions, however. Addition to xylulose in the borate-carbonate buffer did. This is reminiscent of an acetoxy derivative of 3-pentulose which was reported to yield, in acid (in the absence of borate), a mixture of xylulose and ribulose, where the compounds were assigned by thin layer chromatography.

Amidst reports that calcium could potentially influence reactions by catalyzing rearrangements as some molybdenum species are reported to do,[31, 32] reactions were run both in the presence, and absence, of calcium (Fig. 2-3 & Fig. 2-4). In the absence of any measurable difference between the resulting reactions, we concluded that the presence of calcium did not affect the products of the reactions. These experiments would become the basis of a first look into the kinetics of the system.

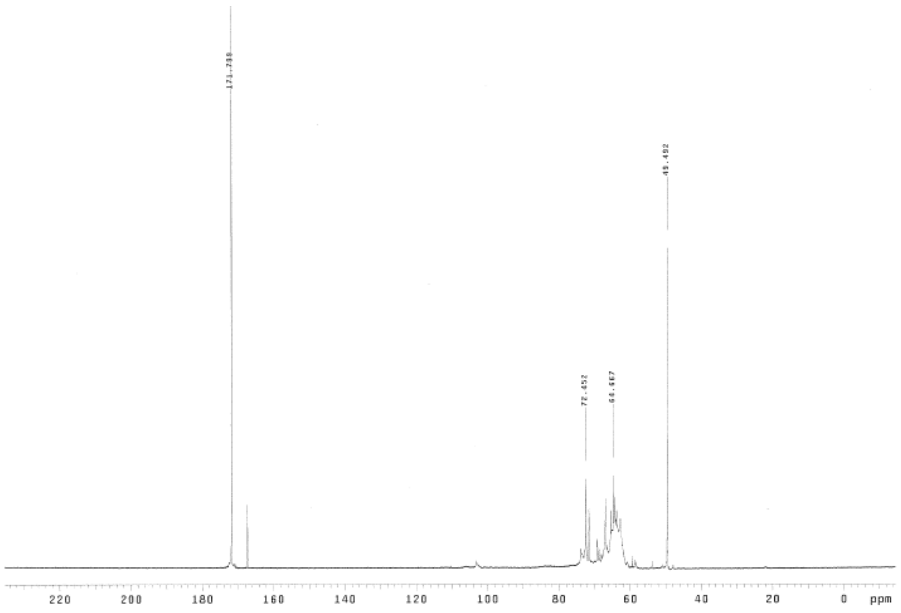


Figure 2-3. Reaction of dihydroxyacetone (DHA) with $H^{13}CHO$ without calcium. Note the peak at 72.4 which was later tentatively assigned to xylulose by superimposition. Spectrum file: dha-100ul_h13cho-50ul_cba_120106_t=54720.pdf

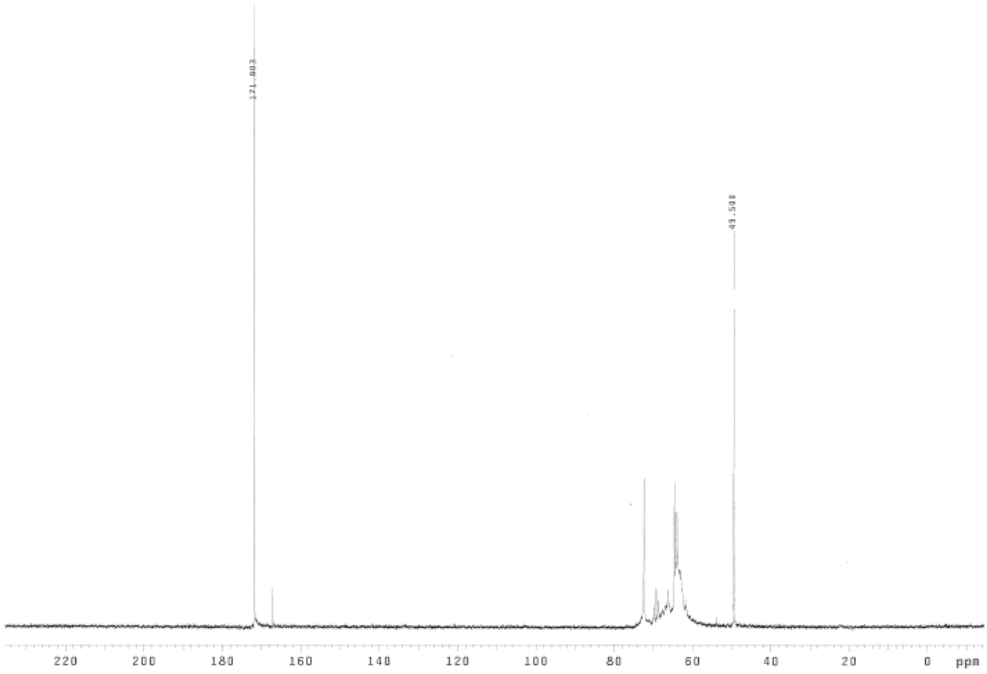


Figure 2-4. Reaction of erythrulose with $H^{13}CHO$. Note the peak at 72.4 which was later tentatively correlated to xylulose via superimposition. Spectrum file: erythrulose_100ul_cba_900ul_hcho50ul_nt=256.pdf

Primitive Catalytic Cycles

Breslow proposed that once even a single molecule of glycolaldehyde is generated, catalytic cycles could set up an abiotic "metabolism" to fix HCHO.[9] As the enolization DHA is compatible with borate, DHA can also catalyze such a metabolism, avoiding the need for the addition of a molecule of formaldehyde to itself to yield a molecule of glycolaldehyde. Further reaction of glyceraldehyde, glycolaldehyde, as well as 1,2,4,5-tetrahydroxypentan-3-one leads to the pentoses.

As stated earlier, another source for dihydroxyacetone is glycerol, which is abundant in meteorites. Glycerol can react via a Fenton style oxidation utilizing H_2O_2 from lightning, UV light and Fe(II) minerals to give dihydroxyacetone[24, 25]. Moreover Fe (II) is compatible with borate as precipitates are not formed. The presence of borate guides product formation as demonstrated in Figure 2-5.

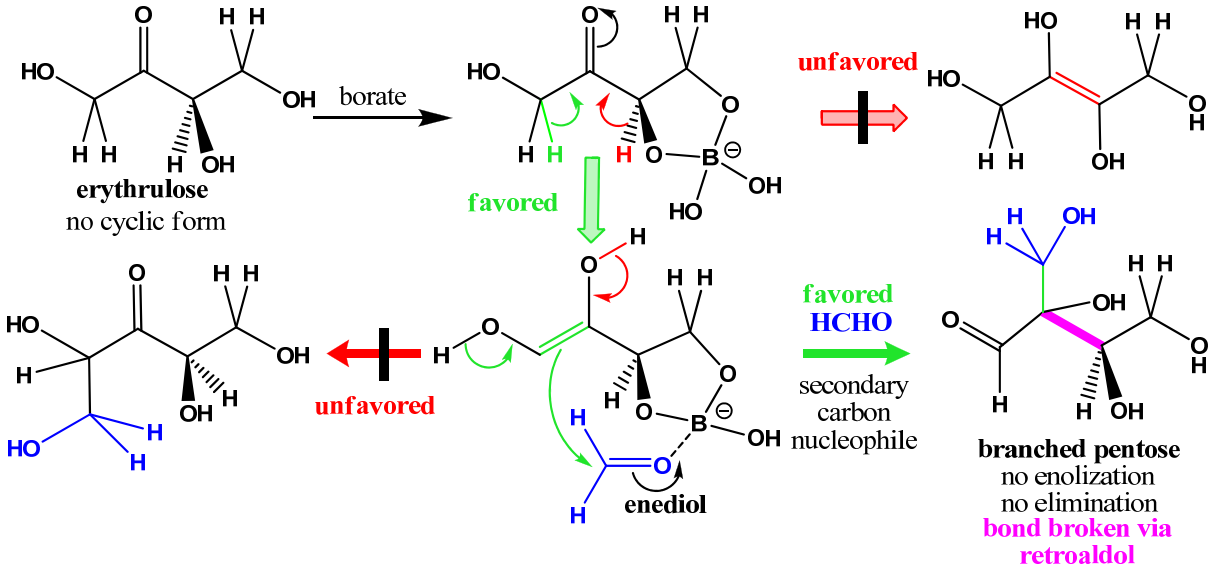


Figure 2-5. The influence of borate on the enolization of erythrulose and regioselectivity of reactions with HCHO. Erythrulose can enolize to form two different products, the 1,2 or the 2,3 enol. Borate binds to the 3 and 4 hydroxyl groups eliminating 2,3 enolization. Further, the borate-mediated reaction adds formaldehyde to the more hindered center.

There appears to be some thermodynamic control by borate on which cyclic forms of pentoses form. When ribose, xylulose and ribulose are incubated in a borate buffer, their spectra display far fewer complexation products in comparison to arabinose, xylose and lyxose. This is attributed to the thermodynamically favourable conformation of one borate-pentose complex, shifting the equilibrium to deplete the others and generating one particular cycle in abundance (Figures 2-6 to 2-11).

Two rules for the abiotic metabolism were established: (a) retroenolization incorporating deuterium is negligible at pH 12.5 as long as the concentration of HCHO is significant, and (b) retroaldol reactions that extrude HCHO are negligible. The first was confirmed by showing that if the formose process is run in D_2O, the C4, C5, C6, and C7 species incorporated negligible amounts of deuterium from solvent. The second is consistent with the general principle that retroaldol reactions are favored when they break sterically crowded bonds having (mostly or entirely) non-hydrogen bulky substituents (Figure 2-12).

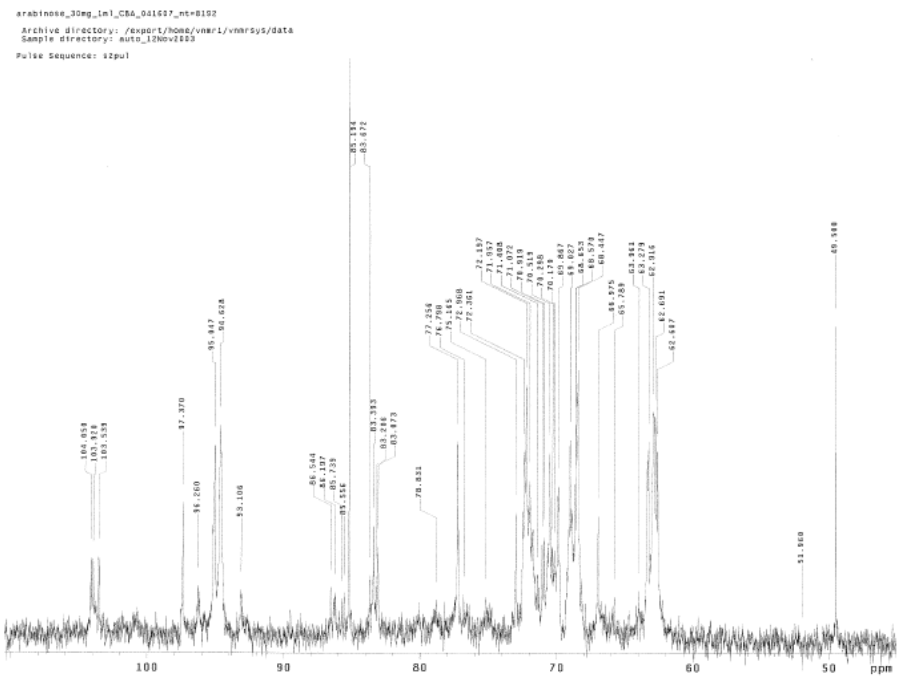


Figure 2-6. ^{13}C NMR spectrum of arabinose (30 mg) in CBA buffer (1 mL), note the complexity. Spectrum file: arabinose_cba_meohref.pdf

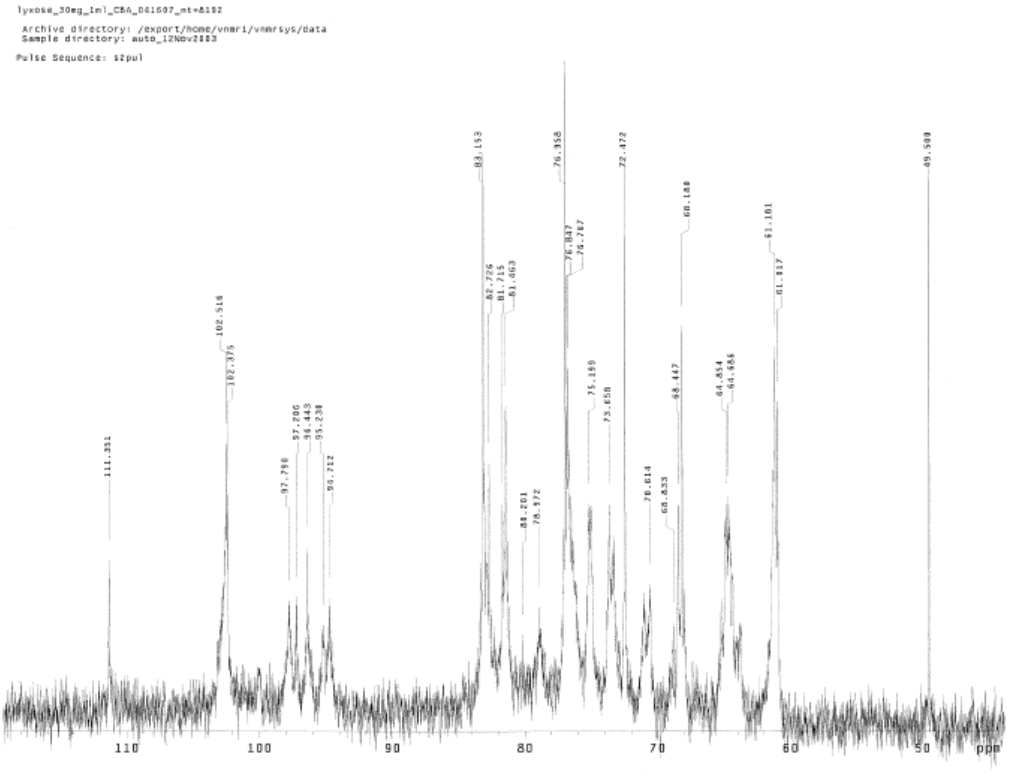


Figure 2-7. ^{13}C NMR spectrum of lyxose (30 mg) in CBA buffer (1 mL), note the complexity. Spectrum file: lyxose_cba_meohref.pdf

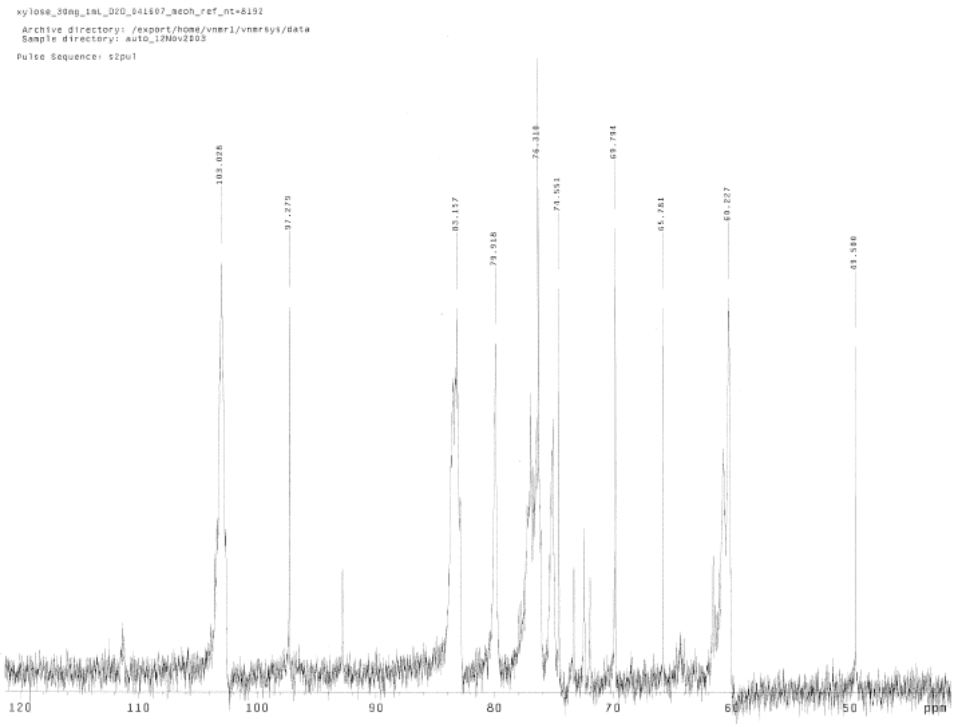


Figure 2-8. ^{13}C NMR spectrum of xylose (30 mg) in CBA buffer (1 mL), note the complexity. Spectrum file: xylose_cba_meohref.pdf

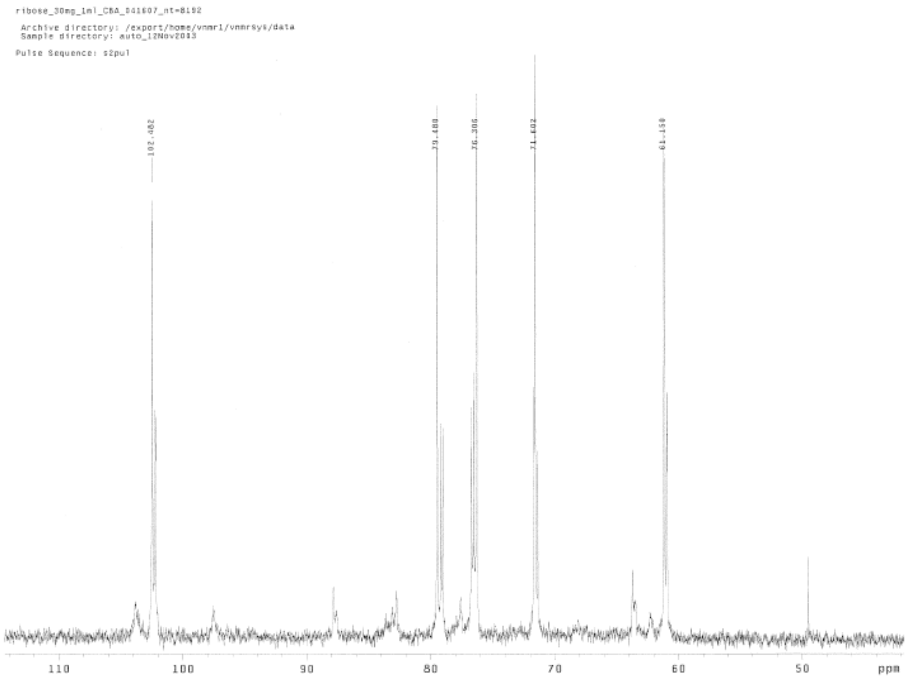


Figure 2-9. ^{13}C NMR spectrum of ribose (30 mg) in CBA buffer (1 mL), note the simplicity indicating a singular cyclic form, assigned by NMR as the alpha furanose. Spectrum file: ribose_cba_meohref.pdf

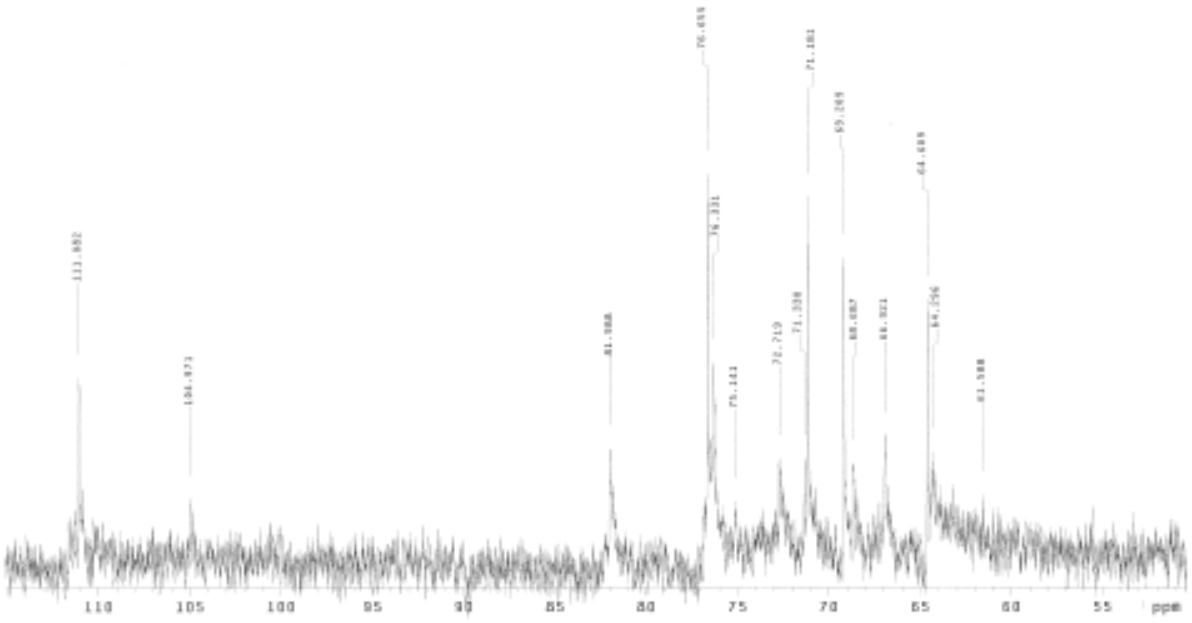


Figure 2-10. ^{13}C NMR spectrum of ribulose (30 mg) in CBA buffer (1 mL), note the complexity. Spectrum file: ribulose_cba_meohref.pdf

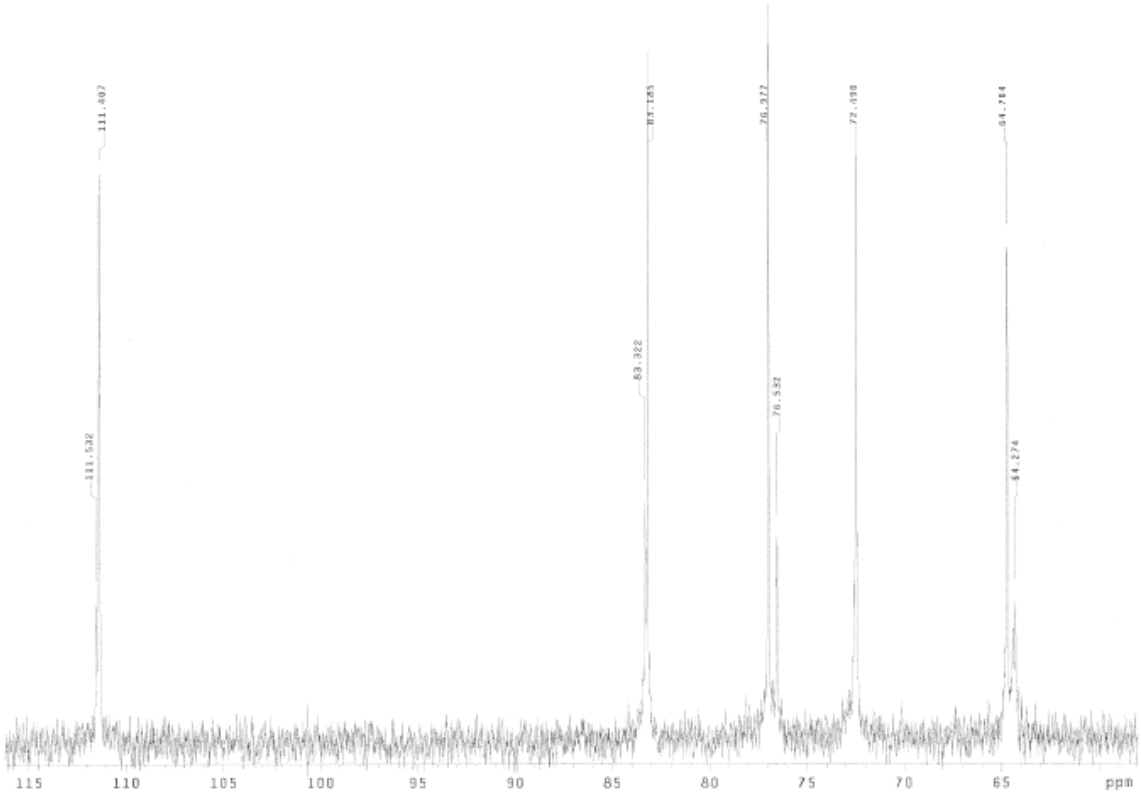
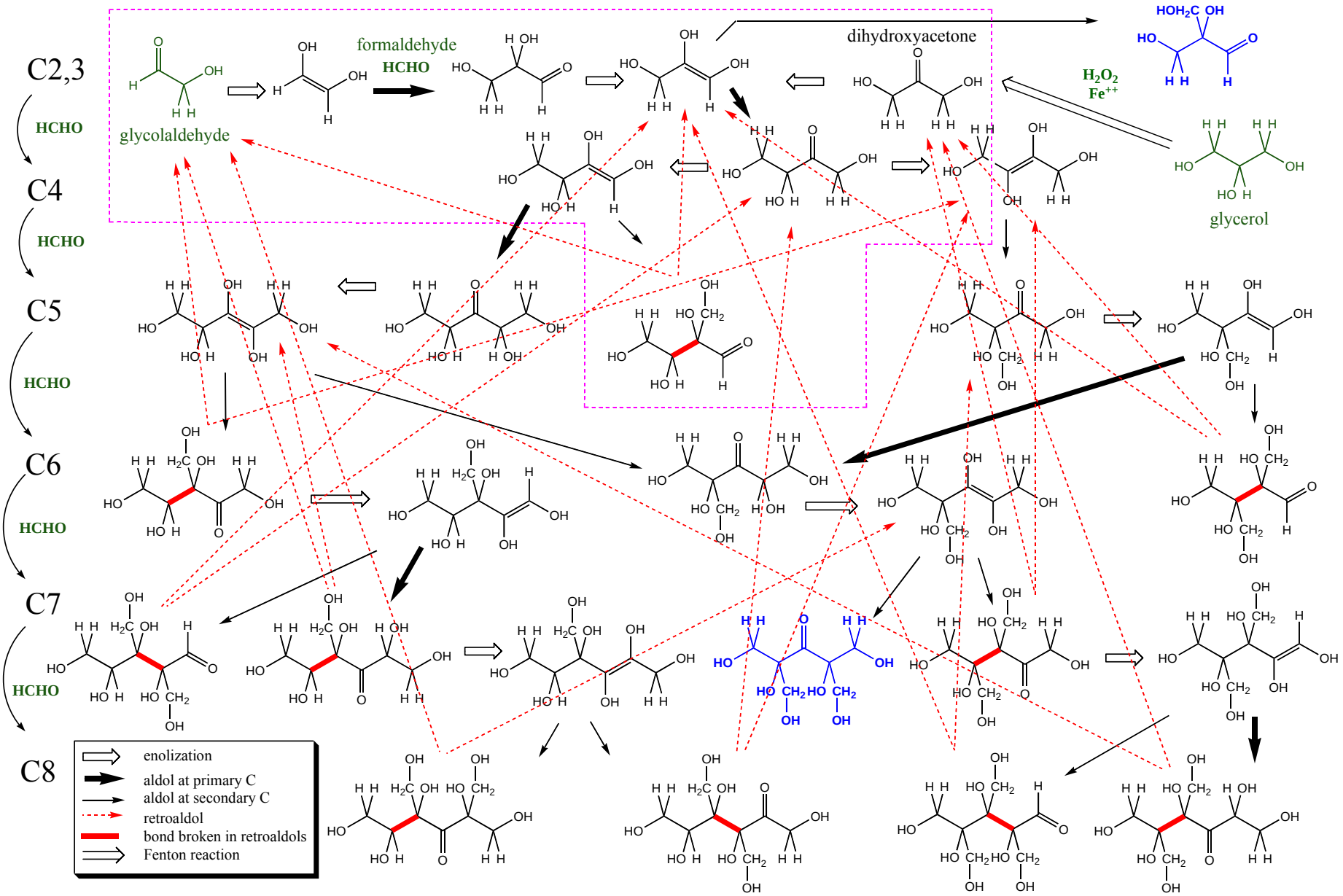


Figure 2-11. ^{13}C NMR spectrum of xylulose (30 mg) in CBA buffer (1 mL). Spectrum file: xylulose_cba_meohref.pdf

Figure 2-12. Pathways of possible aldol/retro-aldol chemistry of carbohydrates. An abiotic "metabolism" that generates higher carbohydrates via repetitive enolization of lower carbohydrates (top) followed by aldol addition of the resulting enediol to formaldehyde (HCHO), followed by fragmentation of the carbohydrates in a cyclic process. Compounds in green are indisputable prebiotic precursors, because of either their established presence in meteorites, their ready formation on Earth via atmospheric electrical discharge, photochemistry, or mineral processes, or their direct observation in interstellar nebulae by microwave spectroscopy. Compounds in blue neither enolize nor fragment (other than to give the same fragments from which they are formed). Bold arrows in black show aldol addition of HCHO to the primary, less hindered center of an enediol; other black solid arrows indicate aldol addition to a more hindered center. Red dotted arrows show retroaldol fragmentation pathways that complete the cycle by cleaving the higher carbohydrate at the red, bold bond. Borate constrains this metabolism to occur only within the dotted box (magenta).


C2,3
HCHO
C4
HCHO
C5
HCHO
C6
HCHO
C7
HCHO
C8
glycolaldehyde
formaldehyde
HCHO
dihydroxyacetone
glycerol
H_2O_2
Fe^{++}
enolization
aldol at primary C
aldol at secondary C
retroaldol
bond broken in retroaldols
Fenton reaction

The Geochemical Synthesis of Threose

In an effort to understand and distinguish known resonances in prebiotic reaction mixtures, attempts to eliminate known starting materials were made by incubating them alone in a Carbonate –Boric Acid (CBA) buffer. While certain species, such as formaldehyde, were not expected to react with themselves, the spectrum arising from incubation of glycolaldehyde was promising. A sample of glycolaldehyde (20 mg, Aldrich) was incubated in CBA buffer (1.0 mL) and an NMR spectrum was acquired. The methanol-standardized spectrum displayed four peaks with resonances at 103.28, 82.33, 76.61 and 71.61, where only two peaks for glycolaldehyde or its dimer were expected.

These results showed that glycolaldehyde formed a four carbon species. This was determined to be threose by the superimposition of the four resonances on the four from authentic threose. Additionally, erythrose was not observed. We hypothesize that borate stabilizes the C1-hydroxy in a beta orientation with the hydroxyl group at the C2 position (Fig. 2-13). Superimposition NMR correlation experiments are pictured in Figure 2-14. Glycolaldehyde (20 mg, Aldrich) was allowed to react with itself in the absence on formaldehyde in CBA buffer (1 mL, 250 mM, pH 10.4) for 24 hours. Aliquots of this reaction were gradually added to a sample of authentic threose (17 mg, Fisher) dissolved in CBA buffer (1 mL, 142 mM, pH 10.4) in increasing ratios from 1:10 up to 10:1 and analyzed via NMR. The peaks in the sample (103, 82, 76 and 71 ppm) corresponded directly to and superimposed upon the authentic threose peaks throughout the concentration gradient.

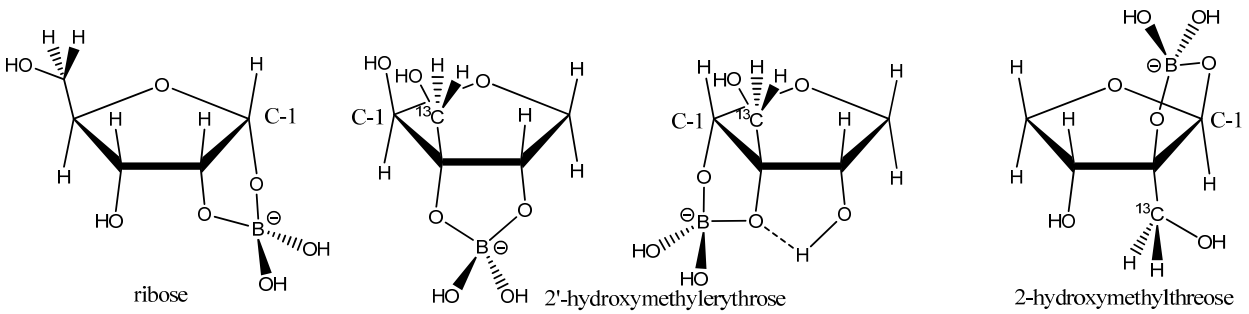


Figure 2-13. Cyclic forms of ribose (in its "alpha" epimer, where the –OH hydroxyl group at C-1 is down; the "D" enantiomer is shown). 2'-Hydroxyerythrose (in both its alpha and beta form; the enantiomer shown is the one synthesized with a ^{13}C label), and 2'-hydroxymethyl threose (in both its alpha and beta form; the enantiomer shown is the one synthesized with a ^{13}C label). The site of borate complexation is known by NMR spectrometry in ribose. The site of complexation of borate to the cyclic forms of the branched pentoses is not established experimentally; experimental data suggests that the erythrose forms two borate complexes, while the threose forms only one.

Compared to previous literature reports, this reaction medium of a carbonate borate buffer offers a prebiotic route to sugars. Weber and Pizzarello reported that a peptide chain placed in a solution of glyceraldehyde demonstrated the synthesis of threose and erythrose in an aqueous prebiotic context.[33] There have been a few other reports of protein mediated carbohydrate synthesis such as one by Cordova which used N,N-dimethylformamide as a solvent.[34, 35] Similarly Ibrahem et al used DMSO with a trace of water for their amino acid-catalyzed formation of carbohydrates.[36]

Both Albert Eschenmoser and Jack Szostak proposed threose to be a precursor to our 'modern' genetic backbone and demonstrated its ability to form a stable and functional biopolymer as the backbone of an RNA-like genetic molecule[37, 38]. Additionally, Szostak demonstrated the ability DNA polymerases to elongate DNA primered strands of threose nucleic acids.[39]

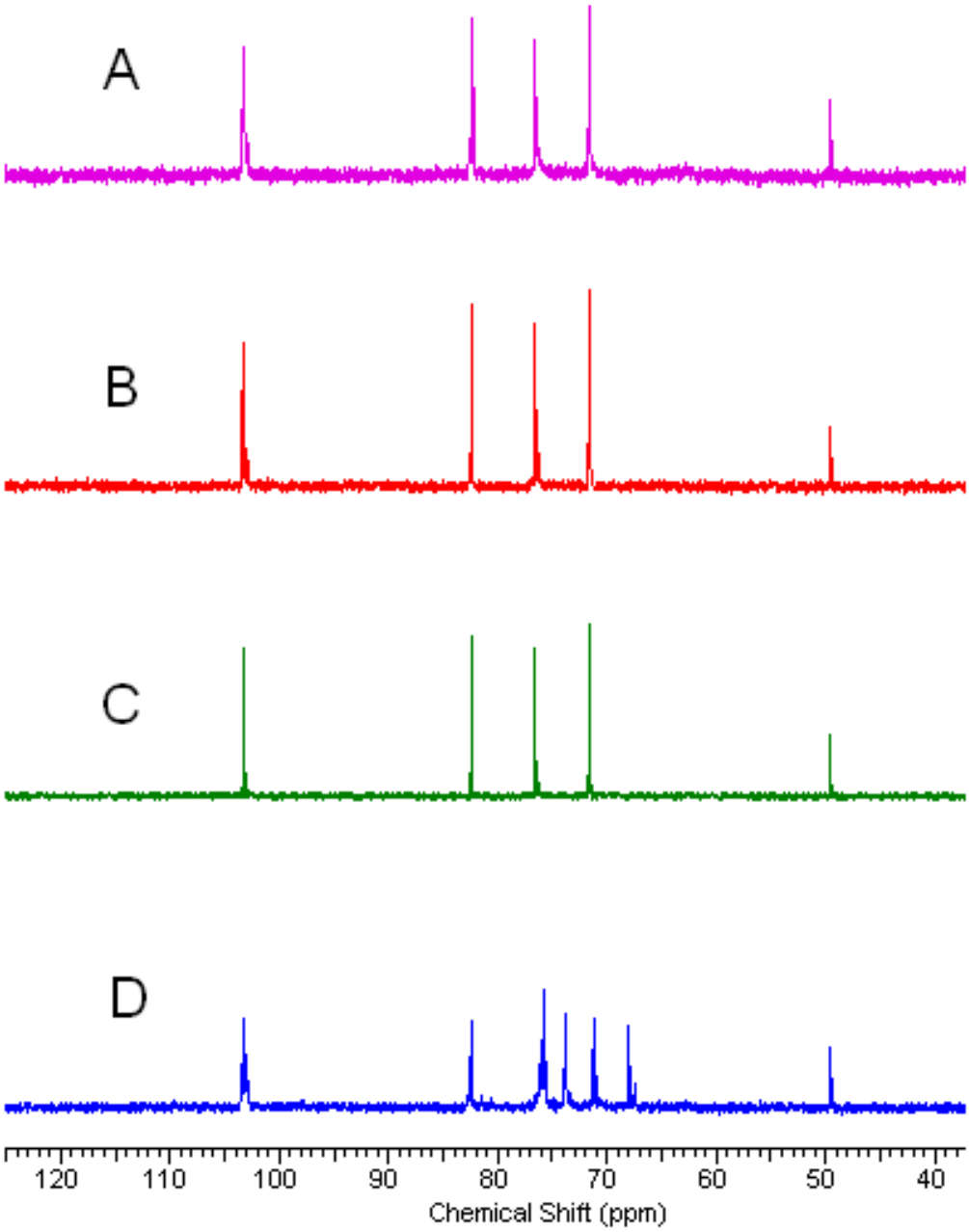


Figure 2-14. NMR analysis of the reaction of glycolaldehyde in CBA buffer, forming threose, in the absence of HCHO. **A**. ^{13}C NMR spectrum of the reaction products of glycolaldehyde (250 mM) with itself. **B**. ^{13}C NMR spectrum of authentic threose (142 mM) in CBA buffer. **C**. ^{13}C NMR spectrum of samples **A** and **B** mixed together, here we show an equimolar mixture. **D**. ^{13}C NMR spectrum of authentic erythrose (167 mM) in CBA buffer. Signal at 49.500 ppm is reference CH_3OH. Erythrose and threose do not undergo reaction in days in CBA buffer. Spectrum files: 070607_12C_GOL_cba_with_authentic_threose_added.pdf, 070523_erythrose_20mg_cba_1ml_nt=4096.pdf

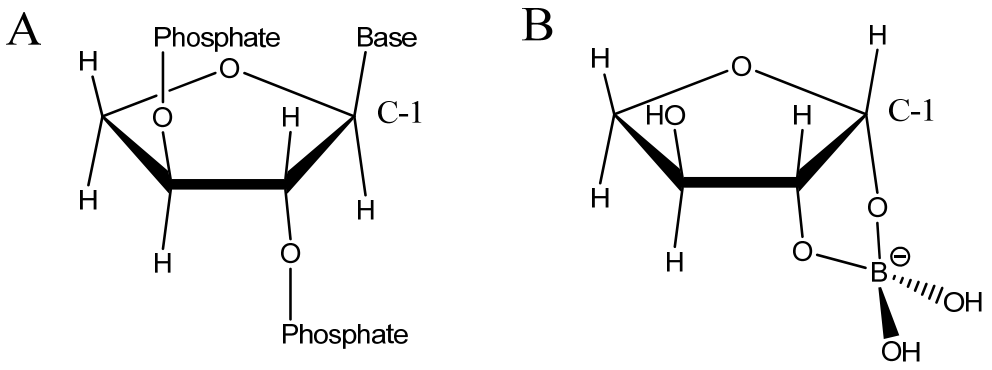


Figure 2-15. A) Phosphate backbone and heterocyclic base locations in a threose nucleic acid (TNA) model. B) Coordination of borate to the alpha anomer of threose.

Kinetics

A kinetics study of the rate of enolization of dihydroxyacetone, glyceraldehyde and erythrulose offered a more detailed view of the borate-catalyzed cycle. Borate in CBA was found to slow the rate of enolization of erythrulose by a factor of 4.48, of dihydroxyacetone by a factor of 9.17, and glyceraldehyde by a factor of 3.33 (Table 2-1).

Table 2-1. Enolization rates of dihydroxyacetone, glyceraldehyde and erythrulose at 25 °C with formaldehyde concentration of 167 mM.

CBA Buffer – pH=10.4

	Slope of line	[enolizable species]	pH	enolization rate constant
Dihydroxyacetone	1.0×10^{-2} min^{-1}	0.317 M	10.4	0.0052 min^{-1}
Glyceraldehyde	3.7×10^{-3} min^{-1}	0.317 M	10.4	0.0018 min^{-1}
Erythrulose	1.2×10^{-2} min^{-1}	0.317 M	10.4	0.0063 min^{-1}

Carbonate Buffer – pH=10.4

	Slope of line	[enolizable species]	pH	enolization rate constant
Dihydroxyacetone	9.5×10^{-2} min^{-1}	0.317 M	10.4	0.0477 min^{-1}
Glyceraldehyde	1.2×10^{-2} min^{-1}	0.317 M	10.4	0.0060 min^{-1}
Erythrulose	5.6×10^{-2} min^{-1}	0.317 M	10.4	0.0282 min^{-1}

When performing the kinetics, it was not necessary to label the higher carbohydrates as ^{13}C labeled HCHO was introduced into the medium at a concentration high enough to support a convenient rate. The concentration of enolizable species must remain well below the

concentration of borate. As a further complication, the product of the reaction is also an enolizing species, meaning that the rate of consumption of $H^{13}CHO$ as the reaction progresses need not slow down. Indeed, if the rate of enolization of the product is faster than the rate of enolization of the starting material, consumption of $H^{13}CHO$ may in fact speed up (Figure 2-16).

The rate law could have the form velocity = k[enolizing species][$H^{13}CHO$]. However, a classical experiment in physical organic chemistry, dating back to experiments in 1904 by Lapworth, showed that the rate of bromination of acetone was independent of the concentration of bromine[40]. This was the first evidence for a slow, rate limiting enolization of acetone as an obligatory step in the bromination reaction, and the enol of acetone as an intermediate in the bromination reaction. Therefore, the reaction process here is assumed to be rate limited by the enolization step and the rate law is expected to be of the form:

Velocity = k[enolizing species].

To obtain rate constants in the standard CBA buffer, a known concentration of the enolizing species was incubated with $H^{13}CHO$, and the initial rate was estimated by following the loss of label assigned to $H^{13}CHO$ relative to the intensity of the formate peak arising from the introduced formaldehyde. This ratio was converted to a logarithm, log ([$H^{13}CHO/H^{13}COO^-$]) (now expected to be linear) normalized by division against the first data point, and plotted against time (Figs. 2-17 to 2-22). This gives a "pseudo" rate constant that was converted to a first order rate constant by dividing by the concentration of enolizable species.

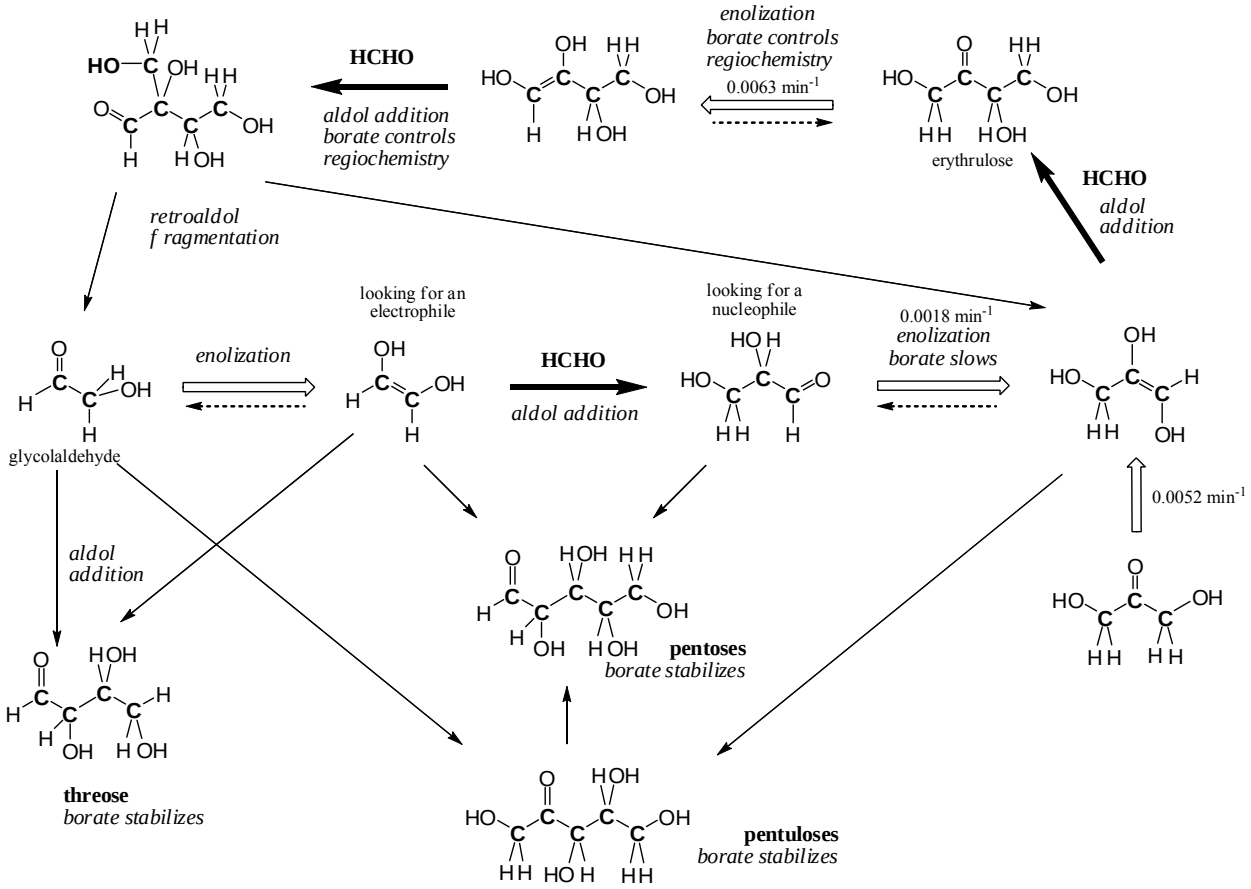


Figure 2-16. The borate-compatible cycle for conversion of formaldehyde into pentoses, pentuloses and threose. Enolization rates of glyceraldehyde, dihydroxyacetone and erythrulose in the presence of borate.

The first order process for the consumption of $H^{13}CHO$ approximation is good only if [$H^{13}CHO$] << [enolizable species], allowing the [enolizable species] to be assumed to remain constant. The slope of a line plotting [$H^{13}CHO$] versus time is equal to k [enolizing species]. To compare these rate constants with rate constants obtained for various enolizing species, however, the slope of the line, and the "pseudo first order" rate constant is converted to a real rate constant by dividing by the concentration of enolizable species. While peak intensities in ^{13}C spectroscopy need not give precise values, these averaged over a dozen measurements proved not to create large variance when the data were plotted.

In the presence of borate, the pseudo first order rate constant for the reaction of DHA (317 mM, two-fold excess over HCHO) with $H^{13}CHO$ is measured to be 0.0052 min^{-1} (the error was calculated by comparing results using MeOH and formate as references, at pH 10.25, 25 °C). This gives a second order rate constant of 0.016 $M^{-1}min^{-1}$. The loss of formaldehyde versus time plot was linear for up to 200 minutes ($R^2 \approx 0.98$).

The corresponding experiments for erythrulose showed a slightly higher rate (pseudo first order rate constant of 0.0063 min^{-1}) and giving a second order rate constant ([erythrulose] ≈ 317 mM) of 0.02 $M^{-1}min^{-1}$. In the absence of borate, the pseudo first order rate constant for the reaction of DHA (317 mM, two-fold excess over [HCHO]) with $H^{13}CHO$ is measured to be 0.0477 min^{-1} (error calculated by comparing results using MeOH and formate as references, at pH 10.25, 25 °C). This gives a second order rate constant of 0.150 $M^{-1}min^{-1}$. The loss of formaldehyde versus time plot was linear for up to 200 minutes ($R^2 \approx 0.98$). The corresponding experiments for erythrulose showed a slower rate (pseudo first order rate constant of 0.0282 min^{-1}) and giving a second order rate constant ([erythrulose] ≈ 317 mM) of 0.089 $M^{-1}min^{-1}$). Given that a "statistical factor" operates (erythrulose has one less primary enolizable hydrogen to abstract than DHA), and assuming that the reaction is under kinetic control (implying that the enolization at the primary center dominates the overall rate), this slower rate constant for the reaction of erythrulose with HCHO is entirely expected. This rate difference is not observed in the presence of borate. Though borate slows down the overall rate of enolization, borate also nearly equates the rates of formaldehyde consumption by dihydroxyacetone and erythrulose respectively. This fits the observation that borate favours the enolization of erythrulose between carbons 2 & 3 over carbons 1 & 2.

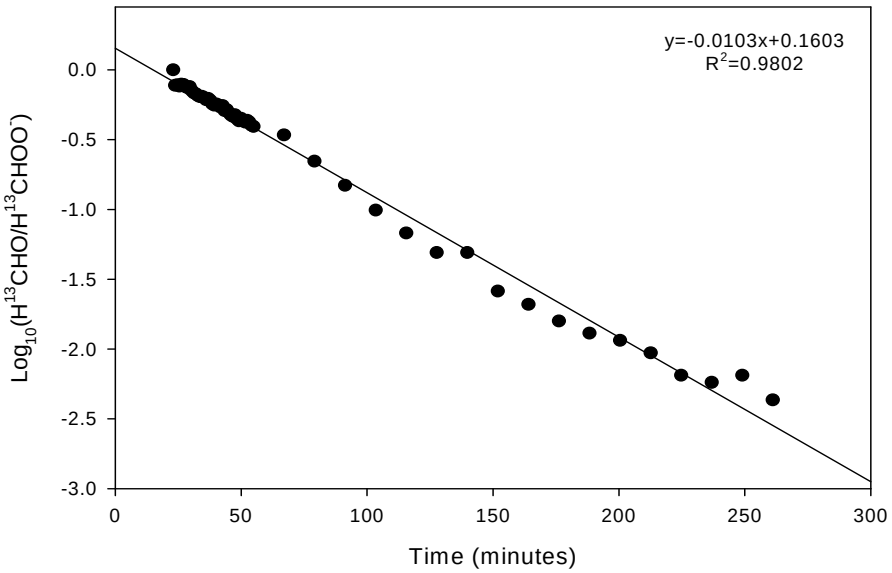


Figure 2-17. Reaction of dihydroxyacetone (0.317 M) with formaldehyde (0.167 M) in the presence of borate (CBA buffer with a measured pH of 10.4 at 25 °C) plotted against time.

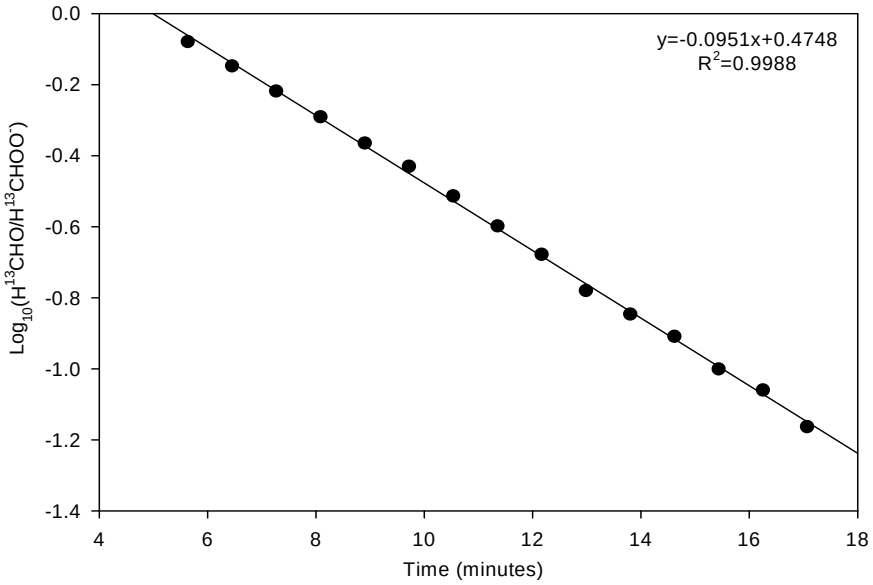


Figure 2-18. Reaction of dihydroxyacetone (0.317 M) with formaldehyde (0.167 M) in the absence of borate (carbonate buffer with a measured pH of 10.4 at 25 °C) plotted against time.

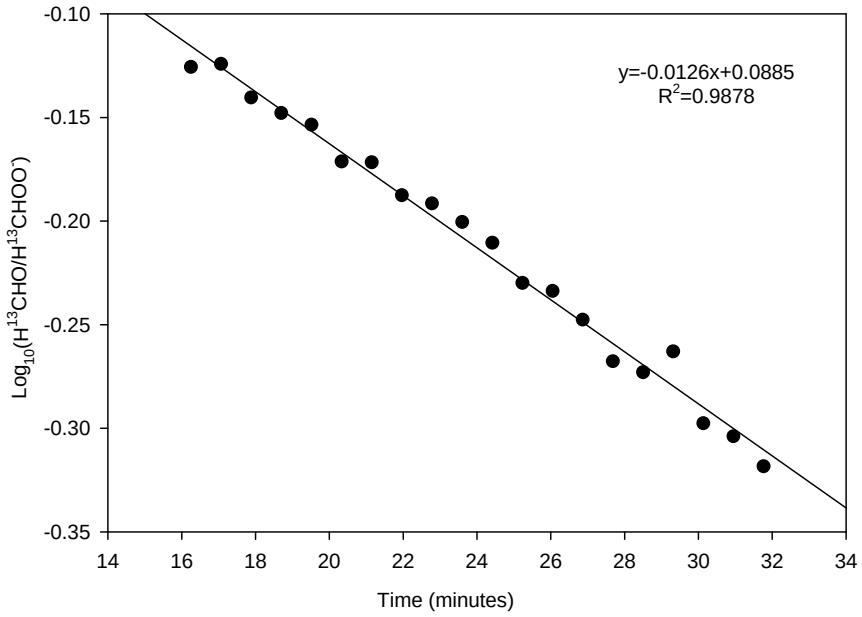


Figure 2-19. Reaction of erythrulose (0.317 M) with formaldehyde (0.167 M) in the presence of borate (CBA buffer with a measured pH of 10.4 at 25 °C) plotted against time.

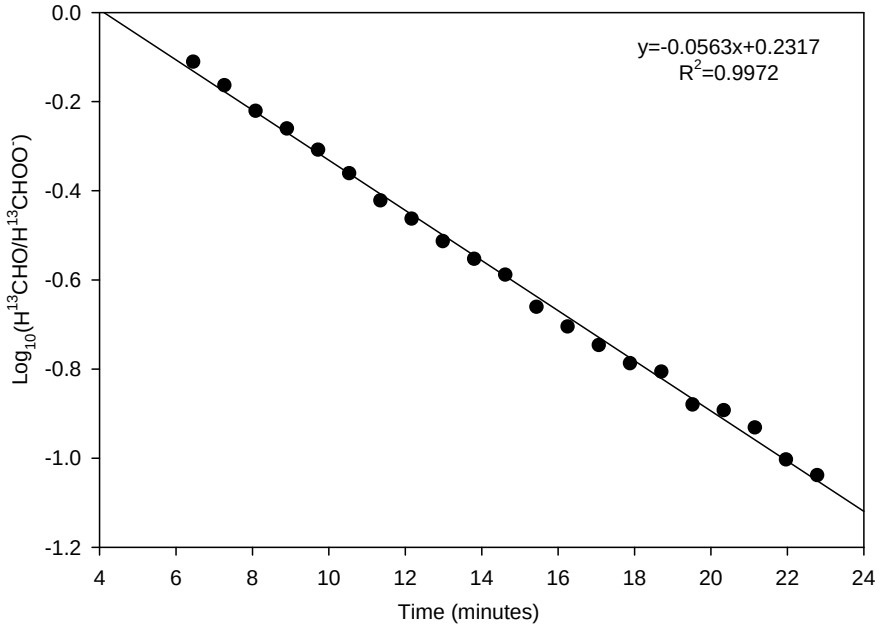


Figure 2-20. Reaction of erythrulose (0.317 M) with formaldehyde (0.167 M) in the absence of borate (carbonate buffer with a measured pH of 10.4 at 25 °C) plotted against time.

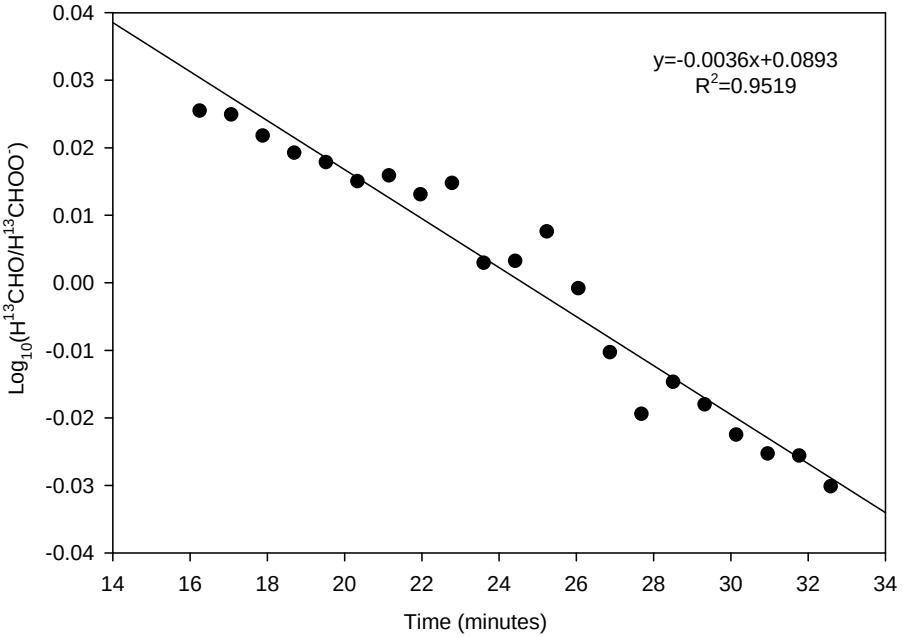


Figure 2-21. Reaction of glyceraldehyde (0.317 M) with formaldehyde (0.167 M) in the presence of borate (CBA buffer with a measured pH of 10.4 at 25 °C) plotted against time.

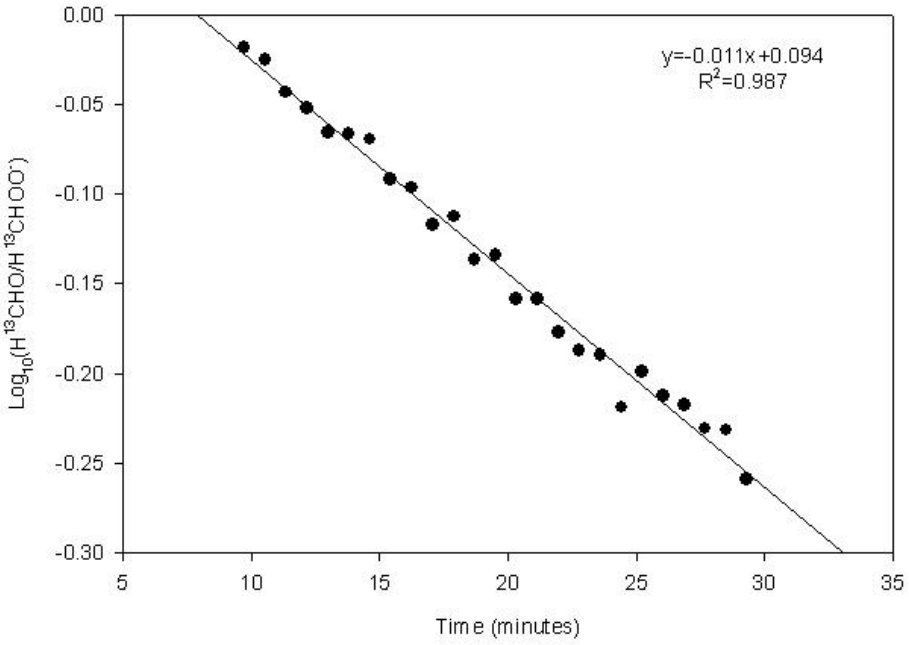


Figure 2-22. Reaction of glyceraldehydes (0.317 M) with formaldehyde (0.167 M) in the absence of borate (carbonate buffer with a measured pH of 10.4 at 25 °C) plotted against time.

Catalytic Cycles

A reaction cycle starting with DHA proved to be compatible with borate, and avoids the slow 1 carbon unit, formaldehyde + 1 carbon unit, formaldehyde → 2 carbon unit, glycoaldehyde reaction. Glycerol from meteorites can form DHA via a Fenton oxidation utilizing H_2O_2 from lightning, UV light and Fe(II) minerals. Additionally Fe(II) is compatible with borate, as is H_2O_2. The presence of borate, in the end, can guide product formation (Figure 2-5).

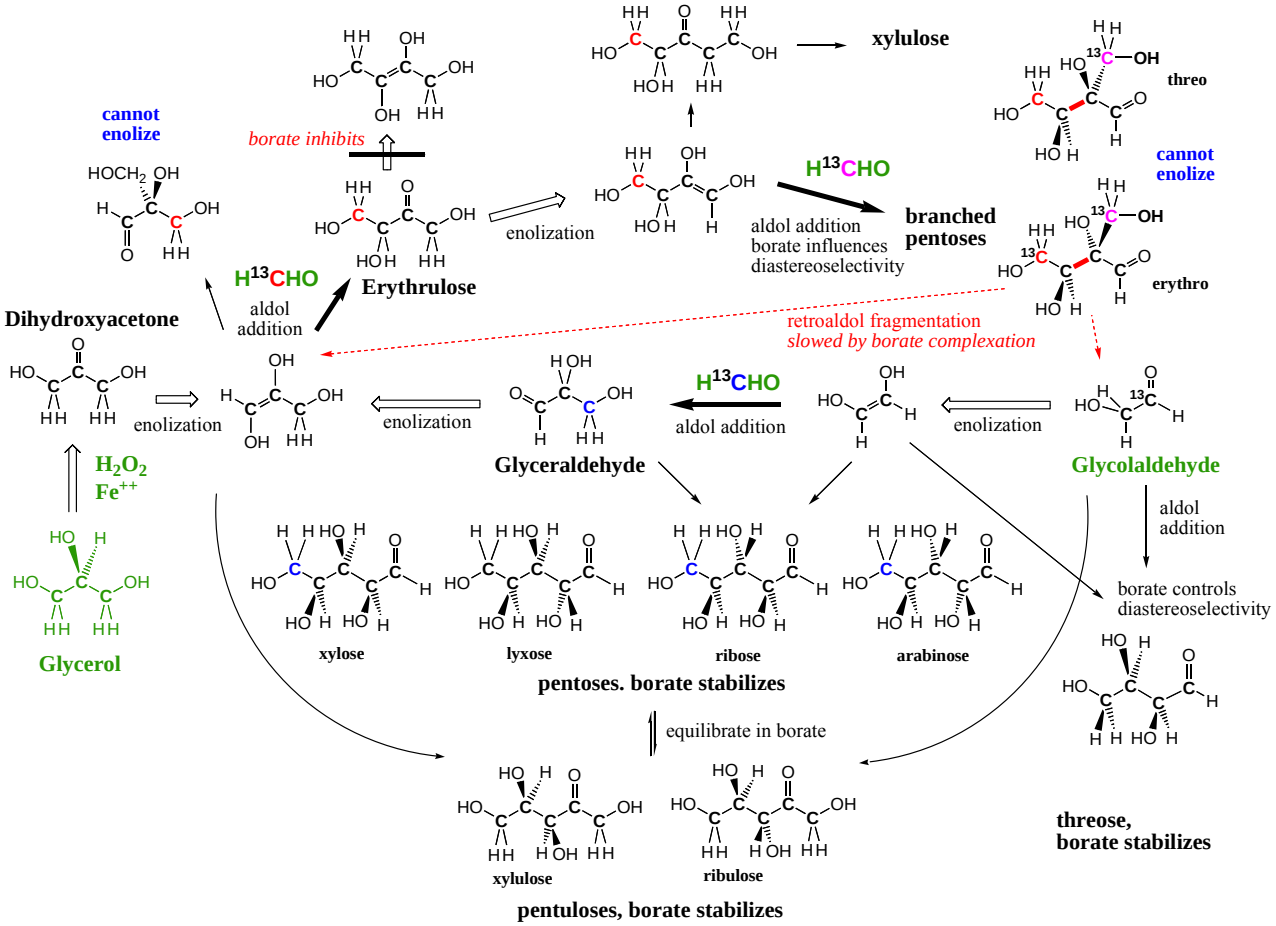


Figure 2-23. A segment of the abiotic metabolism, the compounds in green are known prebiotically in either meteorites, via electrical discharge, photochemistry, or mineral-based processes on Earth, or in the interstellar nebula. Carbon atoms preceded by a superscript 13 have been ^{13}C labeled in higher carbohydrates prepared by laboratory chemical synthesis, to establish the movement of carbon through the pathway. Red, blue, and magenta colored carbons in the higher carbohydrates indicate the position of label introduced from $H^{13}CHO$ in the immediately preceding aldol addition product.

The sudden drop in formaldehyde concentration was hypothesized to stem from late retroaldol reactions undergone by C4, C5, C6, and C7 species that have such crowded bonds (red bonds in Fig. 2-12). Many of these retroaldols generate dihydroxyacetone. The rate of enolization of dihydroxyacetone was measured through its reaction with $H^{13}CHO$, and found to be 0.0103 min^{-1} at 25 °C, pH 10.4 (CBA buffer), certainly competent at higher temperatures and pH to support the observed rapid consumption of HCHO in the crash.

The formose process was repeated using a 2:1 mixture of $H^{12}CHO$ and $H^{13}CHO$ to help assign the products formed. After HCHO was consumed, the characteristic "yellowing" of the formose product began. This mixture generated ^{13}C-NMR resonances that were split (1:2:1) by ^{13}C-^{13}C coupling at 183.64, 69.46, and 21.04 ppm (assigned respectively as C-1, C-2, and C-3 of lactate), 182.68 and 62.70 (assigned respectively as C-1 and C-2 of glycolate), and 59.26 and 37.11 (assigned respectively as C-1 and C-3, and C-2 of 1,3-propanediol). All of these products, formed only after HCHO was consumed, requiring enolization where the enediol was not trapped by HCHO, but rather suffers beta-elimination and protonation. Further, they require Cannizzaro reactions[41], as well as the well-known methylglyoxal-lactate rearrangement[42]. All of these products are "metabolic" dead ends, as lactate, glycolate, and diols are essentially unreactive under a wide range of conditions, including these.

A geological model where borate might be available to stabilize C7, C6, C5, and C4 carbohydrates under conditions where they are formed was revisited. Serpentinization, a process of hydration and metamorphic transformation in ultramafic rock, specifically of olivine-containing igneous rocks[43] is well known to generate highly alkaline solutions having a pH >12. Such a high pH could not be maintainable under an atmosphere of carbon dioxide on primitive Earth, where CO_2 would be absorbed to create a buffered carbonate solution with a much lower

pH. Attempts to run the formose reaction at pH 10 failed to yield detectable products. This is presumably due to the failure of the 1 carbon unit, formaldehyde + 1 carbon unit, formaldehyde → 2 carbon unit, glyceraldehyde reaction (which is remarkably slow even at pH 12.5). This inference was supported by the fact that adding small amounts of glycolaldehyde to HCHO in carbonate buffer at pH 10.4 generated formose behavior, with an induction period followed by rapid loss of HCHO. The induction period shortening as the concentration of glycolaldehyde increased. Small amounts of glycolaldehyde presumably could be obtained prebiotically.

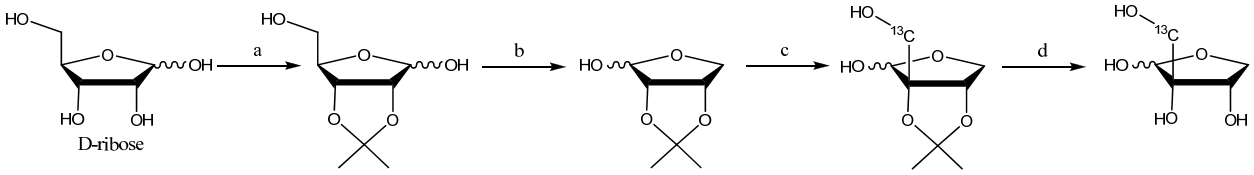
While this allowed the cycles in figure 2-23 to begin even at lower pH, and diminished the amount of dead end products formed by the Cannizzaro reaction, they did not solve a second problem. This problem was encountered when borate was introduced to stabilize the C7, C6, C5, and C4 products. Even at 6 mM, borate shut down the formation of higher carbohydrates from HCHO under formose conditions. Instead, the principal products were lactate, glycolate, and 1,3-dihydroxypropane, with only two other additional compounds formed in assignable amounts. No ^{13}C-resonances were observed at 100-115 ppm, which would represent the formation of aldopentoses or ketopentoses. Borate evidently shut down the cycles that move HCHO to higher carbohydrates.

Figure 2-12 was further studied with the goal of identifying small catalytic cycles that might be initiated by other precursors that would fix HCHO. As an alternative precursor, dihydroxyacetone was particularly attractive, not only because it supported the consumption of HCHO, but also because it is available by the reaction of H_2O_2 (obtained by electrical discharge through moist atmospheres) in the presence of ferrous iron (indisputably present on early Earth) in a Fenton reaction with glycerol, which is well known to survive impact as a major (>100 ppm) component of the soluble fraction of carbonaceous chondrites.

As a model environment, sodium carbonate-bicarbonate buffer at pH 10.4 (1.1 M, essentially saturating, as expected under a CO_2 atmosphere) was compared with the sodium carbonate-bicarbonate buffer at pH 10.4 containing sodium borate at various concentrations up to 278 mM.

The reaction of erythrulose with $H^{13}CHO$ was found to generate species that were tentatively assigned as the 2'-^{13}C-2-hydroxymethyltetrose, a branched sugar arising from reaction of the 1,2-erythrulose enediol with its *more* hindered center (Hyo-Joong Kim unpublished results). This assignment was confirmed by synthesizing both the *erythro* and *threo* diastereoisomers with label at both C-4 and C-2' (for the *erythro* diastereoisomer) and at C-2' (for the *threo* diastereoisomer), and demonstrating that the signals from the authentic materials superimposed on the signals arising in the reaction (Figure 2-24).

A. 2'-^{13}C-2-hydroxymethylerythrose



B. 4-^{13}C-2-hydroxymethylerythrose

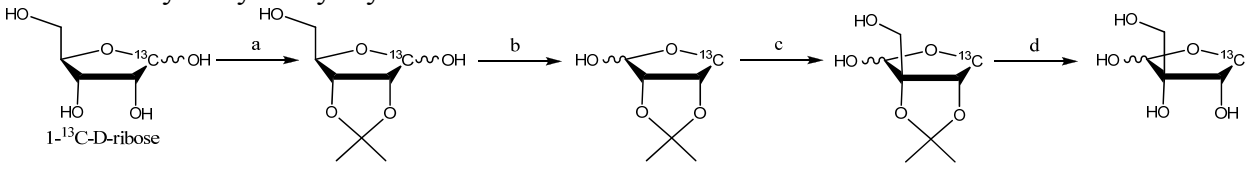


Figure 2-24. Synthesis of erythro branched pentose (A) and threo-branched pentose (B). Reagents and conditions: Scheme A: a. 2,2-dimethoxypropane, p-TsOH, acetone, 80%; b. 1. $NaBH_4$, H_2O. 2. $NaIO_4$, AcOH, H_2O, 71%; c. $H^{13}CHO$, K_2CO_3, CH_3OH, 65 °C, 70%; d. Dowex 50Wx8 (H+), H2O, 80 °C, 95%.Scheme B: a. 2,2-dimethoxypropane, p-TsOH, acetone, 80%; b. 1. $NaBH_4$, H_2O. 2. $NaIO_4$, AcOH, H_2O, 70%; c. HCHO, K_2CO_3, CH_3OH, 65 °C, 55%; d. Dowex 50Wx8 (H+), H2O, 80 °C, 95%. These compounds were synthesized by Hyo-Joong Kim at the Foundation for Applied Molecular Evolution.

These results suggested that borate controls the regiochemistry of two microscopic steps in the formose process starting with erythrulose. First, borate appears to direct the enolization of erythrulose to give its 1,2-enediol in preference to its 2,3-enediol. This is interpreted as the consequence of borate coordinating to the C3 and C4 alcohol groups of erythrulose; the anionic nature of the borate complex is expected to suppress enolization at carbons 3 and 4. Once the 1,2-enediol is formed, however, the borate appeared to direct the incoming HCHO electrophile to the more hindered center carbon-2; the intrinsic regiochemistry in the absence of borate is expected to involve nucleophilic attack at carbon-1. Diastereocontrol of aldol additions by borate is well known in synthetic chemistry[44], especially in non-aqueous media, but was unexpected in water, however.

Relevant to the reactivity of the pentoses formed from $H^{13}CHO$ and erythrulose in the presence of borate, was the observation that these branched sugars had no enolizable proton alpha to the C=O aldehyde unit. They were therefore stable against enolization. Further, they formed cyclic hemiacetals via the reaction of their aldehyde units with the 4-hydroxyl group; hemiacetal formation appears to slow the enolization of sugars generally. Further, they appeared to be stabilized by borate, although the breadth of the ^{13}C resonances of the borate complexes at pH 10.4 suggested that they were (at least) conformationally dynamic. They could, however, undergo slow retroaldol reaction to give glycolaldehyde and glyceraldehyde, the C2 and C3 species that have already been shown to give pentoses and pentuloses in the presence of borate.

The outcome of the reaction between glycolaldehyde and glyceraldehyde formed by the retroaldol reaction is also controlled by borate. The initial products of the retroaldol reaction are glycolaldehyde and the 1,2-enediol of glyceraldehyde. If these react directly as the electrophile and the nucleophile respectively, the results are the corresponding pentuloses, ribulose and

xylulose. If, however, the 1,2-enediol of glyceraldehyde undergoes a retroenolization to give glyceraldehyde, and glycolaldehyde undergoes an enolization to give the 1,2-enediol of glycolaldehyde, then glycolaldehyde and glyceraldehyde may enter the 2 + 3 = 5 reaction as the nucleophile and the electrophile respectively to give one or more of the four diastereoisomeric pentoses ribose, arabinose, xylose and lyxose.

Discussion

These experiments establish many features of the reaction of formaldehyde (HCHO) and lower carbohydrates under conditions expected following the aqueous erosion of igneous rocks containing both olivine and tourmalines. All of these lead to stable pentoses. As with any prebiotic experiment, it is difficult to select environmental parameters (temperature, pH, reaction time) that strike a defensible compromise between the need for reactions that proceed rapidly enough to measure in real time and outcomes that are likely to emerge after thousands or millions of years. Here, we have chosen a pH of 10.4 that is higher than that likely to be sustained (at least on the surface of the early Earth under a CO_2 atmosphere) and at temperatures (65 °C) that may be higher than plausible on early Earth. The choice of harsher-than-perhaps-plausible conditions strengthens, however, the inference that these processes generated stable borate-pentoses, including ribose, as organic minerals on early Earth. A previous study measured the stability of various borate-pentose complexes[45]. The relatively high affinity of ribose for borate suggested that after the pentose equilibrium is achieved, borate-ribose would predominate as an organic mineral.

A final parameter is, of course, the relative concentration of organic species. Excess HCHO (which is expected under current prebiotic models) drives the cycle in Figure 2-24 in a clockwise fashion. This may be an example of what is sought by individuals who advocate a "metabolism first" model for the formation of life, rather than a "genetics first" model. Here, the

borate complexes of the branched sugars would accumulate as organic minerals, with their retroaldol products consuming HCHO. A kinetics study of the enolization of dihydroxyacetone, glyceraldehyde and erythrulose found that borate slows the rate of enolization of erythrulose by a factor of 4.5, of dihydroxyacetone by a factor of 9.2, and glyceraldehyde by a factor of 3.3. Dihydroxyacetone was also found to enolize faster than erythrulose in carbonate by a factor of 1.69, whereas in the presence of borate, erythrulose enolizes by a factor 1.2 faster than dihydroxyacetone. The preliminary establishment of kinetic rates for the cycle allows for future *in-silico* modeling of this system.

The reaction of glycolaldehyde in the absence of HCHO, here again, demonstrates the ability of borate to control diastereoselectivity. Incubation of glycolaldehyde in carbonate-borate buffer generated just four detectable signals, assigned as threose, not erythrose. These were also assigned by their superimposition on the signals arising from authentic threose. This result is more than remarkable, as threose (not erythrose), as noted above, is one of very few replacements for backbone ribose that support rule-based molecular recognition. The borate complex of threose as an "organic mineral" also proved to be very stable against base-catalyzed decomposition. It is remarkable that the two sugars (ribose and threose) that have emerged from synthetic biological experiments as being especially able to create useful backbones are also the two that emerge from borate-moderated formose processes.

The concept of an "organic mineral" may prove to be useful in prebiotic chemistry generally. Although a few organic minerals are known on contemporary Earth, and one (mellite) has been proposed to be abundant on the surface of Mars, organic minerals are generally consumed in the biosphere by the life that inhabits it. On a prebiotic Earth, of course, a wider range of organic minerals are expected. Organic minerals involving borate and carbohydrates are

reasonably soluble in water; however, if they were important on early Earth, and if high concentrations were needed, such organic minerals would be useful only in evaporite basins. Such basins appear to be abundant on Mars, whether they could have existed on early Earth remains unknown.

Experimental

Buffer and Stock Solution Preparations

Depolymerizaton of paraformaldehyde: ^{13}C-labeled paraformaldehyde (1 g) Cambridge Isotope Laboratories, Andover MA) was suspended in D_2O (4 mL) and sodium deuteroxide (NaOD, 1.0 mL, 10 M, Cambridge Isotope Laboratories, in two 0.5 mL portions, the second 1 h after the first) at 4 °C. The paraformaldehyde is depolymerized under these conditions to give $H^{13}CHO$, half of which undergoes the Cannizzaro reaction to give labeled methanol ($H_3{}^{13}COH$, set in all NMR experiments to 49.500 ppm) and labeled formate ($H^{13}COO^-$, 171.6 ±0.02 ppm). These served as internal standards for both chemical shift and (approximated) concentration of products. The material was stored at -10 °C. The final concentration of total ^{13}C was 3.22 M. A ^{13}C NMR spectrum of $H^{13}CHO$ showed, in addition to the prominent peak at 82.30 ± .02 (assigned to the hydrate $H_2{}^{13}C(OH)_2$), small peaks at 90.18 (intensity relative to 82.30 = 0.037) and 86.31 (intensity relative to 82.30 = 0.012) , and 55.29 (intensity relative to 82.30 = 0.022).

Buffer B: A sodium borate (anhydrous) and boric acid buffer was prepared by mixing in D_2O (Cambridge Isotope Laboratories, 10 mL), $Na_2B_4O_7$ (Aldrich (anhydrous), 0.503 grams, 2.5 mmol contributing 250 mM to total borate concentration), and H_3BO_3 (61.4 mg, 1 mM, contributing 100 mM to total borate concentration). The final pD of this was 9.53.

CBA Buffer (Buffer C): CBA buffer was prepared by dissolving Na_2CO_3 (Fisher Scientific, 1.18 g, 11.1 mmol) and H_3BO_3 (Fisher Scientific, 0.172 g, 2.78 mmol) in 10 mL D_2O. This buffer has a pH of 10.4 with a final borate concentration of 278 mM.

Carbonate Solution: The carbonate solution was prepared by dissolving Na_2CO_3 (1.18 g) in D_2O (Cambridge Isotope Laboratories, 10 mL).

Carbonate Buffer: Carbonate Buffer with pH 10.4 was prepared by adding a solution of sodium bicarbonate (1.11 M) to a solution of sodium carbonate (1.11 M) until the pH reached 10.4, the same as the pH of CBA buffer.

Carbohydrate stock solutions: Dihydroxyacetone dimer (Aldrich, 3.0 g, 33.3 mmoles, 3.33 M) was dissolved in D_2O (Cambridge Isotope Laboratories, 10 mL), and the solution was incubated at room temperature for four days. Separate experiments showed that this generated the monomer, with minimal formation of self-aldol products.

General Experiemental Conditions

Detailed experimental conditions not reported in this section can be found in Appendix A.

Experimental A: Dihydroxyacetone and $H^{13}CHO$ in CBA buffer

Dihydroxyacetone dimer (Aldrich, 3.3 M, 100 μL) was dissolved in CBA buffer (1 mL). To this solution, $H^{13}CHO$ (Cambridge, 3.3 M, 50 μL) was added. The mixture was agitated vigorously using a vortex stirrer. A portion of the reaction mixture (500 μL) was transferred to an NMR tube. Acquisition of an NMR spectrum began after four minutes at 25 °C. Multiple time points were acquired. The following data represents the reaction composition after 9250 minutes (6.42 days).

^{13}C NMR (D_2O): δ 171.799, 167.313, 103.228, 73.990, 73.879, 72.460, 71.613, 71.564, 71.037, 70.038, 69.305, 69.214, 68.706, 68.058, 67.978, 66.986, 66.887, 65.445, 64.674, 64.346, 64.030, 63.801, 62.824, 61.775, 60.818, 59.334, 58.693, 58.510, 53.860, 49.962, 49.645, 49.500 (MeOH). Spectrum file: dha-100ul_h13cho-50ul_cba_120106_t=9250.pdf

Experimental B: Erythrulose and $H^{13}CHO$ in CBA buffer

Erythrulose (3.33 M, 100 μL) was mixed with CBA buffer (0.900 mL) and $H^{13}CHO$ (Cambridge Isotope Laboratories, 6 M, 50 μL). The mixture was agitated vigorously using a vortex stirrer. A sample of the reaction mixture (500 μL) was transferred to an NMR tube. Acquisition of an NMR spectrum began after four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.779, 166.698, 94.044, 72.461, 72.411, 69.325, 66.372, 64.671, 64.339, 63.839, 63.694, 63.450, 63.271, 63.149, 61.776, 49.500 (MeOH).

Spectrum file: erythrulose_100ul_cba_900ul_hcho50ul_nt=16_47sec_10spectra.pdf

Experimental C: Glycolaldehyde in CBA buffer

Glycolaldehyde dimer (20 mg) was dissolved in 1 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.042, 103.268, 82.318, 76.599, 71.598, 49.500 (MeOH).

Spectrum file: 070515_12C-glycolaldehyde_20mg_cba_1ml_refmeoh.pdf

Erythrulose in CBA buffer: Erythrulose (30 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O solution as an internal reference. Acquisition of an NMR spectrum began within four minutes and ended after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.026, 129.955, 110.287, 106.045, 103.195, 98.942, 79.151, 76.557, 71.625, 66.903, 62.828, 61.787, 60.891, 49.500 (MeOH), 33.593.

Spectrum file: 070510_erythrulose_28mg_cba_1ml_refmeoh.pdf

Dihydroxyacetone with CBA buffer: Dihydroxyacetone dimer (18 mg, Aldrich) was dissolved in CBA buffer (1 mL). To this was added 10 μL of 10% MeOH in D_2O as an internal reference. The solution was agitated using a hand vortexer and transferred to an NMR tube. Spectrum acquisition started within 4 minutes and ended after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.904, 111.961, 110.920, 106.617, 85.522, 84.095, 83.012, 82.875, 79.220, 77.324, 75.470, 73.861, 73.170, 69.916, 64.358, 63.603, 62.706, 60.143, 58.911, 56.355, 49.500 (MeOH). Spectrum file: 070530_dha_cba_meohref.pdf

Kinetics

Kinetic runs: Runs were initiated by mixing CBA buffer (0.85 mL), carbohydrate solution (3.17 M, 0.1 mL, final concentration 317 mM) and $H^{13}CHO$ (3.33 M, 0.05 mL, final concentration 167 mM) The final pH was measured to be 10.4. Previous independent runs with unadjusted carbonate buffer were run at pH 11.2. The reaction proceeded at room temperature with a half life measured in minutes, while the half life at pH 9.5 was measured in hours. Subsequently the pH of the carbonate solution was adjusted to 10.4 for kinetic runs.

To determine whether calcium had any impact on this rate, a solution of 1 M $CaCl_2$ in H_2O was treated with NaOH until its pH was 7.0. In an analogous run, including calcium at varying concentrations, no significant deviations were found.

CHAPTER 3
2'-DEOXYCYTIDINES CARRYING AMINO AND THIOL FUNCTIONALITY: SYNTHESIS AND INCORPORATION BY VENT (EXO-) POLYMERASE

The synthesis of 2'-deoxycytidine nucleosides bearing amino and thiol groups appended to the 5-position of the nucleobase via a butynyl linker is described. The corresponding triphosphates were then synthesized from the nucleoside and incorporated into oligonucleotides by Vent (exo-) DNA polymerase. The ability of Vent (exo-) polymerase to amplify oligonucleotides containing these functionalized cytidine derivatives in a polymerase chain reaction (PCR) was demonstrated for the amine-functionalized derivative.

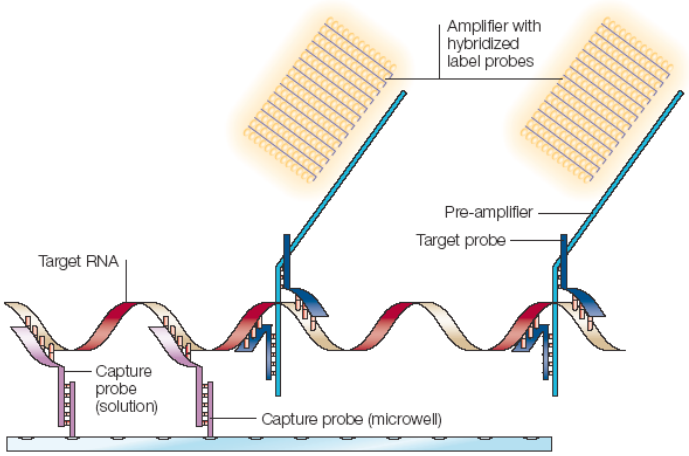


Figure 3-1.The Versant bDNA diagnostic test marketed by Bayer Diagnostics.[46]

Deoxyribonucleosides carrying functionality appended at the 5-position of uracil were introduced three decades ago to complement the functionality that DNA carries intrinsically.[47, 48] Fluorescent appendages attached to 2',3'-dideoxynucleotides have been the key to automated DNA sequencing.[49] Functionalized appendages may also increase the power and versatility of nucleic acids as receptors, ligands, and catalysts.[50] Modified standard nucleotides have been incorporated into in vitro evolution experiments.[51-56] The C5-position of the pyrimidine

nucleobases is an appropriate place to introduce functionality, as the site lies in the major groove of the duplex, where appendages do not interfere with Watson-Crick pairing. Appendages at this site are also well tolerated by RNA and DNA polymerases, which do not interact with the nucleobases in the major groove.[57-60]

Most of the previous work in this area has functionalized 2'-deoxyuridine, although some work has also appended functionality through the 5-position of 2'-deoxycytidine analogues.[61] We report here the synthesis of 2'-deoxycytidine nucleosides carrying amino or thiol terminal functionality appended at position C5 via a four-carbon alkynyl linker. We also report the successful incorporation of these as triphosphates into DNA using Vent (exo-) DNA polymerase and PCR amplification.

Reports suggest that protected 2'-deoxycytidine nucleosides carrying a C5 acetylene group readily undergo undesired cyclization.[62] Anticipating this, we explored chemistry to attach substituents to the 5-position of an unprotected cytidine nucleoside heterocycle to circumvent any additional side products. Trifluoroacetyl protected 1-aminobut-3-yne was synthesized from but-3-yn-1-ol via a facile route adapted from the literature.[63, 64] Mesylation of the free hydroxyl group of compound **1** allowed the conversion to the corresponding azide in DMF using sodium amide. Reduction of the azide to the corresponding amine was achieved via treatment with PPh_3. Acidification go the reaction mixture, followed by the extraction of side products yielded the free amine **5**, isolated by the neutralization of its acid salt. A final protection reaction with trifluoroacetic anhydride gave **6** in 51% yield (Figure 3-2).

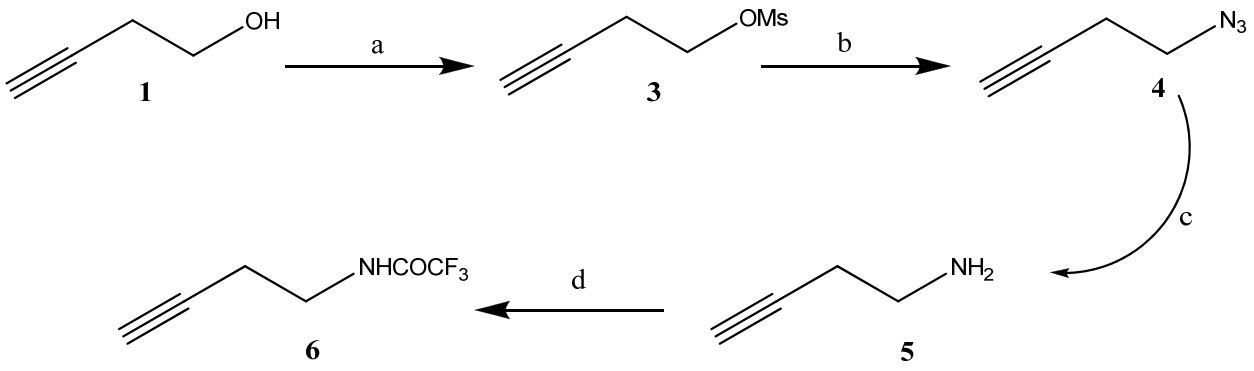


Figure 3-2. Synthesis of the trifluoroacetyl protected 1-aminobut-3-yne linker. Conditions: (a) MsCl/anhydrous ether/TEA/3 h/0ºC/H2O; (b) NaN3/anhydrous DMF/3.5 h/67ºC/H2O; (c) PPh3/anhydrous ether/H2O/18 h/0 ºC to rt/10% HCl(aq); 10% NaOH (aq); (d) anhydrous MeOH/CF3CO2CH3/18 h/0ºC to rt.

Sonogashira coupling of the linkers with 5'- tritylated 5-iodo-2'-deoxycytidine (**8**) in the presence of palladium(0) catalyst gave the desired products **9** and **10** (Figure 3-3). The 5'-tritylated nucleoside was used in preference to the unprotected 5'-hydroxy nucleoside because of ease in handling with respect to monitoring and its greater solubility. This resulted in a significant conservation of solvents during column chromatography purification. Substitution of the benzoyl-protecting group on **9** with a tert-butylthio group was achieved in a one-step reaction[65] with di-t-butyl-1-(t-butylthio)-1,2-hydrazine-dicarboxylate in the presence of LiOH providing the more stable compound **11**. Protection of **10** and **11** via treatment with acetic anhydride yielded mixtures of both mono (**14**, **15**) and diacetylated products (**13**, **15**) with the major products being the desired compounds **14** and **15**. Attempts to selectively deacetylate **12** and **13** with an equimolar amount of the Lewis acid $ZnBr_2$ gave the desired **14** and **15**, together with the unreacted starting compound **12** and **13**, in nearly equivalent amounts.

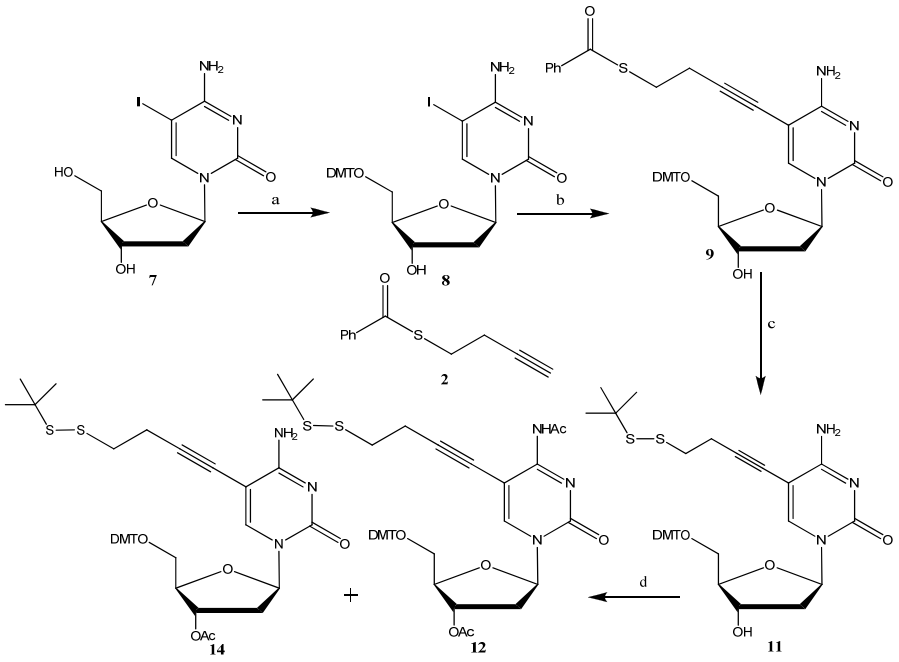


Figure 3-3a. Synthesis of 3'-O-Acetyl-5'-O-(4,4'-Dimethoxytrityl)-5-(1-butynyl)-2'-deoxycytidine tert-butyl disulfide (**14**). Conditions: (a) DMTCl/TEA/DMAP/Py anhydrous/6 h/0ºC to rt; (b) **2**/CuI/TEA /$Pd(PPh_3)_4$/DMF anhydrous/10-14 h/rt; (c) Di-t-butyl-1-(t-butylthio)-1,2-hydrazine-dicarboxylate/LiOH/MeOH-THF/90 min anhydrous. (d) Py/DMAP/TEA/Ac_2O/4 h/0ºC to rt.

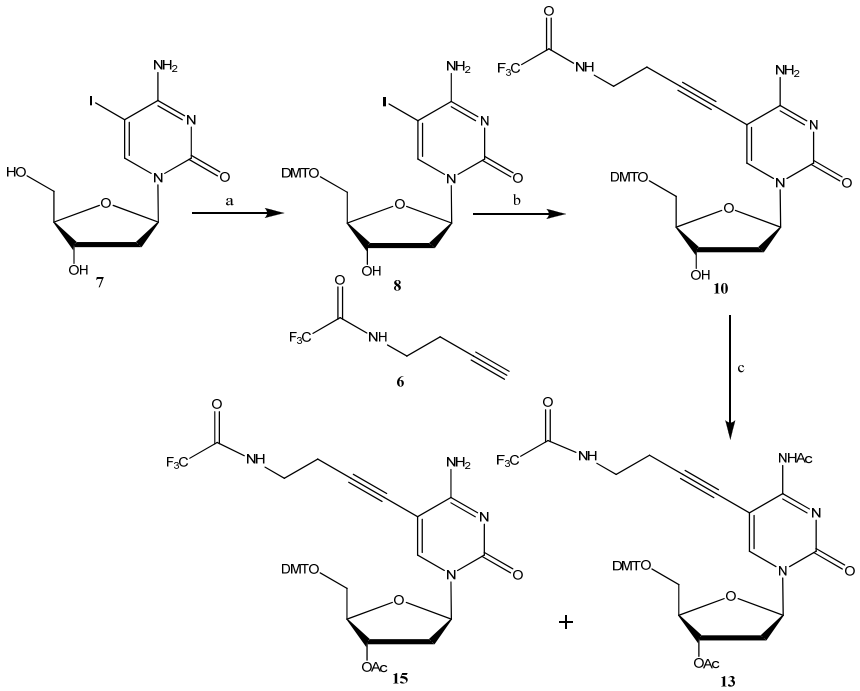


Figure 3-3b. Synthesis of 3'-O-Acetyl-5-(3''-Trifluoroacetamidobutynyl)-5'-O-4,4'-dimethoxytrityl-2'-deoxycytidine (**15**). Conditions: (a) DMTCl/TEA/DMAP/Py anhydrous/6 h/0ºC to rt; (b) **6**/CuI/TEA /$Pd(PPh_3)_4$/DMF anhydrous/10-14 h/rt; (c) Py/DMAP/TEA/Ac_2O/4 h/0ºC to rt.

Treatment with 2 equivalents of $ZnBr_2$ yielded the N-4 deacetylated and deprotected 5'-OH compounds **16** and **17** only. Compounds **16** and **17** were also obtained from **14** and **15** via the commercial deprotecting mixture (2.5% trichloroacetic acid in CH_2Cl_2) for solid phase oligonucleotide synthesis (Figure 3-4). Following literature procedure, triphosphate synthesis was carried out on compounds **16** and **17**. Phosphorous oxychloride in trimethyl phosphate and a proton sponge gave the phosphorodichloridates as intermediates, which were converted in situ with pyrophosphate to the corresponding triphosphates. Deprotection via NH_4OH hydrolysis gave the desired nucleotide triphosphates, **18** and **19** (Figure 3-5). Studies assessing the ability of the functionalized dCTP analogues (**18** and **19**) to serve as substrates for thermostable DNA polymerases under PCR conditions were then conducted. Polymerases often behave differently in the presence of unnatural nucleosides.[53] This prompted us to look at representatives from two evolutionary families of DNA polymerases[66]. Taq DNA polymerase from *Thermus aquaticus* (representing Family A) and Vent (exo-) DNA polymerase from *Thermococcus litoralis* (representing Family B) were examined as potential candidates. Primer extension experiments using PAGE purified 5'-[γ-^{32}P]-radiolabeled primer (5'-GCG TAA TAC GAC TCA CTA TAG-3') and template (5'-GAC ACG CGC TAT AGT GAG TCG TAT TAC GC-3') were performed using primers oligos ordered from Integrated DNA Technologies, Coralville, IA. Taq polymerase did not incorporate **18** nor **19** with any acceptable efficiency (data not shown). Vent (exo-) DNA polymerase, on the other hand, successfully incorporated both triphosphates **18** and **19.** Incorporation of **19** can be seen in Figure 3-6; Lane 4 opposite G in a single base extension. Upon the addition of a complete set of natural triphosphates excluding dCTP, the full-length extension product was obtained (Figure 3-6; Lane 5).

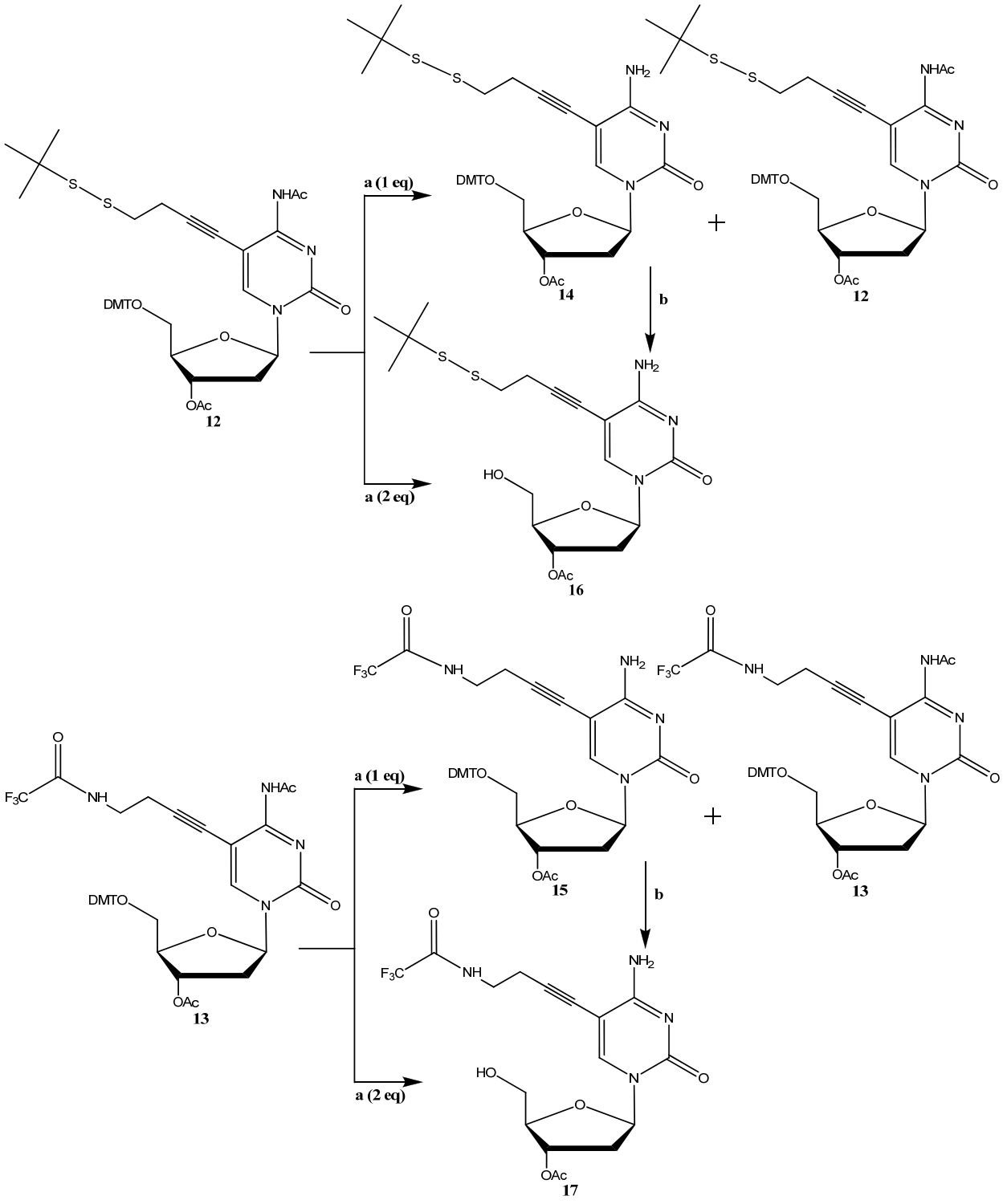


Figure 3-4. Synthesis of 3'-O-Acetyl-5-(3"-Trifluoroacetamidobutynyl)-2'-deoxycytidine (**17**) Conditions: (a) $ZnBr_2$/MeOH/$CHCl_3$/2 h/rt; (b) TCA in CH_2Cl_2/30 min/rt.

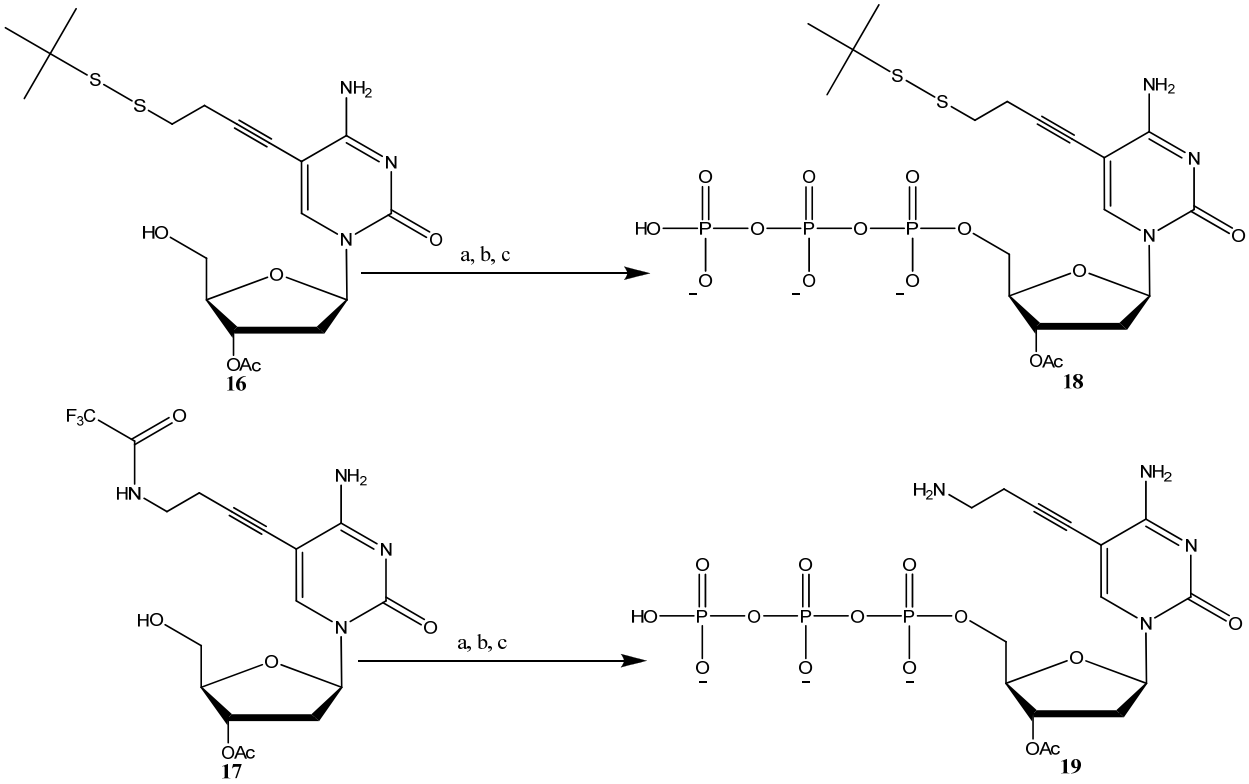


Figure 3-5. Synthesis of 5-(3"-tert-butyl disulfide 1-butynyl)-2'-deoxycytidine-5'-triphosphate (**18**) and 5-(3"-Trifluoroacetamidobutynyl)-2'-deoxycytidine-5'-triphosphate (**19**). Conditions: (a) $POCl_3$/$(MeO)_3P(O)$/proton sponge/2.5 h/0 ºC; (b) Tri-n-butylammonium pyrophosphate/n-tributylamine/DMF/TEAB/2 min/0 ºC to rt; (c) NH_3/rt/18 h.

Incorporation trials using analogue **18**, with a protected thiol functional group, was also successful (data not shown). PCR amplification of an oligonucleotide replacing dCTP with compound **19**, displaying a free amino group, using Vent (exo-) polymerase was also successful (Figure 3-7; Lane 6). The PCR experiment incorporated 22 and 31 functionalized cytidine analogues per strand in the forward and reverse reactions respectively. Low concentrations of triphosphate (2 μM) were used in the previous PCR amplification. Though compound **19** alone supported the synthesis of full-length products at such low concentrations (Figure 3-7; lane 5), doubling the concentration of **19** increased the amount of full-length product formed. This

suggested that **19** was able to support PCR amplification at concentrations similar dNTPs, though at a lower efficiency. In an experiment where a 1:1 mixture of **19** and dCTP was used, reduction in the amount of full-length product was observed relative to dCTP alone which is consistent with this conclusion. As a caveat, it is important to note that though these results are consistent with, but do not absolutely prove, the presence of the functionalized cytidine derivative in the product. Two features of these results are particularly noteworthy.

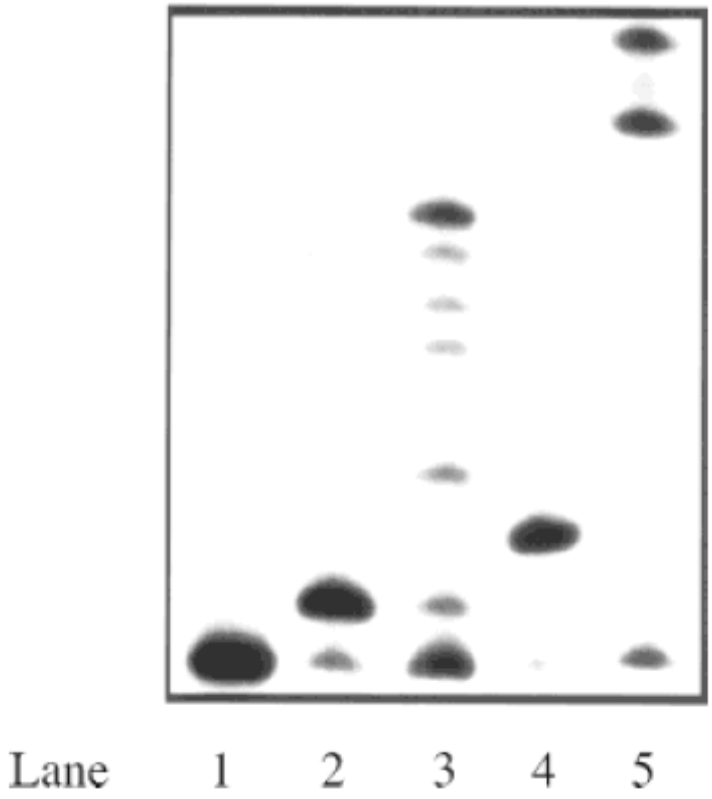


Figure 3-6. Primer extension experiment using amino-functionalized 2'-deoxycytidine triphosphate **19**. Denaturing 20% PAGE-urea gel was used. 5'-Radiolabeled primer was visualized using a phosphoimager. All lanes contain annealed primer and template, buffer, and water. Lane 1: All natural dNTPs (0.1 mM each), no polymerase (negative control). Lane 2: Vent (exo-) DNA polymerase and dCTP only showing pausing after incorporation of dC. Lane 3: Vent (exo-) polymerase and natural dNTPs showing formation of full-length product (positive control). Lane 4: Vent (exo-) polymerase and **19** only, showing incorporation of a single **19**; lower mobility due to positively charged functionality. Lane 5: Vent (exo-) polymerase and **19**, dTTP, dGTP, dATP, expecting incorporation of three **19**s.

First, in primer extension experiments, DNA carrying 3 equivalents of **19** showed a double band of products. Polymerases frequently add a non-templated nucleotide to the product. We believe this is the simplest explanation for this observation.[67] Second, although **19** supported the PCR amplification of DNA, the yield appeared to be lower than with standard dCTP. We considered the possibility that the positively charged amine linker group may disrupt the intercalation of the similarly cationic ethidium bromide.

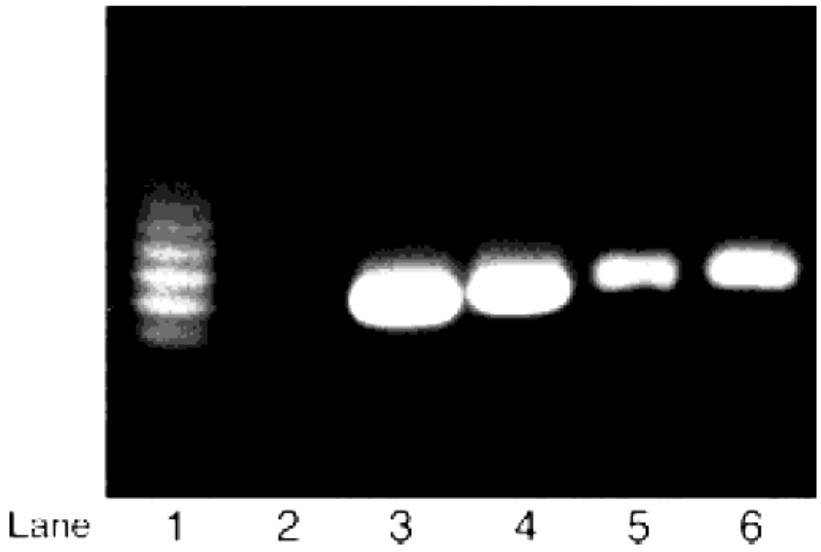


Figure 3-7. PCR amplification with **19** replacing dCTP. Agarose gel (2%) was used and stained with ethidium bromide. A total of 25 PCR cycles were run, with 2 min each incubation. Lane 1: Promega DNA ladder 25-300 nts. Lane 2: Negative control, lacking polymerase. Lane 3: Positive control with standard dNTPs. Lane 4: Positive control including both 2'-dCTP and **19** (1:1 ratio) and standard dNTPs. Lane 5: With 2 μM **19**, dATP, dGTP and dTTP. Lane 6: With 4 μM **19**, dATP, dGTP and dTTP. Template (100mer): 5'-CGC ATT ATG CTG AGT GAT ATC TAT CCA GAC CTA GAA AGA GTG CAC TGA TGC TGT TCG AGC GCA CGG CCT CCA ACA TGC CGT CCA TGC ACC ACT AGA CCT C-3'. Primer (24mer): 5'-GAG GTC TAG TGG TGC ATG GAC GGC- 3'. Reverse primer (24mer): 5'-CGC ATT ATG CTG AGT GAT ATC TAT-3'.

As the intensity of the band increases when the concentration of **19** is doubled, we we forced to reject this hypothesis. Rather, it seems the replacement of dCTP by **19** simply lowers the yield of full-length product. Accessibility to functionalized nucleoside derivatives and polymerases that

accept their triphosphates sufficiently well to support the polymerase chain reaction gives two new tools to those wishing to perform molecular biology with DNA containing thiol and amino functionalities. This is another significant step toward the development of a synthetic biology.[68]

Experimental

General Experimental: Chemical reagents were purchased from Acros, Aldrich, Fischer, Berry & Associates. Reactions were carried under argon (Ar). TLCs were obtained on Whatman, silica gel plates (250 μm Layer) and monitored with 254 nm fluorescence. Room temperature, where mentioned, range from 22- 24 ºC. Silica gel (230-425 mesh, Fischer) was used for column chromatography (CC). AG 1-X8 resin (Bio-Rad) was obtained as the Cl^- form and converted to the HCO_3^- form by washing with 16 volumes of 1M NH_4HCO_3 solution, followed by de-ionized water and finally with 0.5 M NH_4HCO_3 solution; no Cl^- was detected. Ion-exchange chromatography was performed with DEAE Sephadex (Sigma) equilibrated in 0.2M (Et_3NH) HCO_3^- (pH 7.0). NMR spectra was recorded on a Varian XL 300 spectrometer at 300 MHz, using TMS as an external reference for ^1H and ^{13}C while H_3PO_4 for ^{31}P NMRs. UV spectra were measured on a Varian Cary 1 Bio spectrophotometer. Mass spectrometry were recorded by the Spectroscopy Services of the University of Florida Chemistry Department; for LSIMS: Finnigan MAT-95Q apparatus, for HPLC/ESI-MS: Finnigan MAT (San Jose, CA) LCQ in electrospray ionization (ESI) mode and Beckman Instruments (Fullerton, CA) System Gold, model 126 pump with Waters RP18 Symmetry Shield analytical column (2.1 x 150mm + guard). HPLC was performed using a Waters PrepLC 4000 System (1.2 mL Injection Loop) with a 486 tunable absorbance detector. Reversed - phase HPLC separation was done using a Waters Prep Nova - Pak HR C18 Preparative Column (6 μM, 60Å, 25 x 100mm; WAT038510) with Waters Prep Nova - Pak HR C18 Preparative Guard Column (6 μM, 60Å, 25 x 10 mm; WAT038528).

General Procedure for the Synthesis of the Linker: Buty-3-yne-1-methanesulfonate (**3**): Methanesulfonylchloride (34.37 g, 0.3 mol, 1.5 eq.) was added dropwise to a stirred solution of butyl-1-yne-3-ol 1 (14.0 g, 0.2 mol) and TEA (30.35 g, 0.3 mol, 1.5 eq.) in anhydrous ethyl ether (200 mL) at 0 ºC. After 3h, water (100 mL) was added to the S 2 reaction mixture. The organic layer was separated, washed with water (100 mL), dried with Na_2SO_4 and distilled in vacuo to yield 26.50 g (89.6 %) of 5 as dark yellow liquid. ^{1}H NMR ($CDCl_3$) δ : 2.10 (t, 1H, -CH), 2.64 - 2.69 (m, 2H, -CH_2), 3.07 (s, 3H, -CH_3), 4.31 (t, 2H, -CH_2).

4-Azido-but-1-yne (**4**): Sodium azide (16.40 g, 0.25 mol) was added to a solution of the mesylate **3** (15.04 g, 0.1 mol) in anhydrous DMF (80 mL). The mixture was stirred for 3.5 h at 67 ºC. The reaction mixture was poured over water (30 mL) and extracted with ethyl ether (3 x 100 mL). The solution was then dried over Na_2SO_4 and evaporated to yield 8.00 g (83.0%) of **4** as yellow liquid. ^{1}H NMR ($CDCl_3$) δ : 2.08 (t, 1H, -CH), 2.47 - 2.53 (m, 2H, -CH_2), 4.40 (t, 2H, -CH_2).

But-3-yne-1-amine (**5**): To a solution of azide **4** (8.00 g, 0.08 mol) in ethyl ether (50 mL) at 0 ºC, PPh_3 (23.18 g, 0.08 mol) was added and allowed to stir for 1.5 h. Water (3.0 mL) was then added to the reaction mixture and allowed to stir for another 16 h. The reaction mixture was poured over 10% aqueous HCl, extracted with ethyl ether (3 x 100 mL). The aqueous layer was made basic (pH = 9.0) with 10% aqueous NaOH and extracted with ethyl ether (5 x 100 mL). The solvent was dried over Na_2SO_4 and evaporated to yield 1.4 g (24.0 %) of **5** as light yellow liquid. ^{1}H NMR ($CDCl_3$) δ : 2.09 (t, 1H, -CH), 2.64 - 2.69 (m, 2H, -CH_2), 2.84 (t, 2H, -CH_2), 1.61 (bs, 2H, -NH_2).

N-But-3-ynyl-2,2,2-trifluoroacetamide (**6**): To a stirred solution of butyl-1-yne-3-amine **5** (1.4 g, 0.02 mol) in anhydrous MeOH (5.0 mL) at 0 ºC, was added methyl trifluoroacetate (2.45

mL, 0.024 mol 1.2 eq.) drop wise and allowed to stir for next 14-16 h at room temperature. Thereafter, the solvent was evaporated and the residue was diluted with 10 mL chloroform and washed with aqueous $NaHCO_3$ solution (2 x 10 mL) and water (1 x 10 mL). The organic layer was dried over Na_2SO_4 and evaporated to yield an orange residue which upon distillation yielded 6 1.70 g (50.8 %), as light yellow liquid (solidified at -20 ºC). 1H NMR ($CDCl_3$) δ : 2.09 (t, 1H, -CH), 2.47 - 2.53 (m, 2H, $-CH_2$), 3.53 (t, 2H, $-CH_2$), 6.80 (bs, S3 1H, -NH). 13 C NMR ($CDCl_3$) δ : 18.87 ($-CH_2$), 21.98 ($-CH_2$), 70.88 (-CH), 80.21 (-C), 114.03, 117.84, 120.89 ($-CF_3$), 156.91 - 158.92 (-CO). Mass (m/z): 166 (M +1). 3)

General Procedure for the 5'-Tritylation Reaction: 5'-O-4,4'-dimethoxytrityl-5-iodo-2'-deoxycytidine (**8**): 5-iodo-2'-deoxycytidine **7** (0.353 g, 10.0 mmol) was co-evaporated twice with pyridine and then dissolved in anhydrous pyridine (5.0 mL). To this solution at 0 ºC under Ar, Et_3N (0.278 mL, 20.0 mmol, 2.0 eq.), DMAP (0.030 g, 2.5 mmol) and 4,4'-dimethoxytrityl chloride (0.677g, 20.0 mmol, 2.0 eq.) were added and stirred for first 15 min at 0 ºC and then at room temperature for 5 hours. The reaction mixture was quenched with MeOH (2 mL), the solvent was evaporated and the residue was extracted with EtOAc, washed with aqueous $NaHCO_3$ solution. Usual work-up yielded the semi-solid residue which was purified by silica column chromatography ($CHCl_3$: MeOH; 97:3 v/v) to give 0.260g (42 %) of, **8** (R_f. 6.5; $CHCl_3$: MeOH; 82:18, v/v) as white solid. ^{1}H NMR (DMSO-d_6) δ: 2.03 - 2.28 (m, 2H, 2'), 3.15-3.20 (m, 2H, 5'), 3.74 (s, 6H, OMe), 3.89- 3.90 (m, 1H, 4'), 4.18-4.22 (m, 1H, 3'), 5.26 (d, 1H, 3'-OH), 6.10 (t, 1H, 1'), 6.60 (bs, 1H, -NH), 6.89 (d, 4H, aromatic), 7.22 - 7.41 (m, 9H, aromatic), 7.85 (bs, 1H, NH), 7.96 (s, 1H, H-6). LRMS-FAB (NBA): 693.71 (M+) 4)

General Procedure for Sonogashira Coupling: Compound **8** (0.353 g, 10.0 mmol) was dissolved in anhydrous DMF (10 mL) and degassed for 10minutes. Compound **2** (0.09 g, 10.0 m

mol, 1.0 eq.) or 6 (0.173 g, 15.0 m mol, 1.5 eq.), TEA (0.276 mL, 20.0 mmol, 2.0 eq.), CuI (0.034 g, 2.0 mmol, 0.2 eq.) and $(Ph_3P)_4Pd$ (0.155 g, 1.0 mmol, 0.1 eq.) were added to the reaction mixture with constant stirring at room temperature under Ar. After 18 h of stirring, the reaction mixture was diluted with EtOAc (3 mL), the solvent was removed in vacuo, the residue was washed with aqueous $NaHCO_3$, extracted with EtOAc (3 x 25 mL), dried over Na_2SO_4 and evaporated to yield a semi-solid residue which was then purified by silica column chromatography.

5'-O-4, 4'-dimethoxytrityl-5-(4-benzoylthio-1-butynyl)-2'-deoxycytidine (**9**): Elution at: 96:4 ($CHCl_3$: MeOH; v/v) to yield 0.254 g (42.0 %) as foamy yellow solid. R_f: 0.5 (88:12; $CHCl_3$: MeOH, v/v). ^{1}H NMR (CD_3OD) δ : 2.12 - 2.24 (m, 1H, 2'), 2.51 (t, 2H, - CH_2), 2.71 - 2.80 (m, 1H, 2'), 2.94 -3.04 (m, 2H, -CH_2), 3.24 - 3.38 (m, 2H, 5'), 3.75 (s, 6H, -OMe), 4.17 - 4.20 (m, 1H, 4'), 4.46 - 4.54 (m, 1H, 3'), 5.92 (bs, NH), 6.60 (t, 1H, 1'), 6.82 (d, 4H, aromatic), 7.18 - 7.36 (m, 12H, aromatic), 7.90 (d, 2H, ArH), 8.14 (d, 1H, H-6). ^{13}C NMR (CD_3OD) δ :21.2(3"), 27.8(4"), 36.1(2'), 55.1(OCH_3), 63.7(5'), 72.1(3'), 77.5 (1"), 86.3(1'), 87.0(4'), 88.1($OC(PH_3)_3$), 90.1(2"), 94.5(5), 127.0, 12128.0, 129.0, 130.2, 133.8, 135.8, 136.4(Ar), 143.6(6), 154.1(2), 158.2(4), 191.1(SCOPh). HRMS-FAB (NBA): m/z = 717.8134 (M+ + 1).

5'-O-4, 4'-dimethoxytrityl-5-(3"-Trifluoroacetamidobutynyl)-2'-deoxycytidine **(10):** Elution: 93:7; $CHCl_3$: MeOH; (v/v) to yield compound 0.164 g (42.0 %) as foamy yellow solid. Rf: 0.6 (88:12; $CHCl_3$: MeOH, v/v) ^{1}H NMR (CD_3OD) δ : 2.20 - 2.32 (m, 1H, 2'), 2.39 (t, 2H, - CH_2), 2.45 -2.53 (m, 1H, 2'), 3.25 - 3.32 (m, 2H + 2H, 5' + -CH_2), 3.75 (s, 6H, -OMe), 4.05 - 4.10 (m, 1H, 4'), 4.45 - 4.50 (m, 1H, 3'), 6.18 (t, 1H, 1'), 6.84 (d, 4H, aromatic), 7.18 - 7.36 (m, 9H, aromatic), 8.19 (d, 1H, H-6). HRMS-FAB (NBA): 694.2587 (M+)

General procedure for the replacement of the thiobenzoyl group: 5'-O-(4,4'-Dimethoxytrityl)- 5-(1-butynyl)-2'-deoxycytidine tert-butyl disulfide (**11**): To a stirred solution of **9** (0.113 g, 0.17 mmol) in THF/MeOH (both anhydrous) (3:1, 4.0 mL), ditert- butyl 1-(tert-butylthio)-1,2-hydrazine-dicarboxylate (0.07 g, 0.22 mmol) and LiOH. H_2O (0.14g, 0.34 mmol, 1.5 eq.) were added quickly, causing the reaction mixture to temporarily turn lilac. The reaction mixture was stirred for 1 h 20 min, diluted with Et_2O and washed with brine.[S5] The lilac middle layer formed was kept aside, diluted with CH_2Cl_2 and washed with brine. Combined organic phases were dried over Na_2SO_4 and concentrated to yield the crude product, which was purified by chromatography on silica gel ($CHCl_3$/CH_3OH 97:3), to yield 11 (0.90 g 68%) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ : 1.29 (s, 9H, t- butyl), 2.07 - 2.14 (m, 2H, 2'), 2.58 (t, 2H, -CH2), 2.71 -2.82 (m, 2H, -CH2), 3.29 - 3.40 (m, 2H, 5'), 3.77 (s, 6H, -OMe), 4.10 - 4.12 (m, 1H, 4'), 4.50 - 4.58 (m, 1H, 3'), 5.80 (bs, NH), 6.29 (t, 1H, 1'), 6.83 (d, 4H, aromatic), 7.19 - 7.40 (m, 9H, aromatic), 8.13 (d, 1H, H-6). ^{13}C NMR (CD_3OD) δ :21.2(3"), 27.8(4"), 30.1($C(CH_3)_3$), 38.1(2'), 48.0(C(CH3)3), 57.1(OCH_3), 63.7(5'), 72.4(3'), 77.5 (1"), 86.8(1'), 87.4(4'), 91.8($OC(PH_3)_3$), 94.5(2"), 113.0(5), 127.0, 128.0, 130.2, 136.1(Ar), 144.2(6), 154.8(2), 164.8(4). HRMS-FAB (NBA): 702.8277 (M+)

General Procedure for Acetylation Reaction: Compound **10** or **11** (1.0 mmol) was coevaporated with pyridine and then dissolved in anhydrous pyridine (2.0 mL), TEA (2.0 mmol, 2.0 eq.), DMAP (0.25 mmol, 0.25 eq.) and Ac_2O (2.5 mmol, 2.5 eq.) were added to the reaction mixture and allowed to stir at 0ºC under Ar for 30 min. After 3 h stirring at room temperature, the reaction was quenched with MeOH (5 mL). The solvent was removed in vacuo, the residue obtained was washed with water and extracted with EtOAc (3 x 20 mL). The organics were dried

over Na_2SO_4 and concentrated to afford a residue which was purified by silica gel Column Chromatography to yield the di-**12**, **13** and mono-**14**, **15** acetylated products respectively.

3'-O-4-N-Diacetyl-5'-O-(4,4'-dimethoxytrityl)-5-(1-butynyl)-2'-deoxycytidine tert-butyl disulfide (**12**): Eluting at 98:2; $CHCl_3$: MeOH; (v/v) and 9 (0.020 g; 54.80 %) R_f: 0.8 ($CHCl_3$: MeOH; 88: 12, v/v). ^{1}H NMR ($CDCl_3$) δ : 1.29 (s, 9H, t- butyl), 2.06 (s, 3H, -OCH_3), 2.26 - 2.40 (m, 2H, 2'), 2.50 (t, S6 2H, -CH_2), 2.69 (s, 3H, -NCH3), 2.78 -2.88 (m, 2H, -CH_2), 3.36 - 3.80 (m, 2H, 5'), 3.76 (s, 6H, -OMe), 4.22 - 4.25 (m, 1H, 4'), 5.35 - 5.42 (m, 1H, 3'), 6.30 (t, 1H, 1'), 6.83 (d, 4H, aromatic), 7.22 - 7.41 (m, 9H, aromatic), 8.35 (d, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ : 21.2(C(CH_3)), 23.5(3"), 26.8(4"), 30.1(C(CH_3)$_3$), 38.1(2'), 41.0(C(CH_3)$_3$), 57.1(OCH_3), 63.7(5'), 72.0(3'), 75.5 (1"), 87.1(1'), 87.4(4'), 91.8(OC(PH3)3), 96.5(2"), 113.8(5), 127.0, 128.0, 129.2, 130.2, 136.1(Ar), 137.0(6), 143.5(6), 154.8(2), 158.3(4), 161.2(OCH3). HRMS-FAB (NBA): 785.9912(M+)

3'-O-4-N-Diacetyl-5'-O-4, 4'-dimethoxytrityl-5-(3"-Trifluoroacetamidobutynyl)- 2'-deoxycytidine (**13**)**:** Eluting at 99:1; $CHCl_3$: MeOH; (v/v) R_f: 0.8 ($CHCl_3$: MeOH; 82: 18, v/v). ^{1}H NMR ($CDCl_3$) δ : 2.09 (s, 3H, -OCH_3), 2.12 - 2.18 (m, 2H, 2'), 2.39 (t, 2H, -CH2), 2.98 (s, 3H, -NCH3), 3.22 - 3.37 (m, 2H + 2H, 5' + -CH_2), 3.75 (s, 6H, -OMe), 4.21 - 4.26 (m, 1H, 4'), 5.31 - 5.36 (m, 1H, 3'), 6.25 (t, 1H, 1'), 6.80 (d, 4H, aromatic), 7.19 - 7.39 (m, 9H, aromatic), 8.35 (d, 1H, H-6). HRMS-FAB (NBA): 780.7981(M+)

3'-O-Acetyl-5'-O-(4,4'-Dimethoxytrityl)-5-(1-butynyl)-2'-deoxycytidine tert-butyl disulfide (**14**) : Eluting at 96:4; $CHCl_3$: MeOH (v/v) R_f: 0.6 ($CHCl_3$: MeOH; 88: 12, v/v). ^{1}H NMR ($CDCl_3$) δ : 1.30 (s, 9H, t- butyl), 2.05 (s, 3H, -OCH_3), 2.20 - 2.31(m, 1H, 2'), 2.58 (t, 2H, -CH_2), 2.66-2.75(m, 1H, 2'), 3.34 - 3.41(m, 2H + 2H, 5' + -CH_2), 3.79(s, 6H, -OMe), 4.18 – 4.21

(m, 1H, 4'), 5.18 - 5.20 (m, 1H, 3'), 5.95 (bs, 1H,-NH), 6.18(t, 1H, 1'), 6.84(d, 4H, aromatic), 7.20 - 7.43(m, 9H, aromatic), 8.18(d, 1H, H-6). HRMS-FAB (NBA): 743.9544 (M+)

3'-O-Acetyl-5-(3"-Trifluoroacetamidobutynyl)-5'-O-4,4'-dimethoxytrityl-2'-deoxycytidine (**15):** Eluting at 98:2; $CHCl_3$: MeOH (v/v) R_f: 0.7($CHCl_3$: MeOH; 82 : 18, v/v).[S7] ^{1}H NMR ($CDCl_3$) δ : 2.05(s, 3H, -OCH_3), 2.20 - 2.31(m, 1H, 2'), 2.42(t, 2H, -CH_2), 2.62-2.71(m, 1H, 2'), 3.24 - 3.40(m, 2H + 2H, 5' + -CH_2), 3.78(s, 6H, -OMe), 4.16 - 4.19(m, 1H, 4'), 5.31 – 5.36(m, 1H, 3'), 6.09(bs, 1H,-NH), 6.30(t, 1H, 1'), 6.82(d, 4H, aromatic), 7.23 - 7.43(m, 9H, aromatic), 8.16(d, 1H, H-6). HRMS-FAB (NBA): 736.2674 (M+)

General Procedures for De-protection

Method A: To a stirred solution of compounds **12** or **13** (0.2 mmol) in anhydrous MeOH and $CHCl_3$ (4:1; 2 mL) under Ar, was added $ZnBr_2$ (0.4 mmol; 2.0 eq.) and allowed to stir for 2 hr at room temperature. The solvent was removed in vacuo and the residue was purified over silica gel CC to give compounds **14** or **15** respectively. Method B: To a stirred solution of compound **14** or **15** (0.5 mmol) in anhydrous CH_2Cl_2 (2 mL) under Ar, was added deprotecting solvent (2.0 mL) (trichloroacetic acid in dichloromethane). It was allowed to stir for 30 min at room temperature. Thereafter, a drop of TEA was added to the reaction mixture, the solvent removed in vacuo and the residue purified over CC to give compounds **16** or **17** respectively.

3'-O-Acetyl-5-(1-butynyl)-2'-deoxycytidine tert-Butyl Disulfide (**16):** Elution at 94:6; $CHCl_3$: MeOH (v/v), gave compound **16** (0.012 g, 52.17%). R_f: 0.5 ($CHCl_3$: MeOH; 82:18, v/v). ^{1}H NMR ($CDCl_3$) δ: 1.34 (s, 9H, t- butyl), 2.09 (s, 3H, -OCH_3), 2.31 - 2.41 & 2.48 - 2.58 (m, 2H, 2'), 2.78 - 2.80 (m, 2H, -CH_2 x 2), 3.89 - 3.96 (m, 2H, 5'), 4.10 - 4.15 (m, 1H, 4'), 5.33 - 5.38 (m, 1H, 3'), 6.08 (bs, 1H, NH), 6.19 (t, 1H, 1'), 7.98 (d, 1H, H-6). 13C NMR ($CDCl_3$) δ : 20.2(3"), 21.0($CO(CH_3)$), 30.1($C(CH_3)_3$), 38.1(4"), 38.5(2'), 48.2($C(CH_3)_3$), 63.7(5'), 73.0(1"), 75.5 (2'),

86.0(1'), 88.0(4'), 92.0(2"), 95.5(5), 136.0(6), 143.5(2), 154.1(2). LRMS-FAB (NBA): 442.15 (M+)

3'-O-Acetyl-5-(3"-Trifluoroacetamidobutynyl)-2'-deoxycytidine (**17**)**:** Elution at 96:4; $CHCl_3$:MeOH (v/v), gave compound **17** (0.012 g, 52.17%). R_f: 0.5($CHCl_3$:MeOH; 82: 18, v/v). ^1H NMR ($CDCl_3$) δ: 2.07(s, 3H, -OCH_3), 2.16 - 2.24 & 2.45 - 2.55(m,2H, 2'), 2.70(t, 2H, -CH_2), 3.52(t, 2H, -CH_2), 3.79 - 3.81(m, 2H,5'), 4.10 - 4.15(m,1H, 4'), 5.25 - 5.30(m, 1H, 3'),6.22(t, 1H, 1'), 8.23(d, 1H, H-6). LRMS-FAB: 434.13 (M+). ^{13}C NMR ($CDCl_3$) δ : 19.8(3"), 22.5(CO(CH_3)), 38.8(4"), 39.0(2'), 61.8(5'), 72.0(3'), 75.5(1'), 74.5 (1"), 86.0(1'), 87.5(4'), 92.7(2"), 95.5(5), 143.0(6), 155.8(2), 154.1(2), 168.0(4), 171.8(CO(CF_3)), 172.0(CO(CH_3)).

General Procedure for triphosphate synthesis: The following precautions were taken: a) Compound **16** or **17** was co-evaporated with pyridine and dried over P_2O_5 overnight before the reaction. b) Tribuylammonium pyrophosphate in DMF (anhyd.) and n-tribuylamine were kept under Ar atmosphere over 4 Å molecular sieves (activated) for 48 h. Compound **16**, **17** (0.18 mmole) was stirred in trimethylphosphate (0.70 mL) with 1,8-bis (dimethylamino) naphthalene (proton sponge, 0.27 mmol) at 0 ºC for 5 min. Phosphorous oxychloride (0.27 mmol) was added and the mixture was allowed to stir at 0-4 ºC for 2.5 h. The solution of tri-n-butylammonium pyrophosphate (10.0 mmol), tri-n-butylamine (10.0 mmol) in anhyd. DMF (2.0 mL) was added quickly to the reaction mixture at 0 ºC. After 1 min, an aqueous solution of triethylammonium bicarbonate (20 mL; 0.2 M) was added and allowed to stir for next 10 min. After evaporation (in vacuo), the residue was treated with aqueous ammonia (2 mL), and stirred overnight at room temperature. Ammonia is removed in vacuo and the residue was subjected to the purification protocols which follow. Purification: This was carried out in two different steps, ion-exchange, followed by RP-HPLC. i) Ion-exchange: The crude sample, after the removal of NH_4OH, was

dissolved in water (MQ grade) and charged on a DEAE (diethylaminoethyl) cellulose (fast flow; fibrous form, 25.0 g in a 26.0 x 5.0 cm column which was previously equilibrated in 0.2 M TEAB, pH 7.0). Starting from 0.2 M TEAB, the triphosphate was collected at 0.4 to 0.5 M. The fractions (UV active) were pooled and lyophilized. ii) RP-HPLC (Carried out by Dr. A. P. Kamath): HPLC was done at a flow rate of 0.75 mL/ min. using 10mM (Et_3NH)OAc (pH 7.0) (mobile phase A) and 20% MeCN in mobile phase A (mobile phase B) in the following linear gradient as: 95% A/ 5% B(10 min), 65% A/ 35% B(30 min), 100% B(10 min) and finally 95% A/ 5% B(10 min); retention time 24 min. The fractions were pooled and lyophilized to yield the pure triphosphate.

5-(3"-tert-butyl disulfide 1-butynyl)-2'-deoxycytidine-5'-triphosphate (**18**): UV (water) λ_{max} = 237, 297 nm. ^{31}P NMR (D_2O) δ: -9.80, -9.90 (d, 1P, J= 19.2 Hz, γ), −10.04 −10.06 (d, 1P, J= 19.2 Hz, α), −22.12 (t, 1P, J= 19.0 Hz, -β). ^{1}H NMR (D_2O) δ: 1.34 (s, 9H, t-butyl), 2.18 - 2.20 (m, 1H, 2/), 2.22 - 2.35 (m, 1H, 2/), 2.75 (t, 2H, J= 4.6Hz, 2"), 2.80 (t, 2H, J= 4.6Hz, 1"), 4.02 - 4.08 (m, 2H, 5'), 4.42 - 4.49 (m, 2H, 4'), 4.46 - 4.54 (m, 2H, 3'), 6.12 (t, 1H, J= 6.6Hz, 1'), 7.90 (s, 1H, H-1). HPLC MS: 640 (M+H)

5-(3"-Trifluoroacetamidobutynyl)-2'-deoxycytidine-5'-triphosphate (**19**): UV (water) λ max = 234.5, 295.0 nm ^{31}P NMR (D_2O) δ: -9.09, -9.25 (d, 1P, J= 19.2 Hz, -γ), −10.42, −10.57 (d, 1P, J= 19.2 Hz, -α), −21.75 (t, 1P, J= 19.0 Hz, -β). ^{1}H NMR (D_2O) δ: 2.10 - 2.20 (m, 1H, 2/), 2.29 - 2.39 (m, 1H, 2/), 2.73 (t, 2H, J= 4.6Hz, 2"), 3.05 (t, 2H, J= 4.6Hz, 1"), 3.68 - 3.72 (m, 2H, 5'), 4.08 - 4.11 (m, 2H, 4'), 4.31 - 4.40 (m, 2H, 3'), 6.07 (t, 1H, J= 6.6Hz, 1'), 7.88 (s, 1H, H-1). HPLC MS: 535 (M+H)

General Experimental: Biochemical:

The primer (5'-GCG TAA TAC GAC TCA CTA TAG –3') and template (5'-GAC ACG CGC TAT AGT GAG TCG TAT TAC GC–3') were purchased from IDT. The primer was 5'

radiolabeled with ^{32}P using T4 polynucleotide kinase (Promega) and Redivue [γ–^{32}P] ATP (Amersham Pharmacia Biotech). The radiolabeled primer was purified using QIAquick nucleotide removal kit (Qiagen). A solution of annealed primer was purified and template was prepared (9:1 ratio of unlabeled and labeled primer with a 20% excess of template) by incubating at 95 ºC for 5 min then cooling slowly to room temperature. The concentration for the functionalized dCTPs were approximated assuming $\lambda = 9000\ M^{-1}cm^{-1}$. Reactions were prepared [10 μL, 2 μM unlabeled primer, 0.2 μM γ–^{32}P labeled primer, 2.4 μM template, 1X thermopol reaction buffer, 1 μM dNTP(s), 2 units Vent (exo-) DNA polymerase (NEB)], incubated for 2 min at 72 ºC and quenched with 5 μL quenching buffer (95% formamide, 40 mM EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue). The reactions were analyzed by running 3 μL of each on 20% denaturing polyacrylamide gels. The reaction ran for 30 minutes at 37 °C. Quantity Reagent 1 mL 1 nmol primer 1 mL 5 units T4 polynucleotide kinase 1 mL adenosine 5'-[γ -^{32}P] triphosphate, triethyl ammonium salt 1 mL 10T T4 kinase buffer 6 mL water 10)

Acknowledgments

I am grateful to Dr. Jodie V. Johnson and the personnel at the mass spectroscopy lab at the Department of Chemistry for acquisition of the mass spectra. Useful discussions with Drs. C. R. Geyer, T. R. Battersby, S. C. Jurczyk, and H. A. Held are acknowledged. Extensive help in the HPLC purification by Dr. A. P. Kamath is highly acknowledged. This work was partially supported by grants from the Department of Defense 6402-202-LO-B and the National Science Foundation (CHE-0213575).

CHAPTER 4
FUTURE WORK

Though significant progress towards understanding the origins of life is shown here, much work remains to be done. These include GC/MS analysis of the carbohydrate formation reactions, exploring phosphate-buffered reaction mediums and the inclusion of trace elements such as magnesium, manganese and other DNA 'friendly' minerals. GC/MS studies could help further confirm and determine low concentration carbohydrates. Phosphate buffered solutions may yield phosphorylated carbohydrate products, perhaps with the aid of other trace minerals.

Molybdenum mineralogy may help us use dead end species, which do not have enolizable hydrogens or whose retro-aldols only yield their parent molecules. The branched species do not release HCHO easily. This may not be entirely bad, as they are storehouses of relatively stable carbohydrates.

The mineral molybdite (MoO_3) can equilibrate these, in water (pH 4.2, 70 °C) to linear carbohydrates via a Bilik style reaction (Fig. 4-1)[32]. As ribose and other pentoses are stabilized by borate under the conditions where they are formed, further analysis of the borate concentration landscape should also be pursued.

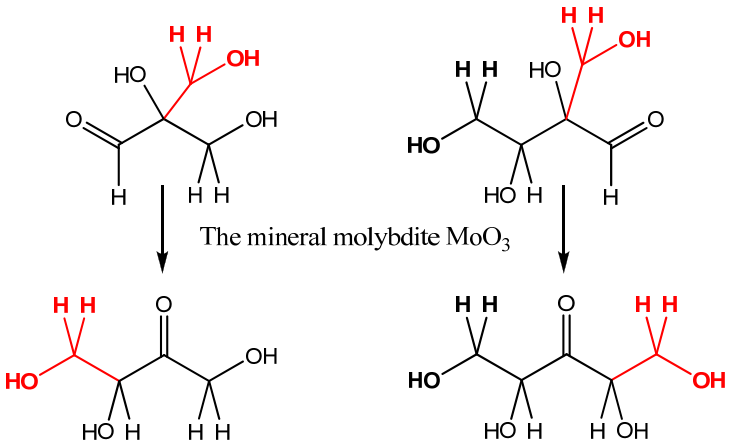


Figure 4-1. The role of molybdenum in carbohydrate interconversion.

In samples containing only glyceraldehyde in CBA buffer incubated for 5 minutes at 25 °C, peaks at 103-111 ppm were observed. These were tentatively assigned, but not yet confirmed, to ketohexoses (Figure 4-2). This class of saccharides includes d-psicose, d-fructose, d-sorbose and d-tagatose (Figure 4-3). A similar pattern is also observed when dihydroxyacetone is dissolved in CBA buffer (Figure 4-4).

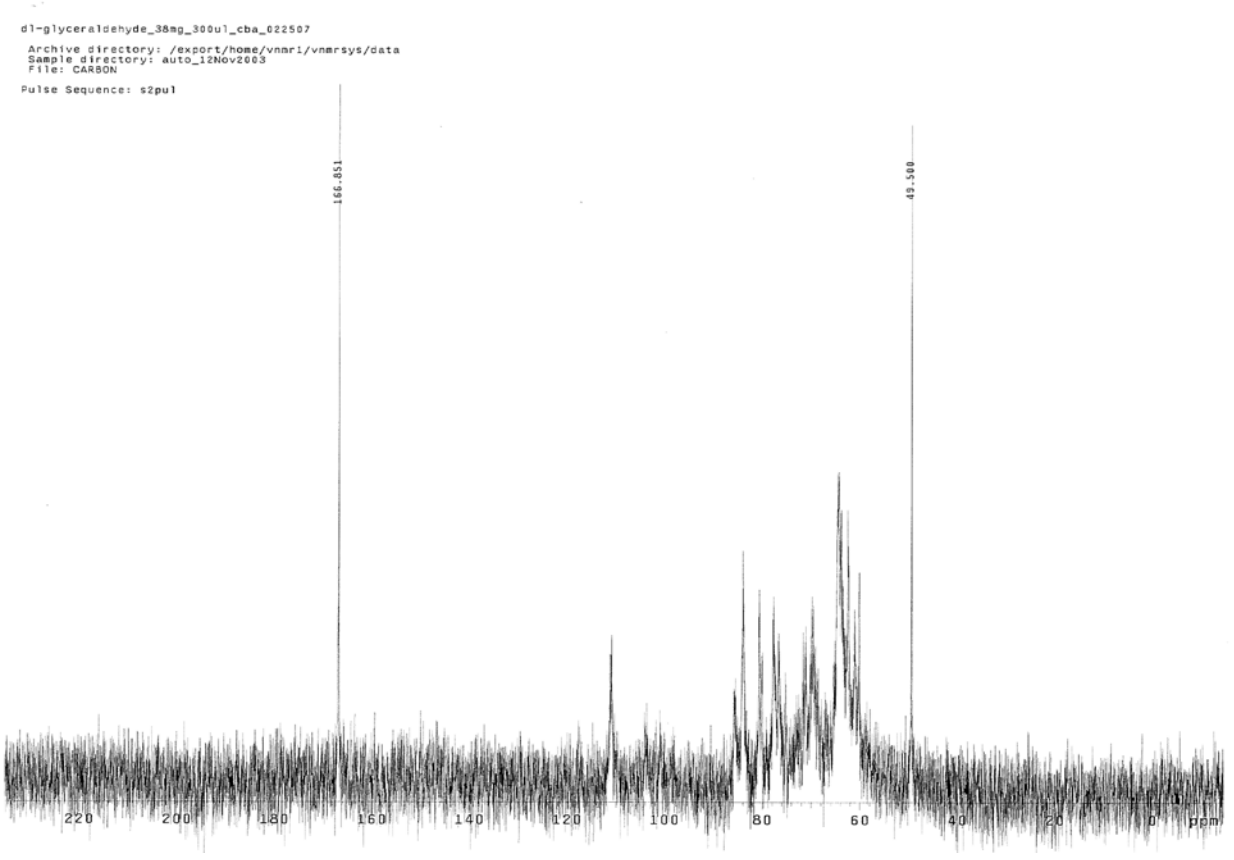


Figure 4-2. A sample of DL-glyceraldehyde (Aldrich, 38 mg) in CBA buffer (0.30 mL) incubated for 5 minutes at 25 ◦C. Spectrum file: dl_glyceraldehyde_38mg_300ulcba_022507.pdf

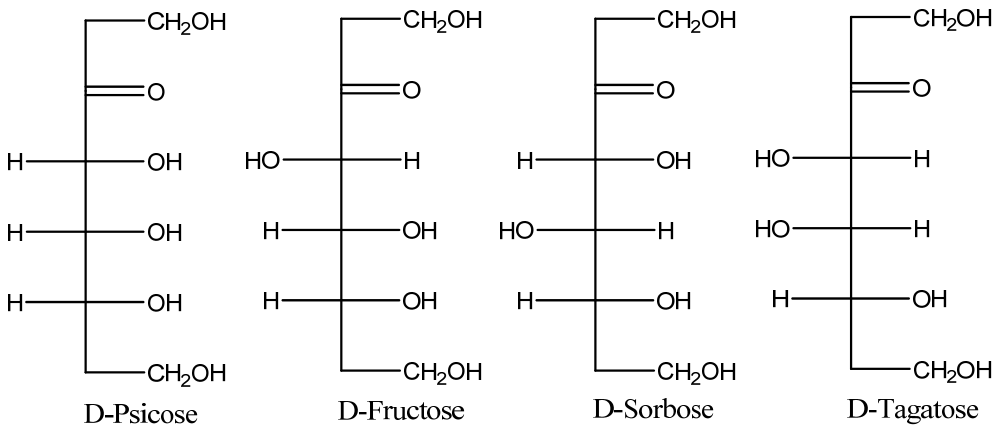


Figure 4-3. The ketohexoses (psicose, fructose, sorbose & tagatose) are presumably formed by the reaction of either dihydroxyacetone or glyceraldehyde with themselves.

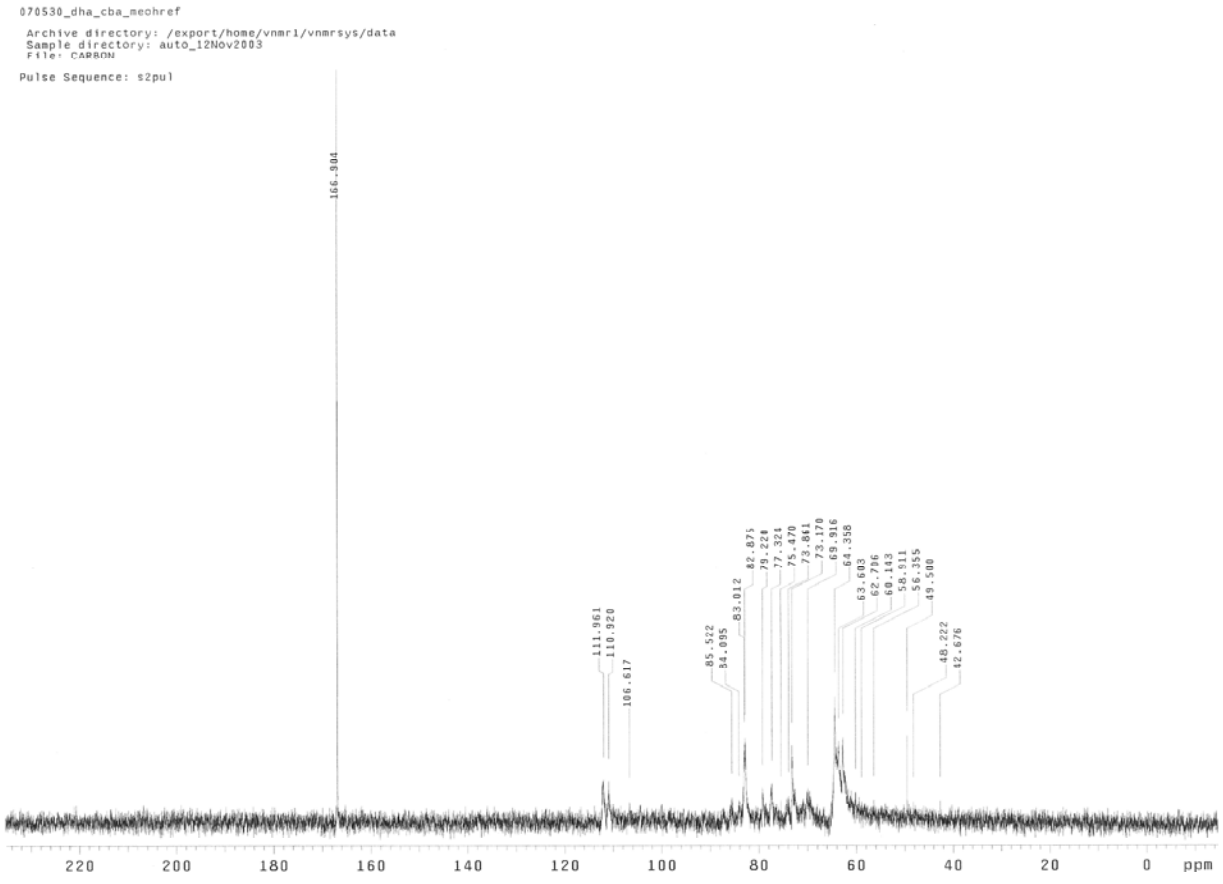


Figure 4-4. A sample of dihydroxyacetone (Aldrich, 30 mg) in CBA buffer (0.50 mL) incubated for 3 hours at 25 °C. Spectrum file: 070530_dha_cba_meohref.pdf

The prebiotic accumulation of material into these six carbon sugars is significant as they provide even more complex carbohydrates to the available prebiotic building blocks believed necessary for life to occur. For example, these carbohydrates could oligomerise to form structures, they could simply complex with various minerals and act as catalysts, or they could regulate the formation of further carbohydrates by restricting their reaction environment.

Here we have demonstrated significant progress into understanding the processes and products of the borate mediated prebiotic synthesis of sugars, formed as stable products from formaldehyde and lower carbohydrates such as glycolaldehyde and dihydroxyacetone. Further work in this area needs to tie up loose ends regarding the formation of nucleosides and oligomers. How the heterocycle, for example adenine, becomes attached to the C-1 of ribose in a prebiotic environment is a key step in origins of life chemistry.

APPENDIX A
NMR SPECTRA OF FURTHER EXPERIMENTS AND CARBOHYDRATES

The following compilation of reactions and their NMR signals are a provided as a supplement and reference for the reader. An electronic version of this is available.

Reactions involving glycolaldehyde

Glycolaldehyde in CBA

Spectrum Name: 070510_12C-glycolaldehyde_20mg_cba_1ml_refmeoh.pdf

Glycolaldehyde dimer (20 mg) was dissolved in 1 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.091, 103.272, 82.478, 82.231, 76.599, 71.602, 49.500 (MeOH).

Four sharp signals were observed. These signals superimposed on those arising from authentic threose [103.2, 82.3, 76.6, 71.6].

Formation of threose from glycolaldehyde

Threose has the following peaks in CBA buffer: 103.279, 82.333, 76.607, 71.614.

Glycolaldehyde in CBA

Spectrum Name:070515_12C-glycolaldehyde_20mg_cba_1ml_refmeoh.pdf

Glycolaldehyde dimer (20 mg) was dissolved in 1 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.042, 103.268, 82.318, 76.599, 71.598, 49.500 (MeOH).

1-^{13}C-Glycolaldehyde in D_2O

Spectrum Name: 070511_1-13C-glycolaldehyde_0.2ml_d2o_0.8ml_refmeoh.pdf

1-^{13}C-Glycolaldehyde (0.200 mL) was mixed with 0.800 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 205.104, 201.721, 181.850, 175.442, 139.961, 135.689, 130.024, 118.816, 110.314 ,109.615, 102.516, 99.827, 99.148, 97.763, 95.375, 93.056, 91.114, 90.649, 90.332, 78.339, 73.918, 72.834, 65.422, 65.113, 64.782, 54.646, 51.796, 49.500 (MeOH), 47.452, 36.393, 46.093, 45.273, 42.023, 35.393, 34.928, 30.263, 27.920, 25.052, 23.347, 18.658, 8.239

1-^{13}C-Glycolaldehyde in CBA (t = 0)

Spectrum Name: 070511_1-13C-glycolaldehyde_0.5ml_cba_0.5ml_refmeoh-166.889_t=0.pdf

1-^{13}C-Glycolaldehyde (0.500 mL) was mixed with 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 512 transients (ca. 20 minutes total acquisition at 25°C).

^{13}C NMR (D_2O): δ 177.536, 171.680, 166.889, 165.516, 143.684, 123.169, 105.988, 103.222, 98.286, 93.262, 93.094, 90.317, 87.773, 82.600, 82.054, 76.901, 76.653, 76.367, 69.344, 62.298, 60.822, 49.500 (MeOH), 31.159, 29.797

1-^{13}C-Glycolaldehyde in CBA (t = 1 hour)

Spectrum Name: 070511_1-13C-glycolaldehyde_0.5ml_cba_0.5ml_refmeoh_1hourlater.pdf

1-^{13}C-Glycolaldehyde (0.500 mL) was mixed with 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.673, 166.889, 105.984, 103.222, 98.282, 93.266, 93.094, 90.378, 82.604, 82.268, 82.051, 76.308, 76.649, 76.359, 75.981, 75.390, 71.545, 71.156, 69.447, 69.332, 65.266, 49.500 (MeOH), 40.562, 39.399, 23.900

1-^{13}C-Glycolaldehyde in CBA (t = 6 hours)

Spectrum Name: 070511_1-13C-glycolaldehyde_0.5ml_cba_0.5ml_refmeoh_6hourslater.pdf

1-^{13}C-Glycolaldehyde (0.500 mL) was mixed with 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.885, 103.222, 98.278, 93.277, 92.377, 90.321, 82.604, 82.318, 82.051, 76.908, 76.653, 76.359, 75.985, 75.768, 75.413, 73.861, 71.545, 71.389, 71.156, 70.877, 69.363, 68.013, 65.117, 49.500 (MeOH), 40.566, 23.904

1-^{13}C-Glycolaldehyde in CBA (t = 8 hours)

Spectrum Name: 070511_1-13C-glycolaldehyde_0.5ml_cba_0.5ml_refmeoh_8hourslater.pdf

1-^{13}C-Glycolaldehyde (0.500 mL) was mixed with 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.696, 171.673, 166.885, 103.371, 103.222, 98.283, 93.281, 90.344, 82.604, 82.321, 82.054, 76.908, 76.653, 76.359, 76.172, 75.985, 71.389, 71.137, 70.874, 69.676, 69.336, 68.020, 65.060, 49.500 (MeOH), 42.405, 40.566, 23.904

1-^{13}C-Glycolaldehyde in CBA (t = 6 hours)

Spectrum Name: 070515_1-13C-glycolaldehyde_0.5ml_cba_0.5ml.pdf

1-^{13}C-Glycolaldehyde (0.500 mL) was mixed with 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 182.068, 171.673, 166.862, 106.014, 103.359, 103.222, 103.058, 102.863, 100.678, 98.282, 82.604 82.321, 82.054, 81.440, 76.912, 76.653, 76.359, 76.187, 75.985, 75.772, 75.661, 75.413, 73.849, 71.549, 71.247, 72.167, 70.877, 70.641, 68.016, 66.899, 63.111, 62.909, 61.818, 49.500 (MeOH), 40.566, 23.904

Glycolaldehyde + $H^{13}CHO$

Spectrum Name: 070617_gol_1.4mg_h13cho_6ul_cba_nomeoh.pdf

Glycolaldehyde (1.4 mg) was dissolved in CBA (1 mL) buffer. To this was added 6 μL of $H^{13}CHO$ (6.66 M) and agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.000 (carbonate), 82.095, 70.403, 63.240

Glycolaldehyde + $H^{13}CHO$

Spectrum Name: 070617_gol_1.8mg_h13cho_48ul_cba_nomeoh.pdf

Glycolaldehyde was dissolved in 1 mL of CBA buffer. To this was added 48 μL of $H^{13}CHO$ (6.66 M) and agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.056, 167.000 (carbonate), 105.099, 104.565, 89.187, 88.232, 86.449, 86.133, 85.025, 83.522, 83.248, 82.111, 81.030, 80.708, 80.574, 79.156, 77.767, 77.647, 73.759, 73.267, 68.965, 68.733, 65.252, 63.335, 62.647, 67.517

Spectrum Name: 071130_gol_8mg_ICN_cba_0.5ml_nomeoh_t=4min-1.5hr.pdf

Glycolaldehyde (8 mg, 0.133 mmol) was dissolved in CBA buffer (0.5 mL). The tube was shaken using a hand vortexer and was transferred to an NMR tube. To this was added 10 μL of a 10% MeOH in D_2O was added as a reference. The tube was maintained at 25 ºC and an NMR spectrum was acquired after 4 minutes.

^{13}C NMR (D_2O): δ 167.198, 103.279, 93.033, 82.329, 76.607, 71.613, 69.477, 49.500 (MeOH). Threose can already be observed emerging at 103.2, 82.3, 76.6 and 71.6.

Spectrum Name: 071130_gol_8mg_ICN_cba_0.5ml_nomeoh_t=1.5hr-3hr.pdf

Glycolaldehyde (8 mg, 0.133 mmol) was dissolved in CBA buffer (0.5 mL). The tube was shaken using a hand vortexer and was transferred to an NMR tube. To this was added 10 μL of a 10% MeOH in D_2O was added as a reference. The tube was maintained at 25 ºC and an NMR spectrum was acquired after 1.5 hours.

^{13}C NMR (D_2O): δ 167.205, 103.279, 93.048, 82.329, 76.603, 76.054, 75.752, 73.868, 71.613, 69.378, 49.500 (MeOH).

Spectrum Name: 071130_gol_31mg_aldrich_cba_1.5ml_meohref_t=3hr-4.5hr.pdf

Glycolaldehyde (8 mg, 0.133 mmol) was dissolved in CBA buffer (0.5 mL). The tube was shaken using a hand vortexer and was transferred to an NMR tube. To this was added 10 μL of a 10% MeOH in D_2O was added as a reference. The tube was maintained at 25 ºC and an NMR spectrum was acquired after 3 hours.

^{13}C NMR (D_2O): δ 167.160, 103.279, 82.329, 76.607, 76.039, 75.753, 73.872, 71.610, 71.114, 68.036, 49.500 (MeOH).

Spectrum Name: 071130_gol_31mg_aldrich_cba_1.5ml_meohref_t=4.5hr-6hr.pdf

Glycolaldehyde (8 mg, 0.133 mmol) was dissolved in CBA buffer (0.5 mL). The tube was shaken using a hand vortexer and was transferred to an NMR tube. To this was added 10 μL of a

10% MeOH in D_2O was added as a reference. The tube was maintained at 25 ºC and an NMR spectrum was acquired after 4.5 hours.

^{13}C NMR (D_2O): δ167.163, 103.279, 82.329, 76.607, 71.613, 49.500 (MeOH).

All four peaks correspond to the formation of threose, with carbonate at 167.16 and the spectrum referenced to methanol at 49.500.

Spectrum Name: 070716_GOL_300ul_HCHO_35ul_SCBA_200ul_firstspectrumtaken070719.pdf

A 0.110 M solution of 1-^{13}C-glycolaldehyde (300 µL, Omicron Biochemicals) and HCHO (35 µL, Fisher) were mixed in 0.200 mL SCBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.634, 166.611, 111.332, 103.207, 98.271, 62.600, 82.314, 82.047, 77.011, 76.920, 76.664, 76.371, 75.981, 75.386, 73.845, 72.449, 72.163, 71.526, 71.160, 70.893, 67.997, 49.500 (MeOH), 40.555

Spectrum Name: 071115_gol_aldrich_25mg_cba_1ml_071113.pdf

Glycolaldehyde (Aldrich, 25 mg) was dissolved in CBA buffer (1.0 mL) and agitated vigorously using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube and 10 µL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was stored at 25 ºC and an NMR spectrum was acquired after 2 days.

^{13}C NMR (D_2O): δ 167.003, 103.264, 82.302, 76.596, 71.598, 49.500 (MeOH).

This spectrum exhibited the expected peaks for the product threose after 2 days. Compare this with the spectrum from 071113_gol_aldrich_25mg_cba_1ml.pdf

Spectrum Name: 071115_gol_34mg_carbonate_1ml.pdf

Glycolaldehyde (Aldrich, 34 mg) was dissolved in carbonate buffer (1.0 mL) and agitated vigorously using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube and 10 μL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was stored at 25 ºC and an NMR spectrum was acquired. The spectrum was referenced to carbonate.

^{13}C NMR (D_2O): δ 167.000, 114.812 (minor), 88.391, 73.590, 73.209, 69.226, 67.883, 60.658, 59.648, 49.500 (MeOH), 44.900, 32.346.

All peaks in the above spectrum are minor except for 167.000 (carbonate), 73.590 and 73.209. The methanol peak is not resolved and remains in the noise.

Reactions starting with glyceraldehyde

1,2,3-^{13}C-glyceraldehyde + H^{13}CHO. 2:1

Spectrum Name:

070331A_glyceraldehyde_H13CHO_start0130am040207end0730am_nt=8192.pdf

CBA buffer (0.900 mL, pH 10.25) and 1,2,3-^{13}C-glyceraldehyde (0.100 mL, final concentration 11.1 mM) were mixed. Then H^{13}CHO (3.5 μL, 6.7 M final concentration 23.3 mM, giving a 2.1:1 ratio) was added and the mixture was agitated vigorously using a vortex stirrer. NMR data acquisition was begun within four minutes, and was completed after 8192 transients (ca. 6 hours total acquisition at 25°C). Three manifolds of signals at 62, 70 and 95-110 ppm were observed, with some H^{13}CHO remaining.

^{13}C NMR (D_2O): δ171.767, 167.220, 105.502, 104.968, 104.789, 104.415, 104.262, 103.759, 103.438, 102.942, 102.359, 102.263, 101.832, 101.737, 100.814, 100.337, 99.490, 98.956, 98.891, 96.324, 95.813, 94.588, 82.305 (HCHO), 81.672, 81.145, 79.803, 77.060, 76.522, 75.976, 74.161, 73.642 73.043, 71.792, 71.300, 69.358, 67.123, 66.608, 65.787, 65.082, 64.387, 63.853, 63.102, 62.530, 61.923, 61.389, 58.936, 58.440, 49.217, 33.725, 31.104, 26.622, 26.527, 25.974, 25.878, 20.832, 15.602.

The peaks at 74.161 and 69.358 correspond to C4 of the erythro-branched pentose. A peak at 63.853 is interpreted as the C2' of the erythro-branched pentose

1,2,3-^{13}C-Glyceraldehyde + HCHO. 2:1

Spectrum Name: 070331B_070410_glyceraldehyde_H12CHO_nt=4096_0300-0600.pdf

CBA buffer (0.900 mL, pH 10.25) and 1,2,3-^{13}C-glyceraldehyde (0.100 mL, final concentration 11.1 mM). Then H^{12}CHO (12 M, 2 μL, final concentration 24 mM, giving a 2.2:1 ratio) was subsequently added and agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.767, 167.208, 105.506, 104.945, 104.781, 104.381, 104.262, 103.751, 103.400, 102.981, 102.343, 102.252, 101.726, 99.418, 98.887, 96.324, 95.809, 84.685, 84.300, 82.305 (HCHO), 81.355, 80.810, 79.867, 79.562, 77.991, 77.468, 77.125, 76.762, 76.213, 74.245, 72.753, 72.196, 71.365, 70.842, 68.484, 67.035, 66.623, 65.719, 65.005, 64.410, 63.872, 49.434, 26.527, 25.958, 25.859

Spectrum Name: dl_glyceraldehyde_38mg_300ulcba_022507.pdf

DL-Glyceraldehyde (38 mg, Aldrich) was dissolved in CBA buffer (0.3 mL). To this was added one drop of MeOH as an internal reference. The solution was agitated using a hand vortexer, transferred to an NMR tube and spectrum acquisition started in 4 minutes.

^{13}C NMR (D_2O): δ 166.851, 111.282, 110.928, 103.653, 100.998, 85.488, 84.061, 80.647, 80.078, 77.782, 76.741, 75.276, 71.629, 71.179, 69.867, 68.619, 64.412, 63.931, 62.573, 61.177, 60.204, 49.500 (MeOH).

Spectrum Name: 071014_dl-12C-GLY_25mg_CBA_1ml_meohref.pdf

DL-^{12}C-Glyceraldehyde (Aldrich, 25 mg) was dissolved in CBA buffer (1.0 mL) in an eppendorf tube. An aliquot (0.5 mL) was transferred to an NMR tube and 10 μL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 167.084, 111.046, 100.949, 94.822, 92.137, 85.404, 84.240, 80.407, 77.256, 73.048, 71.656, 70.263, 68.600, 67.097, 64.213, 63.469, 62.588, 61.161, 49.500 (MeOH).

Spectrum Name: 071014_d-12C-GLY_28mg_CBA_0.5ml_meohref.pdf

D-^{12}C-Glyceraldehyde (Fluka, 28 mg) was dissolved in CBA buffer (0.5 mL) in an eppendorf tube. The sample was transferred to an NMR tube and 10 μL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 166.851, 110.977, 110.047, 103.294, 100.716, 94.837, 90.256, 85.675, 83.916, 80.632, 80.395, 80.113, 77.263, 76.596, 74.524, 72.819, 71.640, 71.110, 69.832, 67.116, 65.346, 64.633, 63.397, 62.554, 61.360, 61.142, 60.147, 49.500 (MeOH), 23.984

Spectrum Name: 071014_12C_GLY_25mg_CBA_1ml_nomeoh.pdf

D-^{12}C-Glyceraldehyde (Fluka, 25 mg) was dissolved in CBA buffer (0.5 mL) in an eppendorf tube. The sample was transferred to an NMR tube and 10 μL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 167.000, 110.894, 110.219, 110.203, 110.016, 100.850, 94.758, 83.852, 80.392, 77.183, 72.957, 68.513, 66.998, 64.118, 63.462, 62.344, 60.788, 49.500 (MeOH).

Spectrum Name: 071015_d-gly_25mg_1-13c-gol_200ul_cba_1ml_meohref.pdf

DL-^{12}C-Glyceraldehyde (Aldrich, 25 mg) was dissolved in 1-^{13}C-glycolaldehyde (Omicron Biochemicals, 200 μl) in an eppendorf tube. To this was added CBA buffer (1.0 mL) and An aliquot (0.5 mL) was transferred to an NMR tube and 10 μL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.779, 166.950, 103.249, 102.471, 98.305, 95.036, 94.872, 94.647, 93.064, 90.225, 82.596, 82.043, 80.529, 76.981, 76.863, 76.779, 76.615, 76.279, 72.468, 72.178, 71.274, 71.179, 69.161, 64.778, 63.424, 49.500 (MeOH), 40.577, 23.965

Spectrum Name: 071015_d-gly_25mg_1-13c-gol_200ul_cba_1ml_nomeoh.pdf

DL-^{12}C-Glyceraldehyde (Aldrich, 25 mg) was dissolved in 1-^{13}C-glycolaldehyde (Omicron Biochemicals, 200 µl) in an eppendorf tube. To this was added CBA buffer (1.0 mL) and An aliquot (0.5 mL) was transferred to an NMR tube. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.722, 166.950 (carbonate), 110.985, 103.233, 102.455, 100.922, 98.278, 97.290, 95.020, 93.067, 90.218, 83.332, 82.573, 82.035, 80.403, 76.962, 76.847, 76.760, 76.271, 72.453, 72.163, 71.259, 71.167, 70.099, 69.271, 68.589, 64.679, 63.534, 62.443, 40.566, 23.953

Spectrum Name: 071020_d-12c-gly_25mg_cba_1ml_meohref_5dayslater.pdf

DL-^{12}C-Glyceraldehyde (Aldrich, 25 mg) was dissolved in CBA buffer (1.0 mL) in an eppendorf tube. An aliquot (0.5 mL) was transferred to an NMR tube and 10 µL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was maintained at 25 ºC and an NMR spectrum was acquired 5 days later.

^{13}C NMR (D_2O): δ 167.767, 110.966, 103.897, 100.891, 85.564, 84.061, 80.399, 77.717, 77.248, 75.798, 72.922, 71.637, 71.175, 70.214, 68.463, 66.735, 64.423, 63.740, 62.577, 61.135, 60.193, 49.500 (MeOH), 23.987

Spectrum Name: 071020_d-12c-gly_25mg_cba_1ml_meohref_6dayslater_071014.pdf

DL-^{12}C-Glyceraldehyde (Aldrich, 25 mg) was dissolved in CBA buffer (1.0 mL) in an eppendorf tube. An aliquot (0.5 mL) was transferred to an NMR tube and 10 µL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was maintained at 25 ºC and an NMR spectrum was acquired 6 days later.

^{13}C NMR (D_2O): δ 166.790, 110.989, 110.310, 103.916, 100.914, 85.419, 84.084, 80.418, 80.109, 77.740, 77.267, 75.821, 72.766, 71.659, 71.194, 69.836, 68.428, 66.754, 64.259, 63.725, 62.600, 61.135, 60.215, 49.500 (MeOH), 24.007

Reactions of glycolaldehyde and glyceraldehyde

Glycolaldehyde dimer + Glyceraldehyde in CBA (t = 6 hours)

Spectrum Name: 070519_GOL_40mg_GER_21mg_CBA_refmeoh_6hourslater_nt=8192.pdf

Glycolaldehyde dimer (40 mg) and glyceraldehyde (21 mg) were dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.923, 166.374, 149.197, 125.042, 111.256, 103.253, 103.069, 102.902, 97.359, 85.179, 83.477, 83.336, 83.157, 82.638, 82.558, 82.474, 82.302, 79.933, 79.041, 77.813, 70.954, 76.752, 76.588, 76.359, 76.264, 76.161, 75.890, 73.704, 72.949, 72.449, 71.579, 71.522, 71.320, 69.786, 69.020, 68.680, 67.440, 66.964, 64.682, 64.351, 62.630, 60.894, 60.486, 60.189, 60.036, 58.709, 51.365, 49.500 (MeOH), 48.665, 34.154, 30.369, 22.504, 11.269

Glycolaldehyde dimer + Glyceraldehyde in CBA (t = 0-3 hours)

Spectrum Name: 070519_GOL_40mg_GER_21mg_CBA_refmeoh_t=4.pdf

Glycolaldehyde dimer (40 mg) and glyceraldehyde (21 mg) were dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.931, 103.249, 103.119, 102.947, 94.849, 93.029, 90.164, 82.302, 80.658, 76.954, 76.584, 76.378, 74.532, 73.700, 72.445, 71.583, 69.668, 64.597, 64.377, 63.446, 63.340, 62.569, 49.500 (MeOH).

Spectrum Name: 070716_1-13C_GOL_280ul_0.11M_2-13C-GLY_28ul_CBA_200ul_nomeohref.pdf

A 0.110M solution of 1-^{13}C-glycolaldehyde (280 µL, Omicron Biochemicals) and a 0.110 M solution of 2-^{13}C-glyceraldehyde (280 µL, Omicron Biochemicals) was dissolved in 0.200 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.279, 166.301 (carbonate), 102.851, 97.926, 92.933, 90.061, 82.240, 81.951, 81.691, 80.161, 76.564, 76.309, 76.015, 75.630, 71.197, 69.072, 64.826

Spectrum Name: 070716_1-2-3-13C_GLY_60ul_0.11M_1-13C_GOL_200ul_0.11M_CBA_meohre.pdf

A 0.110M solution of 1,2,3-^{13}C-glyceraldehyde (60 µL, Omicron Biochemicals) and 0.110 M solution of 1-13C-glycolaldehyde (200 µL, Omicron Biochemicals) was dissolved in 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.772, 167.126, 103.260, 98.316, 95.230, 94.673, 93.228, 90.968, 82.615, 82.066, 80.948, 80.494, 79.937, 76.893, 76.634, 76.344, 75.711, 69.325, 65.064, 63.885, 63.332, 49.500 (MeOH).

Spectrum Name: 070716_2-13C-GLY_28ul1-13C-GOL_280ul_CBA_firstspectrum070719.pdf
A 0.110M solution of 2-^{13}C-glyceraldehyde (28 µL, Omicron Biochemicals) and a 0.110 M solution of 1-^{13}C-glycolaldehyde (280 µL, Omicron Biochemicals) was dissolved in 0.700 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).
^{13}C NMR (D_2O): δ 171.634, 166.611, 111.332, 103.207, 98.271, 62.600, 81.314, 82.047, 77.011, 69.920, 76.664, 76.371, 75.981, 75.386, 73.845, 72.449, 72.163, 71.526, 71.160, 70.893, 67.997, 49.500 (MeOH), 40.555

Spectrum Name:
070716_2-13C_GLY_60ul_0.11M_1-13C_GOL_200ul_0.11M_CBA_meohre.pdf
A 0.110M solution of 2-^{13}C-glyceraldehyde (60 µL, Omicron Biochemicals) and a 0.110 M solution of 1-^{13}C-glycolaldehyde (200 µL, Omicron Biochemicals) was dissolved in 0.740 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.768, 167.129, 104.920, 104.378, 103.821, 103.260, 100.708, 98.316, 93.205, 90.496, 88.524, 82.615, 82.341, 82.066, 81.463, 80.494, 76.992, 76.893, 76.638, 76.344, 76.027, 71.595, 69.466, 68.280, 64.133, 62.790, 59.689, 58.094, 56.614, 49.500 (MeOH), 49.477

Spectrum Name:

070724_1-2-3-13C-GLY_280ul_1-13C-GOL_280ul_SCBA_400ul_nomeoh.pdf

A 0.110M solution of 1,2,3-^{13}C-glyceraldehyde (280 µL, Omicron Biochemicals) and a 0.110 M solution of 1-^{13}C-Glycolaldehyde (280 µL, Omicron Biochemicals) was dissolved in 0.400 mL SCBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 180.171, 171.286, 170.668, 162.173, 105.632, 104.503, 103.980, 102.859, 99.715, 97.915, 96.595, 92.929, 92.868, 92.765, 90.133, 88.420, 80.310, 80.272, 80.188, 80.142, 80.070, 76.675, 76.328, 76.030, 75.759, 74.188, 62.782, 49.500 (MeOH), 40.187, 39.028, 23.510

Spectrum Name: 070724_1-2-3-13C-GLY_28ul_1-13C-GOL_280ul_SCBA_700ul_nomeoh.pdf

A 0.110M solution of 1,2,3-^{13}C-glyceraldehyde (28 µL, Omicron Biochemicals) and a 0.110 M solution of 1-^{13}C-glycolaldehyde (280 µL, Omicron Biochemicals) was dissolved in 0.700 mL SCBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.340, 162.505, 102.851, 97.907, 92.143, 82.229, 81.680, 76.530, 76.271, 75.981, 75.633, 71.166, 68.290, 65.040, 62.721, 49.500 (MeOH), 40.161

Spectrum Name: 070724_2-13C-GLY_28ul_1-13C-GOL_280ul_SCBA_700ul_nomeoh.pdf
A 0.110M solution of 2-^{13}C-glyceraldehyde (28 μL, Omicron Biochemicals) and a 0.110 M solution of 1-^{13}C-glycolaldehyde (280 μL, Omicron Biochemicals) was mixed in 0.700 mL SCBA buffer. The mixture was agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).
^{13}C NMR (D_2O): δ 171.340 (carbonate), 162.520, 102.847, 97.907, 92.140, 91.136, 88.760, 82.229, 81.943, 81.676, 76.526, 76.271, 75.977, 75.622, 71.163, 68.167, 65.036, 64.025, 62.271, 54.527, 40.157, 38.990, 25.234, 23.514

Spectrum Name: 071117_from071014_d-gly_28mg_cba_0.5ml.pdf
D-Glyceraldehyde (Fluka, 28 mg) was dissolved in CBA buffer (0.5 mL) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. The tube was kept in a car for 34 days, with temperatures fluctuating between 37-85 ºF
Acquisition of an NMR spectrum (nt=8192) was acquired 34 days later.
^{13}C NMR (D_2O): δ 187.685, 166.595, 111.229, 102.589, 97.191, 91.042, 88.642, 87.086, 83.878, 83.195, 80.616, 79.899, 77.488, 76.477, 74.520, 73.227, 72.346, 71.125, 69.760, 69.619, 67.994, 65.697, 63.851, 62.363, 61.497, 61.047, 60.124, 49.500 (MeOH).

Spectrum Name: 071116_12c-d-gly_100ul_0.110m_camh13cho_3.5ul_cba_900ul_nt=8192.pdf

D-Glyceraldehyde (Fluka, 100 μL, 0.110 M solution) was mixed with formaldehyde (Cambridge, 3.5 μL, 6.6 M) in an eppendorf tube. To this was added CBA buffer (900 μL) and a 0.5 mL aliquot was transferred to an NMR tube. An NMR spectrum was started after 4 minutes with an acquisition time of 6.5 hours (nt=8192).

^{13}C NMR (D_2O): δ 171.852, 167.320, 82.360, 76.397, 64.282, 63.652, 62.391, 53.985, 49.500 (MeOH).

A peak at 62.391 was observed and is assigned to the branched tetrose with the assumption that any erythrulose produced would be moved towards a pentose product. Accumulation of the branched tetrose is also favored as it has no enolizable centers and is considered a dead end product. Formaldehyde is less than 2% consumed and appears at 82.360.

Spectrum Name:

071117_24h_071116_12c-d-gly_100ul_0.110m_camh13cho3.5ul_cba900ul.pdf

Glyceraldehyde (Aldrich, 100 μL, 0.110M) was dissolved in CBA buffer (1.0 mL) and to this was added $H^{13}CHO$ (Cambridge, 3.5 μL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 25 ºC. An aliquot (0.5 mL) was transferred to an NMR tube and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.852, 167.312, 82.356, 74.002, 72.224, 71.644, 66.330, 65.525, 64.190, 63.805, 62.909, 58.766, 53.898, 50.271, 49.500 (MeOH).

Reactions involving dihydroxyacetone

Dihydroxyacetone in CBA

Spectrum Name: 070530_dha_cba_meohref.pdf

Dihydroxyacetone (90 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.904, 111.961, 110.920, 85.522, 84.095, 83.012, 82.875, 79.220, 77.324, 75.470, 73.861, 73.170, 69.916, 64.358, 63.603, 62.706, 60.143, 58.911, 56.355, 49.500 (MeOH), 48.222, 42.676

Dihydroxyacetone + Glycolaldehyde in CBA

Spectrum Name: 070530_dha_gol_cba_meohref.pdf

Dihydroxyacetone (90 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.782, 111.416, 111.290, 111.229, 103.028, 102.883, 83.309, 82.535, 82.390, 76.725, 76.626, 76.500, 76.329, 76.241, 76.134, 72.384, 71.583, 71.499, 71.240, 64.328, 49.500 (MeOH).

Dihydroxyacetone + Glycolaldehyde in CBA no MeOH

Spectrum Name: 070610_DHA_102mg_GOL_51mg_cba_sametime_nomeohref_ref-111.28.pdf

Dihydroxyacetone dimer (Aldrich, 102 mg) and glycolaldehyde (Aldrich, 51 mg) were dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. Acquisition of an NMR

spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.688 (carbonate), 111.280, 104.547, 104.055, 103.021, 98.714, 98.207, 84.428, 83.311, 83.257, 79.206, 78.539, 76.715, 76.620, 76.513, 73.221, 72.801, 72.439, 72.344, 71.054, 69.864, 68.620, 66.366, 64.325, 63.761, 62.803, 62.635, 61.701, 58.225, 54.426, 54.148

Spectrum Name: 070510_dha_18mg_cba_1ml_refmeoh.pdf

Dihydroxyacetone dimer (18 mg, Aldrich) was dissolved in CBA buffer (1 mL). To this was added 10 μL of 10% MeOH in D_2O as an internal reference. The solution was agitated using a hand vortexer, transferred to an NMR tube and spectrum acquisition started in 4 minutes and continued for 3 hours (nt=4096).

^{13}C NMR (D_2O): δ 230.457, 226.028, 222.290, 217.117, 215.385, 212.468, 208.530, 203.163, 195.350, 193.355, 181.839, 167.061, 99.907, 93.487, 87.921, 85.450, 82.836, 80.231, 78.953, 77.923, 76.969, 74.551, 73.143, 70.389, 68.982, 66.800, 64.473, 62.333, 60.261, 58.339, 54.123, 52.502, 50.759, 49.500 (MeOH).

<u>Dihydroxyacetone + Glycolaldehyde in CBA</u>

Spectrum Name: 070610_DHA_104mg_GOL_67mg_CBA_sametime_refmeoh.pdf

Dihydroxyacetone dimer (Aldrich, 104 mg) and Glycolaldehyde (Aldrich, 67 mg) were dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.664, 111.454, 111.405, 111.279, 104.061, 103.001, 102.341, 98.202, 92.572, 89.447, 87.574, 84.431, 83.309, 82.524, 80.868, 79.956, 77.908, 76.714, 76.619, 76.516, 76.317, 75.882, 73.616, 73.277, 72.434, 72.350, 71.823, 71.236, 70.904, 69.867, 68.631, 68.039, 67.101, 65.323, 65.033, 64.320, 63.584, 62.790, 61.718, 57.957, 56.080, 52.506, 49.916, 49.504 (MeOH), 49.412, 47.501, 46.399, 45.662, 44.652, 43.973

Dihydroxyacetone + Glycolaldehyde in CBA

Spectrum Name: 070610_DHA_40mg_GOL_80mg_CBA_05302007.pdf

Dihydroxyacetone dimer (Aldrich, 40 mg) and glycolaldehyde (Aldrich, 80 mg) were dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.595, 111.210, 83.165, 76.481, 73.178, 72.358, 71.007, 68.879, 68.615, 68.024, 65.724, 63.641, 62.470, 61.741, 49.500 (MeOH).

Dihydroxyacetone + Glycolaldehyde in CBA

Spectrum Name: 070610_DHA_80mg_GOL_42mg_CBA_sametime_refmeoh.pdf

Dihydroxyacetone dimer (Aldrich, 80 mg) and glycolaldehyde (Aldrich, 42 mg) were dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.923, 111.431, 111.305, 83.332, 76.737.72.453, 65.346, 64.339, 49.504 (MeOH)

Dihydroxyacetone + Glycolaldehyde in CBA no MeOH

Spectrum Name: 070610_DHA_first_103mg_GOL_49mg_second_cba_nomeohref.pdf

Dihydroxyacetone dimer (Aldrich, 103 mg) was dissolved in CBA buffer (1 mL) and 49 mg of glycolaldehyde was subsequently added and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.020, 112.276, 111.444, 83.429, 83.154, 82.994, 76.765, 73.385, 72.546, 71.443, 70.192, 70.055, 64.539, 63.822, 62.971, 62.841, 61.891, 49.500 (MeOH).

Dihydroxyacetone + Glycolaldehyde in CBA no MeOH

Spectrum Name: 070611_DHA_30mg_GOL_106mg_nomeoh_cba.pdf

Dihydroxyacetone dimer (Aldrich, 30 mg) and glycolaldehyde (Aldrich, 106 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.851 (carbonate), 111.244, 102.894, 90.149, 82.539, 76.332, 72.388, 71.499, 64.957, 64.339

Dihydroxyacetone + Glycolaldehyde in CBA

Spectrum Name:

070611_DHA_30mg_GOL_106mg_refmeoh_1-13c-xylose_added_nt=3072.pdf

Dihydroxyacetone dimer (Aldrich, 30 mg) and glycolaldehyde (Aldrich, 106 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 1-^{13}C-xylose and 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 3072 transients (ca. 2.5 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 177.166, 166.954, 150.745, 126.003, 111.279, 102.974, 102.757, 97.573, 97.271, 96.493, 92.813, 88.593, 86.140, 83.340, 82.566, 76.367, 76.165, 73.880, 72.411, 71.526, 68.699, 66.994, 65.781, 64.347, 49.500 (MeOH), 27.066

Dihydroxyacetone + Glycolaldehyde in CBA

Spectrum Name:

070611_DHA_30mg_GOL_106mg_refmeoh_1-13c-xylulose_added_nt=3072.pdf

Dihydroxyacetone dimer (Aldrich, 30 mg) and glycolaldehyde (Aldrich, 106 mg) was dissolved in CBA buffer (1 mL)and agitated vigorously using a vortex stirrer. To this was added 1-^{13}C-xylulose and 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 3072 transients (ca. 2.5 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.908, 166.878, 111.706, 111.607, 110.993, 110.886, 102.921, 83.325, 82.547, 76.348, 76.153, 72.388, 71.507, 70.507, 69.634, 65.026, 64.694, 64.309, 63.973, 62.535, 61.757, 49.500 (MeOH).

Dihydroxyacetone + Glycolaldehyde in CBA

Spectrum Name: 070611_DHA_30mg_GOL_106mg_refmeoh_cba.pdf

Dihydroxyacetone dimer (30 mg) and glycolaldehyde (106 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.851, 111.347, 102.883, 90.149, 83.306, 82.543, 76.729, 76.336, 75.291, 73.647, 72.384, 71.503, 64.949, 64.335, 49.500 (MeOH).

Dihydroxyacetone + Glycolaldehyde in CBA no MeOH

Spectrum Name: 070617_dha_5.4mg_gol_100.6mg_cba_nomeoh.pdf

Dihydroxyacetone dimer (Aldrich, 5.4 mg) and glycolaldehyde (Aldrich, 100.6 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.000 (carbonate), 149.313, 125.189, 112.123, 103.196, 103.029, 99.390, 97.160, 83.338, 82.684, 76.461, 76.281, 75.820, 76.265, 72.495, 71.614, 63.475, 62.633, 11.881

Spectrum Name: 20071307_DHA_30_GOL_103_carbonatebuffer.pdf

Dihydroxyacetone dimer (Aldrich, 30 mg) and glycolaldehyde (Aldrich, 103 mg) was dissolved in 1 mL of carbonate buffer. The mixture was agitated vigorously using a vortex stirrer. To this

was added 10 μL of a 10% MeOH in D_2O as an internal reference. An NMR spectrum was acquired immediately.

^{13}C NMR (D_2O): δ 168.361, 100.227, 99.518, 98.442, 97.107, 84.244, 71.707, 78.602, 76.176, 75.627, 75.306, 74.707, 74.219, 72.407, 71.808, 70.935, 70.740, 69.695, 69.226, 66.037, 64.621, 64.080, 63.256, 62.287, 62.073, 61.570, 54.883, 49.500 (MeOH), 48.985, 48.501, 45.765, 40.593

Spectrum Name: 070617_october_sample_h13cho_dha_tetraborate.pdf

Dihydroxyacetone dimer (Aldrich, 30 mg) was dissolved in 1 mL of CBA buffer. To this was added $H^{13}CHO$ (6 μL, 6.66 M) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. A sample prepared in October 2006 was analyzed by NMR.

^{13}C NMR (D_2O): δ 182.390, 171.681, 167.401, 106.454, 105.813, 105.119, 103.229, 102.161, 101.653, 101.006, 99.270, 88.513, 80.474, 79.419, 77.243, 76.174, 75.353, 74.612, 73.998, 73.503, 72.802, 72.402, 71.073, 70.692, 70.031, 69.210, 67.701, 67.080, 66.393, 65.898, 65.017, 64.570, 63.815, 63.441, 61.899, 61.271, 60.150, 59.509, 55.115, 49.500 (MeOH), 49.059, 45.841, 26.758, 26.298, 24.649, 23.921, 22.352, 20.729

Spectrum Name: 070702_DHA_31_GOL_95_CBA_1-13C-xylose_added_meohref.pdf

Dihydroxyacetone dimer (Aldrich, 31 mg) and glycolaldehyde (Aldrich, 95mg) were dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 1-^{13}C-xylose and 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.749, 166.797, 166.756, 111.435, 111.248, 103.047, 102.898, 97.248, 92.793, 83.321, 82.554, 76.748, 76.645, 76.538, 76.355, 76.264, 76.157, 74.211, 72.396, 71.503, 64.343, 63.385, 49.500 (MeOH).

Spectrum Name: 070702_DHA_31_GOL_95_CBA_meohref.pdf

Dihydroxyacetone dimer (Aldrich, 31 mg) and glycolaldehyde (Aldrich, 95 mg) were dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.759, 111.241, 103.043, 83.344, 82.547, 76.626, 76.519, 76.344, 72.403, 71.514, 64.335, 63.385, 63.080, 62.619, 49.500 (MeOH).

Spectrum Name:

070713_DHA_30_GOL_100_meohborateremoval_immediate_white_nt=8192.pdf

Dihydroxyacetone dimer (Aldrich, 30 mg) and glycolaldehyde (Aldrich, 100 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To remove the borate, 30 mL of MeOH was added and evaporated, at room temperature, from the mixture in three portions of 10 mL each. The remaining residue was re-suspended in 1 mL of D_2O. An NMR spectrum was acquired immediately.

^{13}C NMR (D_2O): δ 166.729, 103.066, 102.928, 83.245, 82.569, 76.554, 76.382, 76.214, 72.419, 71.499, 64.377, 63.416, 61.776, 49.500 (MeOH).

Spectrum Name:

070713_DHA_30_GOL_100_meohborateremoval_overnight_yellow_nt=8192.pdf

Dihydroxyacetone dimer (Aldrich, 30 mg) and glycolaldehyde (Aldrich, 100 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To remove the borate, 30 mL of MeOH was added and evaporated at 50 ºC from the mixture in three portions of 10 mL each. The remaining residue was kept under vacuum for 24 hours and subsequently re-suspended in 1 mL of D_2O. An NMR spectrum was acquired immediately.

^{13}C NMR (D_2O): δ 168.178, 160.663, 111.378, 103.279, 103.115, 86.109, 83.515, 82.905, 76.775, 76.561, 76.474, 72.518, 71.610, 64.652, 60.124, 57.633, 55.874, 55.493, 54.814, 53.524, 52.689, 51.625, 50.564, 50.488, 50.236, 49.500 (MeOH), 48.752, 48.440, 47.375, 46.315, 44.446, 43.126, 38.880, 37.472

Spectrum Name:

070716_1-13C_GOL_280ul_0.11M_DHA_28ul_0.11M_CBA_500ul_refmeoh.pdf

A 0.110M solution of 1-^{13}C-glycolaldehyde (280 µL, Omicron Biochemicals) and a 0.110 M solution of dihydroxyacetone (28 µL)in D_2O was dissolved in 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.730, 167.000, 110.268, 103.245, 98.305, 93.216, 90.431, 82.611, 82.062, 76.905, 76.649, 76.355, 71.141, 70.176, 69.317, 49.500 (MeOH), 40.585, 13.497

Dihydroxyacetone and $H^{13}CHO$ in CBA buffer

Dihydroxyacetone dimer (Aldrich, 3.3 M, 100 μL) was dissolved in CBA buffer (1 mL). To this solution was added $H^{13}CHO$ (Cambridge, 3.3 M, 50 μL) was added and agitated vigorously using a vortex stirrer. A portion of the reaction mixture (500 μL) was transferred to an NMR tube and acquisition of an NMR spectrum began within four minutesat at 25°C.

Spectrum Name: dha-100ul_h13cho-50ul_9cba_120106_1955-2003.pdf

^{13}C NMR (D_2O): δ 171.811, 167, 90.087, 89.626, 83.732, 82.981, 82.309, 81.611, 80.902, 65.399, 64.064, 63.465, 62.859, 55.264, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=15.pdf

^{13}C NMR (D_2O): δ 171.811, 167, 90.087, 89.626, 83.732, 82.981, 82.309, 81.611, 80.902, 65.399, 64.064, 63.465, 62.859, 55.264, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=23.pdf

^{13}C NMR (D_2O): δ 171.371, 166.965, 89.651, 89.178, 88.007, 83.292, 82.545, 81.869, 81.179, 80.462, 68.465, 64.963, 63.777, 63.006, 62.434, 54.824, 49.068, 48.915 MeOH ref corr. to 49.500.

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=32.pdf

^{13}C NMR (D_2O): δ 171.803, 167, 90.083, 83.728, 82.977, 82.305, 81.604, 65.331, 64.251, 63.427, 62.862, 62.687, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=41.pdf

^{13}C NMR (D_2O): δ 171.807, 167.394, 90.083,88.447, 83.709, 82.977, 82.305, 81.607, 80.894, 71.617, 69.942, 69.187, 68.924, 68.642, 67.036, 66.421, 65.453, 64.232, 63.423, 62.855, 62.679, 61.710, 55.275, 53.860, 49.653, 49.500 (MeOH), 49.347, 49.035

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=51.pdf

^{13}C NMR (D_2O): δ 171.807, 167.390, 90.106, 82.305, 71.613, 69.206, 68.691, 67.013, 66.429, 65.445, 64.224, 63.423, 62.859, 62.668, 61.703, 53.864, 49.653, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=61.pdf

^{13}C NMR (D_2O): δ 171.803, 167.386, 90, 73.341, 71.609, 69.206, 68.630, 67.051, 66.391, 65.174, 64.232, 63.419, 62.851, 61.707, 49.500 (MeOH), 49.344

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=71.pdf

^{13}C NMR (D_2O): δ 171.803, 167.386, 82.302, 73.395, 71.609, 71.041, 69.199, 68.672, 66.990, 66.410, 65.445, 64.217, 63.427, 62.862, 62.679, 61.733, 56.126, 54.539, 53.856, 53.658, 80.545, 49.694, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=81.pdf

^{13}C NMR (D_2O): δ 171.807, 167.386, 82.302, 73.326, 71.613, 69.199, 68.661, 67.715, 67.032, 66.406, 65.441, 65.186, 64.205, 63.423, 62.847, 62.679, 61.714, 53.856, 49.649, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=91.pdf

^{13}C NMR (D_2O): δ 171.803, 167.386, 82.302, 73.665, 72.891, 72.460, 71.930, 71.609, 70.606, 69.187, 68.672, 66.990, 66.402, 65.453, 64.194, 63.419, 62.840, 61.710, 54.871, 53.856, 54.472, 50.988, 50.187, 49.946, 49.500 (MeOH), 49344, 48.741, 48.005

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=101.pdf

^{13}C NMR (D_2O): δ 171.811, 167.390, 82.305, 73.871, 72.494, 71.613, 69.195, 68.676, 66.994, 66.399, 65.460, 64.266, 63.419, 62.851, 62.660, 61.730, 58.480, 53.860, 49.962, 49.500 (MeOH), 47.997

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=111.pdf

^{13}C NMR (D_2O): δ 171.811, 167.390, 82.305, 73.894, 73.467, 72.471, 71.613, 69.195, 68.642, 66.982, 66.429, 65.445, 64.201, 63.423, 62.840, 62.687, 61.714, 58.563, 53.868, 49.500 (MeOH), 47.997

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=121.pdf

^{13}C NMR (D_2O): δ 171.807, 167.386, 82.302, 73.860, 73.326, 72.475, 71.609, 71.022, 69.187, 68.706, 66.986, 66.402, 65.445, 65.125, 64.293, 63.427, 62.851, 61.730, 53.586, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=131.pdf

^{13}C NMR (D_2O): δ 171.807, 167.386, 82.302, 73.406, 72.487, 71.609, 69.206, 68.649, 66.978, 66.406, 65.456, 64.674, 64.240, 63.426, 62.851, 62.695, 61.688, 53.860, 49.649, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=14310.pdf

^{13}C NMR (D_2O): δ 171.811, 167.310, 103.240, 73.826, 72.460, 71.613, 71.564, 71.052, 70.160, 69.214, 68.684, 68.062, 66.986, 66.891, 65.449, 65.201, 64.674, 64.350, 64.056, 63.805, 62.824, 61.779, 59.334, 58.693, 53.864, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=180.pdf

^{13}C NMR (D_2O): δ 171.379, 166.958, 72.039, 71.181, 68.759, 68.248, 66.554, 65.978, 65.020, 64.223, 63.876, 62.980, 62.411, 62.228, 61.282, 58.066, 53.432, 49.500 (MeOH), 49.220, 49.068, 48.919

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=25935.pdf

^{13}C NMR (D_2O): δ 171.811, 167.275, 110.423, 103.232, 73.807, 72.456 ,71.613, 71.564, 69.969, 69.218, 68.661, 66.982, 65.445, 64.659, 64.335, 64.059, 63.805, 62.843, 61.775, 59.330, 58.945, 58.693, 53.860, 49.653, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=54720.pdf

^{13}C NMR (D_2O): δ 171.811, 167.230, 73.494, 73.192, 72.460, 71.617, 71.571, 69.298, 69.221, 68.726, 66.986, 66.887, 65.445, 64.667, 64.030, 63.797, 62.821, 61.779, 57.334, 53.864, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=9240.pdf

^{13}C NMR (D_2O): δ 171.815, 167.329, 102.3, 72.464, 71.613, 69.206, 66.986, 66.887, 65.441, 65.132, 64.663, 64.327, 64.060, 63.793, 62.637, 61.775, 49.500 (MeOH).

Spectrum Name: dha-100ul_h13cho-50ul_cba_120106_t=9250.pdf

^{13}C NMR (D_2O): δ 171.799, 167.313, 103.228, 73.990, 73.879, 72.460, 71.613, 71.564, 71.037, 70.038, 69.305, 69.214, 68.706, 68.058, 67.978, 66.986, 66.887, 65.445, 64.674, 64.346, 64.030, 63.801, 62.824, 61.775, 60.818, 59.334, 58.693, 58.510, 53.860, 49.962, 49.645, 49.500 (MeOH).

Spectrum Name: dha_100ul_carbonate_900ul_nt=16_47sec-each_10spectra.pdf

Dihydroxyacetone dimer (Aldrich, 3.33 M, 100 µL) was mixed in 1 mL of carbonate buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C). 10 spectra @ nt=16

^{13}C NMR (D_2O): δ 171.195, 82.235, 64.808, 64.526, 64.515, 64.312, 63.424, 63.046, 62.684, 62.310, 49.500 (MeOH).

Reactions involving erythrulose

Erythrulose in CBA

Spectrum Name: 070510_erythrulose_28mg_cba_1ml_refmeoh.pdf

Erythrulose (28 mg) was dissolved in 1 mL of CBA buffer. 10 μL of a 10% MeOH in D_2O was added as a reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.026, 129.955, 110.287, 106.045 , 103.195, 98.942, 79.151, 76.557, 71.625, 66.903, 62.828, 61.787, 60.891, 49.500 (MeOH), 33.593

Erythrulose in CBA

Spectrum Name: 070530_erythrulose_cba_meohref.pdf

Erythrulose (30 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 213.348, 180.431, 178.776, 171.600, 164.245, 110.535, 103.493, 103.054, 99.682, 98.824, 98.446, 85.285, 85.072, 84.095, 82.852, 82.138, 81.757, 81.150, 82.254, 79.575, 76.893, 76.256, 75.436, 74.665, 74.070, 73.754, 73.376, 73.250, 72.991, 72.773, 72.380, 72.285, 72.041, 71.072, 70.801, 70.622, 70.111, 68.859, 68.074, 65.941, 65.655, 64.614, 64.454, 63.336, 63.286, 63.145, 63.054, 62.729, 62.348, 62.088, 61.718, 61.463, 61.329, 60.990, 60.444, 59.529, 59.353, 59.311, 59.021, 57.686, 54.947, 49.500 (MeOH), 40.463, 33.852, 10.297

Spectrum Name: erythrulose_100ul_carbonate_900ul_nt=256_725sec-each_12spectra.pdf

100 µL of a 3.33 M solution of erythrulose was mixed with 0.900 mL of carbonate buffer and 50 µL of $H^{13}CHO$ from Cambridge Isotope Laboratories and was agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C). 12 spectra @ nt=256

^{13}C NMR (D_2O): δ 171.787, 82.306, 76.382, 69.920, 71.991, 69.920, 98.531, 68.242, 67.299, 66.361, 65.308, 64.656, 64.511, 64.331, 64.087, 63.691, 63.588, 63.321, 63.588, 63.321, 63.050, 62.668, 62.603, 61.844, 60.955, 59.716, 58.598, 54.722, 51.117, 49.500 (MeOH).

Spectrum Name: erythrulose_100ul_carbonate_900ul_hcho50ul_nt=16_47sec_10spectra.pdf

100 µL of a 3.33 M solution of erythrulose was mixed with 0.900 mL of carbonate buffer and 50 µL of $H^{13}CHO$ from Cambridge Isotope Laboratories and was agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C). 10 spectra @ nt=16

^{13}C NMR (D_2O): δ 171.779, 82.314, 65.304, 64.515, 64.331, 63.584, 63.321, 63.050, 62.676, 49.500 (MeOH).

Reactions involving erythrose

Spectrum Name: 070523_erythrose_20mg_cba_1ml_nt=4096.pdf

Erythrose (20 mg) was dissolved in CBA buffer (1 mL)and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.034, 103.428, 103.203, 102.883, 82.466, 76.027, 75.734, 73.864, 71.343, 71.118, 68.039, 67.444, 49.500 (MeOH).

Spectrum Name: 1-13c-erythrose_h13cho_noboron.pdf

1-^{13}C-Erythrose (100 μL, 0.085 M, Omicron Biochemicals) was dissolved in carbonate buffer (1.18 g / 10 mL, 0.9 mL). A NMR spectrum was acquired after four minutes. Two spectra were taken on December 23, 2006 (immediately) and January 9, 2007.

^{13}C NMR (D_2O): δ 171.104, 167.000 (carbonate)

Spectrum Name: 1-13c-erythrose_h13cho_withboron_15min_24h_18days.pdf

1-^{13}C-Erythrose (100 μL, 0.085 M, Omicron Biochemicals) was dissolved in CBA buffer (0.9 mL). One drop of MeOH was added as an internal standard. A NMR spectrum was acquired after four minutes.

<u>15 minutes</u>

^{13}C NMR (D_2O): δ 171.830, 167.695, 103.438, 103.259, 82.366, 49.500 (MeOH).

<u>24 hours</u>

^{13}C NMR (D_2O): δ 171.830, 167.703, 106.459, 103.438, 103.240, 82.366, 66.090, 64.579, 64.327, 63.831, 63.458, 63.095, 49.500 (MeOH).

<u>18 days</u>

^{13}C NMR (D_2O): δ 171.834, 167.710, 106.452, 103.240, 82.363, 65.826, 64.331, 63.839, 63.469, 63.065, 49.500 (MeOH).

Spectrum Name: 2-13c-erythrose_h13cho_noboron_60min_24hours_17days.pdf

1-^{13}C-Erythrose (100 µL, 0.099 M, Omicron Biochemicals) was dissolved in carbonate buffer (0.9 mL). One drop of MeOH was added as an internal standard. A NMR spectrum was acquired after four minutes.

60 minutes

^{13}C NMR (D_2O): δ 171.799, 167.642, 110.419, 63.084, 61.791, 49.500 (MeOH), 47.303, 44.953

24 hours

^{13}C NMR (D_2O): δ 171.799, 167.642, 61.787, 49.500 (MeOH).

17 days

^{13}C NMR (D_2O): δ more peaks around 60-65 ppm similar to 24 hours

Spectrum Name: 2-13c-erythrose_h13cho_withboron_35min_36hours_18days.pdf

1-^{13}C-Erythrose (100 µL, 0.099 M, Omicron Biochemicals) was dissolved in CBA buffer (0.9 mL). One drop of MeOH was added as an internal standard. A NMR spectrum was acquired after four minutes.

35 minutes

^{13}C NMR (D_2O): δ 171.386, 167.244, 82.060, 81.923, 75.305, 49.056 note methanol standardization correction is necessary to 49.500 ppm.

36 hours

^{13}C NMR (D_2O): δ 171.830, 167.321, 82.504, 82.366, 75.752, 72.464, 65.372, 63.923, 63.511, 63.149, 49.500 (MeOH), 49.366

18 days

^{13}C NMR (D_2O): δ 171.838, 167.325, 82.366, 63.877, 49.500 (MeOH).

Spectrum Name: 071118_1-13c-erythrose_300ul_cba_900ul_camh13cho50ul_65degC_48h.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with CBA buffer (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 65 ºC for 48 hours. An aliquot (0.5 mL) was transferred to an NMR tube and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.776, 166.973, 105.301, 104.790, 90.138, 82.371, 81.879, 81.257, 80.387, 72.396, 68.928, 68.104, 67.841, 67.719, 67.467,67.135, 66.288, 66.010, 65.514, 65.293, 65.026, 64.587, 64.187, 63.801, 63.546, 63.286, 63.034, 62.771, 62.409, 62.172, 61.818, 61.444, 61.005, 60.662, 53.856, 50.213, 49.500 (MeOH).

Spectrum Name: 071118_2-13c-erythrose_300ul_h12cho_50ul_50mmcba_900ul.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with Buffer B (900 µL) and H^{12}CHO (Fisher, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 25 ºC. An aliquot (0.5 mL) was transferred to an NMR tube and a spectrum was acquired after 4 minutes.

^{13}C NMR (D_2O): δ 168.506, 82.352, 75.692, 49.500 (MeOH).

Spectrum Name: 071118_2-13c-erythrose_h13cho_cba_65degC_2days.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with CBA buffer (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 65 ºC for 48 hours. An an aliquot (0.5 mL) was transferred to an NMR tube and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.722, 166.988, 106.880, 90.134, 86.609, 84.667, 82.367, 80.990, 79.388, 77.969, 76.123, 73.471, 72.380, 69.321, 67.418, 66.296, 64.862, 63.515, 61.814, 60.944, 59.433, 55.459, 53.853, 52.479, 51.487, 49.500 (MeOH), 46.521, 43.541, 39.567

Spectrum Name: 071118_2-13c-erythrose_300ul_h12cho_50ul_50mmcba_900ul_19hours.pdf
In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with Buffer B (900 µL) and H^{12}CHO (Fisher, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 25 ºC. An aliquot (0.5 mL) was transferred to an NMR tube and a spectrum was acquired after 19 hours.
^{13}C NMR (D_2O): δ 221.988, 168.484, 107.086, 102.696, 90.138, 88.959, 84.683, 82.352, 80.662, 76.001, 75.859, 75.768, 75.692, 73.864, 70.862, 68.055, 66.639, 55.164, 55.504, 55.237, 49.500 (MeOH).

Spectrum Name:
071118_2-13c-erythrose_300ul_h12cho_50ul_50mmcba900ul_65degC_24h.pdf
In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with Buffer B (900 µL) and H^{12}CHO (Fisher, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 65 ºC. An aliquot (0.5 mL) was transferred to an NMR tube and a spectrum was acquired after 24 hours.
^{13}C NMR (D_2O): δ 171.722, 167.316, 90.092, 82.333, 81.765, 81.242, 81.059, 80.796, 80.529, 79.674, 78.812, 77.950, 76.676, 76.329, 76.191, 75.577, 74.398, 73.803, 72.522, 72.068, 71.827, 71.026, 70.809, 66.670, 65.083, 62.767, 55.233, 49.500 (MeOH).

Spectrum Name: 071118_2-13c-erythrose_300ul_h12cho_50ul_cba_900ul.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with CBA buffer (900 µL) and H^{12}CHO (Fisher, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 25 ºC. An aliquot (0.5 mL) was transferred to an NMR tube and an NMR spectrum was acquired after four minutes.

^{13}C NMR (D_2O): δ 167.122, 90.126, 82.489, 82.379, 75.718, 74.944, 73.876, 49.500 (MeOH).

Spectrum Name: 071118_2-13c-erythrose_300ul_h12cho_50ul_cba_900ul_13hours.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with CBA buffer (900 µL) and H^{12}CHO (Fisher, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 25 ºC for 13 hours. An aliquot (0.5 mL) was transferred to an NMR tube and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 167.122, 113.560, 103.558, 97.450, 94.578, 94.513, 94.383, 90.126, 84.362, 82.493, 82.379, 80.696, 79.399, 75.718, 74.944, 73.861, 68.036, 67.318, 55.520, 55.253, 49.500 (MeOH).

Spectrum Name: 071118_2-13c-erythrose_300ul_h12cho_50ul_cba_900ul_65degC_24h.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with CBA buffer (900 µL) and H^{12}CHO (Fisher, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 65 ºC for 1 day. An aliquot (0.5 mL) was transferred to an NMR tube and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.741, 166.385, 110.217, 94.380, 90.119, 86.903, 85.121, 85.018, 84.366, 84.023, 82.371, 80.643, 79.396, 78.701, 77.965, 70.813, 49.500 (MeOH).

Spectrum Name: 071118_2-13c-erythrose_h13cho_cba_65degC_2days.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with CBA buffer (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 65 ºC for 2 days. An aliquot (0.5 mL) was transferred to an NMR tube and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.772, 166.988, 90.134, 86.609, 84.667, 82.367, 80.990, 79.388, 77.969, 76.123, 73.471, 72.380, 69.321, 67.418, 66.296, 64.862, 63.515, 61.814, 60.944, 59.433, 55.459, 55.853, 52.479, 51.487, 49.500 (MeOH).

Spectrum Name:

071118_1-13c-erythrose_300ul_cba_900ul_camh13cho_50ul_65deg_2days.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with CBA buffer (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and was maintained at 65 ºC for 2 days. A 0.5 mL aliquot was transferred to an NMR tube and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.776, 166.973, 105.301, 104.790, 90.138, 82.371, 81.879, 81.257, 80.387, 72.396, 98.928, 68.104, 67.841, 67.719, 67.467, 67.135, 66.288, 66.010, 65.514, 62.293, 65.026, 64.587, 64.187, 63.801, 63.546, 63.286, 63.034, 62.771, 62.409, 62.172, 61.818, 61.444, 61.005, 60.662, 55.241, 53.856, 50.213, 49.500 (MeOH).

Spectrum Name: 071127_1-13c-erythrose_300ul_50mmboron_cba_900ul_cam50ul_071113.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with 50 mM CBA buffer (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and an aliquot (0.5 mL) was transferred to an NMR tube. The tube was maintained at 25 ºC for 2 days and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.825, 167.549, 110.306, 82.348, 81.276, 80.723, 79.354, 78.793, 78.324, 75.726, 73.716, 68.089, 67.486, 66.327, 64.793, 63.561, 63.313, 63.027, 62.764, 62.157, 61.802, 61.428, 59.437, 53.879, 51.842, 51.236, 50.187, 49.500 (MeOH), 43.541, 23.995, 20.219, 10.903

Spectrum Name: 071127_2-13c-erythrose_300ul_50mmboron_cba_900ul_cam50ul_071113.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with 50 mM CBA buffer (five fold dilution CBA, 900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and an aliquot (0.5 mL) was transferred to an NMR tube. The tube was maintained at 25 ºC for 14 days and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 181.755, 181.118, 180.378, 171.825, 167.549, 82.341, 79.453, 77.992, 67.795, 67.414, 66.304, 64.808, 63.561, 63.252, 63.023, 62.153, 61.799, 61.497, 61.425, 61.425, 61.211, 59.334, 53.879, 49.500 (MeOH), 48.771, 23.999

Spectrum Name: 071115_1-13c_erythrose_cba_071113.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with CBA buffer (900 µL). The tube was shaken using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube. To this tube was added 10 µL of a 10% MeOH in D_2O as an internal

reference. This tube was stored at 25 ºC and an NMR spectrum was acquired after 2 days. The sample is referenced to carbonate (167.000).

^{13}C NMR (D_2O): δ 169.949, 167.000 (carbonate), 74.666, 72.793, 72.278, 69.879, 69.718, 69.592, 69.375, 68.894, 59.915

The sample is referenced to carbonate (167.000) as the methanol is not resolved over the noise. Major peaks appear at 74.666, 72.793, 72.278, 69.879, 69.375 and 59.915.

Spectrum Name: 071115_E2B_2-13c-erythrose_061223.pdf

A sample from December 23rd 2006 labeled E2B was found and a spectrum was acquired on November 11th 2007. The sample was prepared as follows: In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with Buffer C (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube. This tube was stored at 50 ºC for 18 days, then at 25 ºC for the remainder of time.

^{13}C NMR (D_2O): δ 171.882, 167.316, 113.552, 84.667, 84.008, 82.356, 76.832, 73.399, 72.029, 71.049, 70.317, 68.699, 67.288, 65.304, 63.778, 63.431, 63.061, 61.806, 49.500 (MeOH).

A new peak is observed at 113.552 ppm and remains unassigned. Formaldehyde is faintly present at 82.356 ppm. A broad range of peaks are present: 84-86, 74, 72, 70, 62-68 ppm.

Spectrum Name: 071113_1-13c-erythrose_300ul_cba_900ul_hcho_cam_50ul.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with CBA (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand

vortexer. A 0.5 mL aliquot was transferred to an NMR tube. This tube was maintained at 25 ºC and a NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.764, 167.194, 106.445, 103.417, 103.241, 97.817, 90.130, 88.330, 84.355, 83.554, 82.894, 82.371, 81.837, 81.223, 80.387, 79.392, 76.416, 55.264, 55.237, 53.849, 49.500 (MeOH).

A peak at 103.417 is assigned to 1-^{13}C-erythrose. A signal appearing at 90.130 ppm is believed to be the adduct of methanol with formaldehyde. The formaldehyde signal is observed at 82.371 ppm. A moderate size peak is observed at 55.264 and 55.237 ppm.

Spectrum Name:

071113_1-13c-erythrose_300ul_50mmboronCBA_900ul_h13cho_cam_50ul.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with CBA buffer B (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube. This tube was maintained at 25 ºC and a NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 171.745, 168.571, 106.438, 103.409, 94.879, 92.278, 90.138, 88.307, 84.332, 83.474, 83.218, 82.348, 76.390, 72.419, 69.687, 64.328, 63.942, 63.820, 63.454, 62.996, 55.245, 55.218, 49.500 (MeOH), 46.521

A peak at 103.409 is assigned to 1-^{13}C-Erythrose. A signal appearing at 90.138 ppmis thought to be the adduct of methanol with formaldehyde. The formaldehyde signal is observed at 82.348 ppm. A moderate size peak is observed at 55.245 and 55.218 ppm. Small peaks are seen at 106.438, 88.307, 76.390, 72.419, 69.687, 64.328, 63.942, 63.820, 63.454, 62.996, and 46.521 ppm.

Spectrum Name: 1-erythrose_100ul_cba_900ul_50ulhcho_nt=16_47sec_14spectra.pdf

1-^{13}C-Erythrose (100 µL, 0.120 M, Omicron Biochemicals) was mixed with 0.9 mL of CBA buffer and 50 µL of H^{13}CHO and was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C). 14 spectra @ nt=16

^{13}C NMR (D_2O): δ 171.799, 82.386, 49.500 (MeOH).

Spectrum Name: 1-erythrose_300ul_cba_900ul_50ulhcho_nt=16_47sec_1-10_11-17_725s.pdf

A 0.120 M solution of 1-^{13}C-erythrose (300µL) was mixed with 0.9 mL of CBA buffer and 50 µL of H^{13}CHO and was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C). Started at 21:52 PM, spectra, 17 in a row, 1-10 spectra @ nt=16, 11-17, spectra @ nt=256

^{13}C NMR (D_2O): δ 171.199, 167, 103, 90, 82.379, 49.500 (MeOH).

Spectrum Name: 071115_1-13c-erythrose_carbonate_refmeoh_071113.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with carbonate buffer (900 µL). The tube was shaken using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube. To this tube was added 10 µL of a 10% MeOH in D_2O as an internal reference. This tube was stored at 25 ºC and an NMR spectrum was acquired after 2 days.

^{13}C NMR (D_2O): δ 171.779, 168.835, 142.399, 76.458, 74.116, 71.732, 71.553, 71.347, 71.057, 68.375, 61.837, 49.500 (MeOH).

We must note that the original erythrose degradation was performed in Buffer C.

Spectrum Name: 071115_2-13c-erythrose_300ul_cba_900ul_cam_h13cho_50ul_071113.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.099 M) was mixed with CBA buffer (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube. This tube was stored at 25 ºC and an NMR spectrum was acquired after 2 days.

^{13}C NMR (D_2O): δ 171.802, 167.213, 110.367, 90.126, 83.409, 82.493, 82.363, 81.776, 81.331, 75.726, 55.275, 53.883, 49.500 (MeOH).

A peak at 75.726 2-^{13}C-Erythrose. A signal appearing at 90.126 is thought to be the adduct of methanol with formaldehyde. The formaldehyde signal is observed at 82.363 ppm. Small yet significant peaks are seen at 110.367, 55.275 and 53.883 ppm.

Spectrum Name:

071115_1-13c-erythrose_300ul_50mmcba900ul_cam_h13cho_50ul_071113.pdf

In an eppendorf tube, 1-^{13}C-Erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with CBA buffer B (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube. This tube was stored at 25 ºC and a NMR spectrum was acquired after 2 days.

^{13}C NMR (D_2O): δ171.783, 168.567, 106.442, 104.786, 103.420, 90.126, 84.320, 83.351, 82.913, 82.333, 81.734, 81.295, 78.785, 72.411, 69.882, 69.699, 69.493, 64.969, 64.686, 64.343, 64.011, 63.778, 63.469, 62.939, 55.260, 50.210, 49.500 (MeOH), 48.745

A peak at 103.420 ppm is assigned to 1-^{13}C-erythrose. A signal appearing at 90.126 is thought to be the adduct of methanol with formaldehyde. The formaldehyde signal is observed at 82.333 ppm. Two small peaks are seen at 69.882 and 55.260 ppm as well as a broad range from 62-65 ppm with approximately the same intensity.

Spectrum Name: 071115_1-13c-erythrose_300ul_cba_900ul_cam_h13cho_50ul_071113.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 μL, 0.085 M) was mixed with CBA buffer (900 μL) and H^{13}CHO (Cambridge, 50 μL, 6.6 M). The tube was shaken using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube. This tube was stored at 25 ºC and a NMR spectrum was acquired after 2 days.

^{13}C NMR (D_2O): δ 171.810, 167.221, 106.453, 103.432, 103.245, 97.798, 90.130, 83.405, 82.363, 76.405, 72.430, 55.279, 55.253, 49.500 (MeOH).

Peaks at 103.432 and 103.245 are assigned to 1-^{13}C-erythrose. A signal appearing at 90.130 ppm is thought to be the adduct of methanol with formaldehyde. The formaldehyde signal is observed at 82.363 ppm. Small yet significant peaks are seen at 106.453, 55.279 and 55.253 ppm.

Spectrum Name:071115_2-13c-erythrose_300ul_50mmcba900ul_cam_h13cho_50ul_65c12h.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 μL, 0.099 M) was mixed with CBA buffer B (900 μL) and H^{13}CHO (Cambridge, 50 μL, 6.6 M). The tube was shaken using a hand vortexer and stored at 65 ºC. A 0.5 mL aliquot was transferred to an NMR tube and an NMR spectrum was acquired after 12 hours.

^{13}C NMR (D_2O): δ 171.783, 168.312, 83.077, 77.957, 77.599, 73.325, 72.430, 67.421, 66.574, 65.834, 65.075, 64.930, 64.682, 64.011, 63.820, 62.890, 62.111, 61.760, 61.383, 50.198, 49.500 (MeOH).

A peak corresponding to 2-^{13}C-erythrose has disappeared (~75 ppm). A signal appearing at 90.126 is thought to be the adduct of methanol with formaldehyde. The formaldehyde signal is no longer observed (82.363). Many small yet significant peaks are observed at 72.430 and 61.760. Multiplexes are seen at 82, 78 and 62-68 ppm.

Spectrum Name:

071115_2-13c-erythrose_300ul_50mmcba900ul_cam_h13cho_50ul_071113.pdf

In an eppendorf tube, 2-^{13}C-erythrose (Omicron Biochemicals, 300 μL, 0.099 M) was mixed with CBA buffer B (900 μL) and H^{13}CHO (Cambridge, 50 μL, 6.6 M). The tube was shaken using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube. This tube was stored at 25 ºC and an NMR spectrum was acquired after 2 days.

^{13}C NMR (D_2O): δ 171.779, 168.564, 90.126, 83.859, 82.333, 81.734, 81.284, 75.703, 72.476, 69.699, 66.590, 64.698, 64.331, 64.045, 63.725, 63.439, 63.225, 62.897, 62.645, 61.760, 55.256, 49.500 (MeOH).

A peak at 75.703 2-^{13}C-erythrose. A signal appearing at 90.126 is thought to be the adduct of methanol with formaldehyde. The formaldehyde signal is observed at 82.333. Small yet significant peaks are seen at 69.699, 64.698, 64.331, 63.725, 55.256.

Spectrum Name:

071115_1-13c-erythrose_300ul_50mmcba900ul_cam_h13cho_50ul_65c12h.pdf

In an eppendorf tube, 1-^{13}C-erythrose (Omicron Biochemicals, 300 µL, 0.085 M) was mixed with CBA buffer B (900 µL) and H^{13}CHO (Cambridge, 50 µL, 6.6 M). The tube was shaken using a hand vortexer and stored at 65 ºC. A 0.5 mL aliquot was transferred to an NMR tube and an NMR spectrum was acquired after 12 hours.

^{13}C NMR (D_2O): δ 171.783, 168.339, 83.325, 67.410, 64.942, 62.993, 62.111, 61.760, 61.387, 60.612, 49.500 (MeOH).

A peak corresponding to 1-^{13}C-erythrose has disappeared (~103 ppm). The signal appearing at 90.126 is thought to be the adduct of methanol with formaldehyde is no longer observed. The Formaldehyde signal is barely observed (82.325). Many small yet significant peaks are observed at 72.430 and 61.760. Multiplexes are seen at 80, 74 and 60-67 ppm.

Reactions involving Threose

Threose in CBA

Spectrum Name: 070523_threose_17mg_cba_1ml_nt=4096.pdf

Threose (17 mg) was dissolved in CBA buffer (1 mL)and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.198, 103.279, 82.333, 76.607, 71.614, 49.500 (MeOH).

Spectrum Name: 071115_13c_l-threose_cba.pdf

L-Threose (Aldrich, 18 mg) was dissolved in CBA buffer (1.0 mL) and agitated vigorously using a hand vortexer. A 0.5 mL aliquot was transferred to an NMR tube and 10 μL of a 10% MeOH in D_2O solution was added as an internal reference.

^{13}C NMR (D_2O): δ 167.175, 103.275, 82.318, 76.603, 71.610, 65.182 (minor), 49.500 (MeOH).

This spectrum exhibits the expected peaks for threose. A minor peak at 65.182 is present and unassigned.

Reactions involving pentoses

Ribose

Spectrum Name: ribose_cba_meohref.pdf

Ribose (30 mg, Fluka, final concentration 200 mM) was dissolved in CBA buffer ([B]=278 mM; 1 mL). To this was added 10 μL of 10% MeOH in D_2O as an internal reference. The solution was agitated using a hand vortexer, transferred to an NMR tube and spectrum acquisition started in 4 minutes.

^{13}C NMR (D_2O): δ 103.771, 102.482, 102.246, 102.211, 97.538, 87.834, 83.115, 82.756, 79.480, 79.167, 79.018, 77.595, 76.699, 76.527, 76.306, 71.675, 71.602, 71.392, 63.710, 62.317, 61.150, 60.967, 60.921, 49.500 (MeOH).

Interpretation is that the multiple peaks come from ribose and borate complexes that are 1:1 and 2:1.

For the following ribose spectra

UL-^{13}C-D-Ribose (15 mg, Omicron Biochemicals) was dissolved in CBA buffer (1 mL). As an internal standard, 35 µl of H^{13}CHO was evaporated, dissolved in 35 µL of D_2O, and added to the ribose solution. NMR spectra were acquired over a time period of five months.

Spectrum Name: 120906_13C_RIBOSE_5C_1655-1705.pdf

^{13}C NMR (D_2O): δ 171.811, 167.123, 104.136, 103.515, 102.710, 102.466, 102.244, 102.000, 101.207, 97.297, 96.191, 94.737, 88.359, 88.146, 87.840, 87.608, 87.314, 87.112, 83.618, 83.259, 82.839, 82.641, 82.317, 80.020, 79.708, 79.475, 79.174, 78.926, 78.609, 78.457, 78.006, 77.514, 77.175, 77.015, 76.786, 76.523, 76.290, 76.038, 75.802, 73.429, 72.906, 72.079, 71.884, 71.560, 71.354, 71.041, 70.839, 69.965, 68.973, 68.146, 65.376, 63.976, 63.755, 63.427, 63.210, 62.550, 61.997, 61.417, 61.196, 60.845, 60.627, 49.500 (MeOH).

Spectrum Name: 120906_ul5_13c_ribose_1655-1705.pdf

^{13}C NMR (D_2O): δ 171.811, 167.123, 104.136, 103.515, 102.710, 102.466, 102.244, 102.000, 101.207, 97.297, 96.191, 94.737, 88.359, 88.146, 87.840, 87.608, 87.314, 87.112, 83.618, 83.259, 82.839, 82.641, 82.317, 80.020, 79.708, 79.475, 79.174, 78.926, 78.609, 78.457, 78.006, 77.514, 77.175, 77.015, 76.786, 76.523, 76.290, 76.038, 75.802, 73.429, 72.906, 72.079, 71.884, 71.560, 71.354, 71.041, 70.839, 69.965, 68.973, 68.146, 65.376, 63.976, 63.755, 63.427, 63.210, 62.550, 61.997, 61.417, 61.196, 60.845, 60.627, 49.500 (MeOH).

Spectrum Name: 121006_ul_13c_ribose_1640.pdf

^{13}C NMR (D_2O): δ 171.811, 167.115, 104.121, 103.507, 102.710, 102.450, 102.240, 102.004, 101.207, 97.758, 97.255, 94.127, 88.359, 87.844, 87.627, 87.306, 87.116, 83.610, 83.244, 82.839, 82.633, 82.313, 80.020, 79.704, 79.475, 79.155, 78.922, 78.609, 78.457, 78.025, 77.510, 77.175, 77.015, 76.782, 76.519, 76.290, 76.038, 75.802, 73.406, 72.906, 72.079, 71.869, 71.556,

71.358, 71.041, 70.835, 69.500, 68.989, 68.119, 65.376, 63.976, 63.740, 63.423, 62.546, 61.970, 61.413, 61.192, 60.845, 60.623, 49.500 (MeOH).

Spectrum Name: 010807_ul_13c_ribose_1110.pdf

^{13}C NMR (D_2O): δ 171.811, 102.706, 102.240 ,79.471, 78.922, 76.736, 76.286, 75.802 ,72.067, 71.548, 71.369, 71.626, 71.022, 70.759, 69.496, 68.981, 69.409, 61.180, 60.845, 60.616, 49.500 (MeOH).

Spectrum Name: 022207_ul_13c_ribose.pdf

^{13}C NMR (D_2O): δ 171.802, 112.236, 111.832, 111.702, 111.508, 111.366, 111.218, 110.989, 110.657, 110.489, 104.107, 103.481, 102.707, 102.440, 102.242, 101.982, 101.807, 88.352, 87.826, 87.582, 87.300, 83.618, 83.229, 82.596, 82.192, 81.932, 81.509, 80.571, 80.029, 79.487, 78.934, 78.694, 78.461, 78.244, 77.957, 77.416, 77.214, 76.866, 76.722, 76.371, 76.302, 76.233, 75.843, 75.806, 74.566, 73.323, 73.235, 73.014, 72.838, 72.705, 72.525, 72.194, 72.068, 71.907, 71.770, 71.549, 71.400, 71.263, 71.026, 70.900, 70.759, 69.500, 69.306, 68.985, 68.463, 67.097, 67.006, 66.712, 66.647, 66.430, 66.128, 65.419, 64.991, 64.690, 64.324, 63.981, 63.702, 63.443, 62.577, 62.138, 62.020, 61.840, 61.410, 61.192, 60.852, 60.626, 59.571, 59.079, 49.500 (MeOH).

Spectrum Name: 070702_d-ribose_cba_samplefrom070411_81dayslater.pdf

D-ribose (30 mg, Aldrich) was dissolved in 1.00 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 179.370, 165.404, 76.053, 73.211, 72.418, 70.930, 63.400. No reference.

Spectrum Name: 071019_ul-13c-d-ribose_7mg_cba_1ml_meohref.pdf

D-UL-^{13}C-Ribose (Omicron Biochemicals, 7 mg) was dissolved in CBA buffer (1.0 mL) in an eppendorf tube. An aliquot (0.5 mL) was transferred to an NMR tube and 10 μL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 167.297, 104.271, 103.684, 102.722, 102.253, 97.306, 96.737, 96.268, 88.410, 87.891, 87.372, 83.355, 82.939, 82.756, 82.341, 80.036, 79.487, 78.938, 78.476, 77.671, 76.813, 76.321, 75.829, 73.403, 72.934, 72.110, 71.587, 71.068, 69.096, 68.589, 68.089, 63.969, 63.431, 62.558, 61.993, 61.436, 60.868, 49.500 (MeOH).

Spectrum Name: 20071307_d-ribose_47mg_carbonatebuffer_refmeoh.pdf

D-ribose (47 mg, Aldrich) was dissolved in 1.0 mL of carbonate buffer and was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 168.770, 100.319, 94.818, 94.494, 83.031, 71.610, 71.171, 70.843, 70.610, 69.535, 69.310, 67.719, 67.650, 64.129, 63.481, 62.596, 49.500 (MeOH).

Spectrum Name: 071020_ul-13c-d-ribose_7mg_cba_1ml_nomeoh.pdf

D-UL-^{13}C-Ribose (Omicron Biochemicals, 7 mg) was dissolved in CBA buffer (1.0 mL) in an eppendorf tube. An aliquot (0.5 mL) was transferred to an NMR tube. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 167.000 (carbonate), 103.962, 103.379, 102.418, 101.952, 101.151, 96.943, 96.459, 95.936, 88.116, 87.807, 87.601, 87.052, 83.062, 82.631, 82.005, 79.735, 79.186, 78.637, 78.183, 77.389, 76.516, 76.020, 75.532, 73.083, 72.690, 71.809, 71.286, 70.767, 69.199, 68.795, 68.185, 67.849, 63.668, 63.123, 62.253, 61.684, 61.135, 60.567.

Arabinose

Spectrum Name: arabinose_cba_meohref.pdf

D-arabinose (30 mg, Aldrich) was dissolved in CBA buffer (1 mL). To this was added 10 μL of 10% MeOH in D_2O as an internal reference. The solution was agitated using a hand vortexer, transferred to an NMR tube and spectrum acquisition started in 4 minutes.

^{13}C NMR (D_2O): δ 104.050, 103.920, 103.539, 97.370, 92.260, 95.047, 94.628, 93.106, 86.544, 86.197, 85.739, 85.556, 85.194, 83.672, 83.393, 83.206, 83.073, 78.831, 77.256, 76.798, 75.165, 72.968, 72.361, 72.197, 71.957, 71.408, 71.072, 70.919, 70.519, 70.298, 70.179, 69.867, 69.027, 68.653, 68.570, 68.447, 66.975, 65.789, 63.961, 62.279, 62.916, 62.691, 62.607, 49.500 (MeOH).

Spectrum Name: 070626_2-13C-arabinose_100ul_CBA_500ul_refmeoh.pdf

A 0.120M solution of 2-^{13}C-arabinose (100μL) was dissolved in 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.309, 110.329, 83.329, 80.231, 75.127, 72.235, 70.233, 68.493, 49.500 (MeOH).

Spectrum Name: 070626_5-13C-arabinose_100ul_CBA_500ul_refmeoh.pdf

A 0.120M solution of 5-^{13}C-arabinose (100μL) was dissolved in 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.312, 95.089, 94.769, 78.457, 72.258, 70.546, 68.768, 67.009, 66.086, 64.099, 63.321, 62.878, 52.807, 49.500 (MeOH).

Spectrum Name: 070626_UL-ARABINOSE_100ul_D2O_500ul_refmeoh.pdf

A 0.120M solution of UL-^{13}C-arabinose (100μL) was dissolved in 0.500 mL D_2O and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 101.956, 101.372, 98.068, 97.725, 97.630, 97.546, 97.244, 97.161, 97.065, 96.058, 95.482, 93.495, 93.441, 93.350, 93.258, 93.083, 92.097, 81.141, 83.577, 83.016, 82.043, 81.471, 76.855, 76.630, 76.161, 74.746, 73.731, 73.635, 73.204, 73.113, 72.930, 72.819, 72.644, 72.358, 71.839, 70.019, 69.600, 69.493, 69.409, 69.355, 69.207, 69.161, 69.107, 69.073, 68.993, 68.924, 68.837, 68.726, 68.612, 67.219, 67.006, 66.719, 63.213, 63.202, 62.973, 62.859, 61.997, 61.425, 49.500 (MeOH).

Spectrum Name: 070626_ul_arabinose_50ul_cba_950ul_refmeoh.pdf

A 0.120M solution of UL-^{13}C-arabinose (50μL) was dissolved in 0.950 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH

in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 206.802, 167.000, 161.179, 94.906, 94.441, 71.866, 71.324, 70.618, 70.145, 69.592, 68.539, 68.002, 67.582, 63.149, 62.611, 49.500 (MeOH), 40.174

Xylose

Spectrum Name: xylose_cba_meohref.pdf

Xylose (30 mg, Aldrich) was dissolved in CBA buffer (1 mL). To this was added 10 µL of 10% MeOH in D_2O as an internal reference. The solution was agitated using a hand vortexer, transferred to an NMR tube and spectrum acquisition started in 4 minutes.

^{13}C NMR (D_2O): δ 111.344, 103.569, 103.028, 97.279, 92.816, 83.523, 83.157, 79.918, 77.568, 76.958, 76.73, 76.664, 76.430, 76.310, 75.077, 74.551, 73.307, 72.461, 71.949, 69.897, 49.794, 65.781, 71.570, 61.398, 61.066, 60.730, 60.227, 59.582, 57.656, 54.310, 49.500 (MeOH).

1-^{13}C-Xylose in CBA

Spectrum Name: 070610_1-13C-xylose_50ul_0.120M_cba_950ul.pdf

1-^{13}C-xylose (50 µL, 0.120 M) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added a sample of authentic threose and 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.370, 103.249, 49.500 (MeOH).

Spectrum Name: 070626_1-2-13C-xylose_100ul_CBA_500ul_refmeoh.pdf

A 0.120M solution of 1,2-^{13}C-xylose (100µL) was dissolved in 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.305, 103.489 103.024, 83.622, 83.161, 49.500 (MeOH).

Spectrum Name: 070626_1-2-13C-xylose_100ul_D2O_500ul_refmeoh.pdf

A 0.120M solution of 1,2-^{13}C-xylose (100µL) was dissolved in 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 97.557, 96.947, 96.390, 95.822, 93.167, 92.552, 74.959, 74.349, 72.392, 71.782, 69.874, 65.823, 49.500 (MeOH).

Spectrum Name: 070626_2-13C-xylose_100ul_CBA_500ul_refmeoh.pdf

A 0.120M solution of 2-^{13}C-xylose (100µL) was dissolved in 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.305, 103.489, 103.024, 83.622, 83.161, 49.500 (MeOH).

Spectrum Name: 070626_2-13C-xylose_100ul_D2O_500ul_refmeoh.pdf

A 0.120M solution of 2-^{13}C-xylose (100µL) was dissolved in 0.500 mL D_2O and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 97.550, 96.939, 89.066, 81.219, 77.774, 76.794, 76.218, 75.402, 74.665, 74.364, 73.868, 73.231, 72.094, 69.874, 68.815, 61.558, 55.020, 54.207, 49.500 (MeOH).

Spectrum Name: 070626_UL-xylose_100ul_CBA_500ul_refmeoh.pdf

A 0.120M solution of UL-^{13}C-xylose (100µL) was dissolved in 0.500 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.305, 103.485, 103.028, 83.912, 83.336, 82.871, 77.820, 77.324, 76.767, 75.562, 75.058, 74.574, 61.161, 60.635, 49.500 (MeOH).

Spectrum Name: 070626_ul-xylose_100ul_d2o_500ul_refmeoh.pdf

A 0.120M solution of UL-^{13}C-xylose (100µL) was dissolved in 0.500 mL D_2O and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 97.584, 97.519, 96.985, 96.920, 93.163, 92.568, 76.969, 76.500, 76.462, 76.016, 75.951, 75.188, 74.677, 74.589, 74.547, 74.078, 74.032, 73.502, 73.006, 72.609, 72.544,

72.016, 72.010, 71.961, 71.511, 71.461, 70.538, 70.485, 70.385, 70.343, 70.023, 69.985, 69.859, 69.538, 69.474, 69.355, 69.310, 66.067, 65.537, 61.802, 61.284, 49.500 (MeOH).

Spectrum Name: 070702_xylose_cba_samplefrom070411_81dayslater.pdf

D-xylose (30 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.855, 122.715, 111.282, 83.241, 83.142, 76.962, 76.504, 72.464, 63.965, 49.500 (MeOH).

Spectrum Name: 20071407_d-xylose_cba_20070411.pdf

To a sample, from a reaction prepared on 04/11/2007, of d-xylose (30 mg) in CBA buffer (1 mL) was added 10 μL of a 10% MeOH in D_2O as an internal reference. A NMR spectrum was acquired three days later on 07/14/2007.

^{13}C NMR (D_2O): δ 166.717, 111.309, 83.271, 83.130, 76.954, 76.508, 72.464, 49.500 (MeOH).

Spectrum Name: 071127_2-13c-xylose_2-13c-xylulose_enzyme_50ul_cba_450ul_meohref.pdf

In an eppendorf tube, 2-^{13}C-xylose (500 μL, 0.120 M) was added to a resin bound enzyme. An aliquot (50 μL) was dissolved in CBA buffer (450 μL). To this tube was added10 μL of a 10% MeOH in D_2O as a reference. Acquisition of an NMR spectrum was begun after four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.259, 111.435, 83.386, 74.623, 49.500 (MeOH).

Lyxose

Spectrum Name: lyxose_cba_meohref.pdf

Lyxose (30 mg, Aldrich) was dissolved in CBA buffer (1 mL). To this was added 10 μL of 10% MeOH in D_2O as an internal reference. The solution was agitated using a hand vortexer, transferred to an NMR tube and spectrum acquisition started in 4 minutes.

^{13}C NMR (D_2O): δ 111.351, 102.516, 102.375, 97.790, 97.206, 96.443, 95.230, 94.712, 83.153, 82.726, 81.715, 81.463, 80.201, 78.972, 76.958, 76.847, 76.767, 75.199, 73.658, 72.472, 70.614, 68.833, 68.447, 68.180, 64.854, 64.686, 61.181, 61.017, 49.500 (MeOH).

1-^{13}C-Lyxose in CBA

Spectrum Name: 070611_1-13c-lyxose_cba_refmeoh_nt=1024.pdf

1-^{13}C-Lyxose (50μL, 0.120M) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added a sample of authentic threose and 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.385, 102.574, 99.934, 97.790, 96.359, 95.188, 49.500 (MeOH).

Ribulose

Spectrum Name: 121106_ribulose_cba_start1900-1930.pdf nomeoh

Ribulose (Fluka, 30.5 mg) was dissolved in CBA (0.9 mL) D_2O (0.1 mL). Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 178.005, 167.000 (carbonate), 139.291, 111.082, 76.655, 71.178, 69.209, 66.966, 64.609

Spectrum Name: 121106_ribulose_cba_start1930-0200.pdf

^{13}C NMR (D_2O): δ 167.000 (carbonate), 111.631, 111.090, 104.994, 82.003, 76.869, 76.659, 76.339, 75.145, 73.699, 72.723, 71.353, 71.189, 69.217, 68.671, 66.963, 64.620, 64.338, 64.113

Spectrum Name: 121206_ribulose_cba_1015.pdf

^{13}C NMR (D_2O): δ 167.000 (carbonate), 111.082, 104.971, 81.988, 76.655, 76.331, 75.141, 72.719, 71.330, 71.181, 69.209, 68.687, 66.921, 64.609, 64.296, 61.588, 61.042, 60.726, 55.584

Spectrum Name: ribulose_cba_start121106_050407.pdf

To the sample started on 12/11/06 was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.799, 166.694, 111.096, 105.072, 82.066, 79.945, 76.928, 76.706, 76.420, 75.238, 73.902, 72.808, 72.483, 72.430, 71.270, 70.065, 69.298, 68.779, 65.697, 63.824, 61.791, 61.142, 59.342, 53.856, 49.500 (MeOH).

Xylulose

Spectrum Name: xylulose_CBA_030207.pdf

Xylulose (20 mg) was dissolved in CBA buffer (1 mL)and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 166.980, 111.532, 111.407, 83.322, 83.185, 76.977, 76.532, 72.490, 64.704, 64.274, 49.500 (MeOH).

1,2-^{13}C-Xylulose in CBA

Spectrum Name: 070610_1-2-13C-xylulose_cba_05302007.pdf 2048

1,2-^{13}C-Xylulose (50 μL, 0.120 M) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added a sample of authentic threose and 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.316, 111.752, 111.035, 65.068, 64.343, 49.500 (MeOH).

Spectrum Name: 1-2-13c-xylulose_cba_refmeoh.pdf

A 0.120 M solution of 1,2-^{13}C-Xylulose (50 μL) was mixed with 0.950 mL CBA buffer. The mixture was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.316, 111.790, 111.073, 65.072, 64.354, 49.500 (MeOH).

Spectrum Name: xylulose_CBA_start1500-033107-scanned-033107-2245-0445_nt=8192.pdf

30 mg of d-xylulose was dissolved in 1.0 mL of CBA buffer and was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 167.000, 111.470, 110.753, 106.553, 106.358, 105.939, 102.685, 83.150, 83.092, 82.661, 76.920, 76.665, 76.428, 72.430, 72.213, 71.934, 64.759, 64.454, 64.347, 64.042, 63.737, 63.630, 49.500 (MeOH).

1,2,4,5-tetrahydroxypentan-3-one (3-pentulose)

Spectrum Name: pentulose_d2o_030807_eureka.pdf

1,2,3,4-tetrahydroxy-pentan-3-one (30 mg) was dissolved in D_2O (1 mL). One drop of MeOH was added as an internal standard and a spectrum was acquired after four minutes.

^{13}C NMR (D_2O): δ 222.522, 212.226, 81.799, 76.336, 74.421, 73.483, 73.346, 73.037, 72.399, 71.335, 70.958, 63.374, 63.149, 59.861, 59.075, 49.500 (MeOH), 48.802, 47.741, 43.187, 42.866, 26.799, 25.159, 19.471, 19.318

Spectrum Name: 1,2,3,4-pentan-3-one.pdf

1,2,3,4-tetrahydroxy-pentan-3-one (30 mg) was dissolved in D_2O (1 mL) and a spectrum was acquired after four minutes.

^{13}C NMR (D_2O): δ 211.818, 75.923, 62.959. No reference.

Spectrum Name: 20071307_pentulose_15mg_200ul_d2o_h13cho_30ul_800ul_cba_nt=256.pdf

3-Pentulose (15 mg) was dissolved in 200 μL of D_2O. To this was added 30 μL of $H^{13}CHO$ (6.66 M), 0.800 mL of CBA buffer and was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.787, 168.770, 82.329, 65.335, 64.732, 64.362, 64.087, 63.786, 63.538, 63.065, 62.588, 62.527, 61.989, 61.722, 61.325, 61.036, 49.500 (MeOH).

Spectrum Name: 20071307_pentulose_15mg_200ul_d2o_h13cho_30ul_800ul_cba_nt=8192.pdf

3-Pentulose (15 mg) was dissolved in 200 μL of D_2O. To this was added 30 μL of $H^{13}CHO$ (6.66 M), 0.800 mL of CBA buffer and was agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 171.787, 168.770, 82.329, 65.335, 64.732, 64.362, 64.087, 63.786, 63.538, 63.065, 62.588, 62.527, 61.989, 61.722, 61.325, 61.036, 49.500 (MeOH).

Branched pentose

Branched pentose in borate (0.250 M, t = 0 hours)

Spectrum Name: 070518_branched_pentose_borate-only_refmeoh.pdf

Erythro-branched pentose (30 mg) was dissolved in 1 mL of 0.250 M Sodium tetra-borate in D_2O and agitated vigorously using a vortex stirrer. To this was added 10 μL of a 10% MeOH in

D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25 °C).

^{13}C NMR (D_2O): δ 104.664, 104.603, 104.466, 104.366, 98.953, 98.870, 90.046,85.396, 85.232, 84.572, 84.439, 84.294, 84.023, 82.371, 78.167, 78.007, 77.771, 73.750, 71.253, 71.183, 71.011, 70.187, 69.271, 68.863, 68.787, 68.749, 64.072, 63.931, 63.805, 63.412, 63.366, 63.199, 63.004, 49.500 (MeOH), 48.970, 48.680, 48.394

Branched pentose in borate (0.250 M, t = 13 hours)

Spectrum Name: 070519_branched_pentose_borate-only_refmeoh_13hourslater_nt=8192.pdf

Erythro-branched pentose (30 mg) was dissolved in 1 mL of 0.250 M Sodium tetra-borate in D_2O and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25 °C).

^{13}C NMR (D_2O): δ 104.366, 104.247, 104.186, 104.049, 103.946, 98.537, 98.449, 89.629, 84.983, 84.815, 84.270, 84.155, 84.026, 83.873, 83.605, 81.954, 77.747, 77.590, 73.337, 70.877, 70.758, 70.667, 70.594, 70.152, 69.770, 68.851, 68.443, 68.370, 68.328, 63.652, 63.510, 63.388, 62.992, 62.950, 62.782, 62.583, 49.500 (MeOH), 49.083, 48.553, 48.263, 47.977

Branched pentose in CBA

Spectrum Name: 070523_branched_pentose_30mg_cba_1ml_nt=8192.pdf

Erythro-branched pentose (30 mg) was dissolved in CBA buffer (1 mL) and agitated vigorously using a vortex stirrer. To this was added 10 µL of a 10% MeOH in D_2O as an internal reference.

Acquisition of an NMR spectrum was begun within four minutes, and was completed after 8192 transients (ca. 6 hours total acquisition at 25°C).

^{13}C NMR (D_2O): δ 105.255, 104.778, 104.645, 104.462, 104.355, 98.747, 85.404, 84.687, 84.587, 84.370, 84.027, 82.306, 80.132, 77.366, 73.986, 73.685, 71.347, 71.236, 71.175, 70.916, 69.199, 68.722, 64.446, 64.236, 63.816, 63.420, 49.500 (MeOH).

Spectrum Name: 071119_ery-branched-pentose_cba_refmeoh_nt=8192.pdf

Erythro-branched-pentose (25 mg, 0.167 mmol) was dissolved in CBA buffer (1 mL). The tube was shaken using a hand vortexer and an aliquot (0.5 mL) was transferred to an NMR tube. To this was added 10 μL of a 10% MeOH in D_2O was added as a reference. The tube was maintained at 25 ºC for 2 days and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 167.259, 105.335, 104.813, 85.125, 84.397, 82.341, 80.689, 80.223, 74.223, 74.021, 71.213, 69.230, 64.644, 63.851, 49.500 (MeOH).

Sharp singlets are seen at 104.813, 84.397, 82.341, 74.021, 71.213, 69.230 and 63.851.

<u>Hexoses</u>

Spectrum Name: 071014_d-12C-GLUCOSE_50mg_CBA_1ml_meohref.pdf

Glucose (Aldrich, 50 mg) was dissolved in CBA (1.0 mL) in an eppendorf tube. An aliquot (0.5 mL) was transferred to an NMR tube and 10 μL of a 10% MeOH in D_2O solution was added as an internal reference. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 103.920, 103.218, 103.058, 96.539, 92.659, 82.753, 78.743, 78.137, 77.671, 76.493, 76.157, 75.997, 75.821, 74.658, 73.174, 71.980, 70.080, 69.104, 64.507, 64.217, 61.200, 61.051, 49.500 (MeOH).

Spectrum Name: 071014_d-12C_GLUCOSE_50mg_D2O_1ml_nomeoh.pdf

Glucose (Aldrich, 50 mg) was dissolved in D_2O (1.0 mL) in an eppendorf tube. An aliquot (0.5 mL) was transferred to an NMR tube. This tube was maintained at 25 ºC and an NMR spectrum was acquired.

^{13}C NMR (D_2O): δ 96.057, 92.243, 76.091, 75.908, 74.287, 72.914, 71.632, 71.582, 69.797, 69.751, 60.905, 60.745. No reference.

Formaldehyde in CBA buffer saturated with sodiumtetraborate

Spectrum Name: 070724_H13CHO_SCBA_1ml_nomeoh.pdf

A 6.66 M solution of $H^{13}CHO$ (30 µL) was mixed with 1.00 mL SCBA buffer. The mixture was agitated vigorously using a vortex stirrer. Acquisition of an NMR spectrum was begun within four minutes, and was completed after 4096 transients (ca. 3 hours total acquisition at 25 °C).

^{13}C NMR (D_2O): δ 171.378, 162.673, 92.578, 87.272, 86.189, 85.143, 84.079, 83.736, 83.515, 83.160, 82.473, 81.954, 81.390, 80.718, 80.341, 80.142, 79.830, 79.437, 79.009, 78.769, 77.407, 75.584, 71.334, 49.080. No reference.

For the following six spectra (070331A)

1,2,3-^{13}C-glyceraldehyde (0.1 mL, final concentration 11.1 mM, 11.1 micromoles, Omicron Biochemicals) was mixed with $H^{13}CHO$ (0.0035 mL, 6.66 M, [$H^{13}CHO$] = 23.3 mM final concentration 2.1:1 ratio) in CBA buffer (0.9 mL).

Spectrum Name: 070331A_glyceraldehyde_H13CHO_040507_1015am.pdf

^{13}C NMR (D_2O): δ 171.752, 167.204 (carbonate), 82.305 (HCHO), 71.784, 65.734, 65.028, 63.842, 62.499

070331A_glyceraldehyde_H13CHO_040907_nt=256.pdf

^{13}C NMR (D_2O): δ 171.767, 167.223 (carbonate), 102.374, 101.775, 98.903, 95.832, 82.305 (HCHO), 82.099, 79.989, 76.518, 74.050, 73.596, 72.547, 71.738, 71.265, 70.811, 66.959, 66.611, 65.768, 65.036, 64.262, 63.014, 61.874, 61.385, 58.883, 58.387

Spectrum Name: 070331A_glyceraldehyde_H13CHO_041007_nt=4096_0000-0300.pdf

^{13}C NMR (D_2O): δ 171.767, 167.216 (carbonate), 105.502, 104.964, 104.396, 104.270, 103.755, 103.396, 102.931, 102.256, 101.722, 100.905, 99.490, 99.421, 98.887, 96.301, 95.813, 82.305 (HCHO), 81.642, 81.172, 79.360, 77.361, 77.010, 76.518, 75.973, 74.164, 73.657, 73.081, 72.372, 71.796, 71.300, 69.385, 68.843, 67.065, 66.608, 65.787, 65.013, 63.869, 63.056, 62.465, 62.122, 61.393, 60.798, 58.887, 58.391, 26.618, 25.977

Spectrum Name: 070331A_glyceraldehyde_H13CHO_041507.pdf

^{13}C NMR (D_2O): δ 171.759, 170.512, 167.201 (carbonate), 112.845, 111.380, 104.968, 104.274, 103.434, 102.244, 101.813, 99.410, 98.887, 96.232, 95.828, 85.479, 82.305 (HCHO), 79.837, 76.564, 74.157, 73.657, 72.639, 71.796, 71.262, 69.373, 67.058, 66.608, 65.723, 65.009, 64.056, 61.393, 60.317, 58.871, 58.395, 28.041, 26.542, 25.962, 24.635, 23.162

Spectrum Name: 070331A_glyceraldehyde_H13CHO_CBA_nt=256_start2215-2230.pdf

^{13}C NMR (D_2O): δ 167.227 (carbonate), 151.995, 104.411, 102.359, 101.741, 99.978, 94.520, 82.305 (HCHO), 80.432, 77.373, 73.364, 73.325, 71.414, 65.715, 63.834, 63.110, 62.534, 60.378, 52.810

Spectrum Name: 070331A_glyceraldehyde_H13CHO_CBA_t=2_1300_ref=co3-167.pdf

^{13}C NMR (D_2O): δ 167.000 (carbonate), 102.139, 94.857, 94.319, 82.074, 80.685, 80.136, 79.709, 76.260 73.594, 63.569, 63.008

Spectrum Name:

070331_glyceraldehyde_H13CHO_start0130am040207end0730am_nt=8192.pdf

^{13}C NMR (D_2O): δ 171.767, 167.220 (carbonate), 105.502, 104.968, 104.789, 104.415, 104.262, 103.759, 103.438, 102.942, 102.359, 102.263, 101.832, 101.737, 100.814, 100.337, 99.490, 98.956, 98.891, 96.324, 95.813, 94.588, 84.014, 82.305 (HCHO), 81.672, 81.145, 79.803, 77.060, 76.522, 75.976, 74.161, 73.642, 73.043, 71.792, 71.300, 69.358, 67.123, 66.608, 65.787, 65.082, 64.387, 63.853, 63.102, 62.530, 61.923, 61.389, 58.936, 58.440, 26.622, 26.527, 25.974, 25.878

Experiment **070331A spectra**

U-^{13}C-glyceraldehyde (0.1 mL, final concentration 11.1 mM, 11.1 micromoles) was mixed with H^{13}CHO (0.0035 mL, 6.66 M, [H^{13}CHO] = 23.3 mM final concentration 2.1:1 ratio) and dissolved in CBA buffer (0.9 mL).

Spectrum Name:

070331A_U13C-glyceraldehyde_H13CHO_start0130am040207end0730am.pdf

^{13}C NMR (D_2O): δ 171.767, 167.220 (carbonate), 105.502, 104.968, 104.789, 104.415, 104.262, 103.759, 103.438, 102.942, 102.359, 102.263, 101.832, 101.737, 100.814, 100.337, 99.490, 98.956, 98.891, 96.324, 95.813, 94.588, 84.014, 82.305 (HCHO), 81.672, 81.145, 79.803, 77.060, 76.522, 75.976, 74.161, 73.642, 73.043, 71.792, 71.300, 69.358, 67.123, 66.608, 65.787, 65.082, 64.387, 64.853, 63.102, 62.530, 61.923, 61.389, 58.936, 58.440, 26.622, 26.527, 25.974, 25.878

Spectrum Name:

070331A_U13C-glyceraldehyde_refH13CHO-82.350_nt=2048_040107-1300.pdf

^{13}C NMR (D_2O): δ 171.763, 167.223 (carbonate), 104.976, 104.415, 103.751, 103.442, 102.363, 102.263, 101.925, 100.505, 99.494, 96.347, 95.801, 94.558, 83.991, 82.305 (HCHO), 80.478, 74.164, 73.627, 71.784, 71.296, 65.787, 65.066, 64.449, 63.823, 63.106, 62.556, 26.538, 25.974

070331A_U-glyceraldehyde_H13CHO_CBA_t=2_1300_refco3_-167.pdf

^{13}C NMR (D_2O): δ 167.000 (carbonate), 102.139, 94.857, 94.319, 82.074, 80.685, 80.136, 79.709, 76.260, 73.594, 64.319, 63.569, 63.008

Spectrum Name: 070427_repeatof070331A_withMeOHref.pdf

U-^{13}C-glyceraldehyde (0.1 mL, final concentration 11.1 mM, 11.1 micromoles) was mixed with H^{13}CHO (0.0035 mL, 6.66 M, [H^{13}CHO] = 23.3 mM final concentration 2.1:1 ratio) and dissolved in CBA buffer (0.9 mL). To this was added 10ul of a 10% MeOH in D_2O solution as an internal reference.

^{13}C NMR (D_2O): δ 171.821, 167.248, 113.552, 112.919, 106.796, 106.102, 105.557, 105.030, 104.321, 103.821, 103.043, 102.337, 101.803, 99.549, 99.484, 99.018, 98.953, 98.328, 95.879, 90.142, 85.064, 82.371, 81.696, 81.162, 80.693, 77.076, 76.580, 76.050, 74.215, 73.708, 73.182, 72.419, 71.877, 71.362, 70.996, 70.427, 69.455, 67.124, 66.662, 65.850, 65.785, 65.483, 65.072, 64.057, 63.080, 62.180, 61.455, 59.578, 58.945, 58.464, 49.500 (MeOH), 26.684, 26.028

Spectrum Name:

070427_repeatof070331A_withMolybdicacid_13mg_nomeoh_refhcho82.37.pdf

U-^{13}C-glyceraldehyde (0.1 mL, final concentration 11.1 mM, 11.1 micromoles) was mixed with H^{13}CHO (0.0035 mL, 6.66 M, [H^{13}CHO] = 23.3 mM final

concentration 2.1:1 ratio) and dissolved in CBA buffer (0.9 mL). To this was added 13 mg of molybdic acid (Aldrich).

^{13}C NMR (D_2O): δ 171.810, 165.844, 137.157, 136.879, 136.402, 136.082, 118.755, 118.534, 117.970, 117.611, 114.220, 113.522, 113.289, 112.927, 106.686, 106.072, 105.511, 104.981, 104.267, 103.447, 102.986, 99.518, 99.453, 98.923, 96.829, 95.802, 84.992, 82.371 (HCHO), 81.669, 81.135, 80.597, 77.069, 76.569, 76.031, 74.761, 74.208, 73.681, 73.139, 72.792, 72.346, 71.835, 71.293, 70.946, 70.420, 68.720, 67.112, 66.658, 66.319, 66.208, 65.762, 65.049,63.080, 62.191, 61.463, 61.062, 60.868, 59.582, 59.079, 58.922, 58.434, 54.165, 50.492, 50.213, 50.148, 50.042, 49.931

Experiment **070331B spectra**

Spectrum Name:

070331B_glyceraldehyde_H12CHO_start0730am040207end1330pmnt=8192.pdf

1,2,3-^{13}C-glyceraldehyde (0.1 mL, final concentration 11.1 mM, 11.1 micromoles) was mixed with HCHO (0.002 mL, 12 M, [HCHO] = 24 mM final concentration 2.2:1 ratio) and dissolved in CBA buffer (0.9 mL)

^{13}C NMR (D_2O): δ 171.763, 167.216, 105.502, 104.949, 104.789, 104.400, 104.262, 103.755, 103.412, 102.939, 102.363 102.267, 101.829, 101.737, 100.833, 100.333, 99.486, 99.418, 98.952, 96.328, 95.798, 94.569, 82.305 (HCHO), 79.528, 77.937, 77.434, 76.766, 76.213, 75.763, 74.760, 74.145, 72.745, 72.211, 70.838, 70.155, 68.481, 65.719, 65.002, 64.395, 63.796, 73.014, 61.389, 49.438, 26.618, 26.523, 25.966, 25.867

Experiment **070331D spectra**

1-^{13}C-glycolaldehyde (0.1 mL, final concentration 11.6 mM, 11.6 micromoles) was mixed with H^{13}CHO (Aldrich-Isotec, 0.0054 mL, 6.66 M, [H^{13}CHO] = 35.6 mM final concentration, 3.1:1 ratio) and the mixture was dissolved in CBA buffer (0.9 mL)

Spectrum Name: 070331D_glycolaldehyde_H13CHO_ref=hcho_82.305_nt=256_t=2.pdf

^{13}C NMR (D_2O): δ 167.253, 98.262, 93.059, 82.305

070331D_glycolaldehyde_H13CHO_ref=hcho_82.305_nt=256_t=17.pdf

^{13}C NMR (D_2O): δ 171.767, 167.231, 98.273, 97.220, 92.975, 89.938, 82.305 (HCHO), 80.440, 73.939, 63.533, 53.081, 9.906

Spectrum Name: 070331D_glycolaldehyde_H13CHO_ref=hcho_82.305_nt=256_t=32.pdf

^{13}C NMR (D_2O): δ 167.231, 98.266, 93.005, 92.002, 91.132, 82.305 (HCHO).

Spectrum Name: 070331D_glycolaldehyde_H13CHO_ref=hcho_82.305_nt=256_t=47.pdf

^{13}C NMR (D_2O): δ 167.235, 93.120, 82.305 (HCHO).

Spectrum Name: 070331D_glycolaldehyde_H13CHO_ref=hcho_82.305_nt=256_t=62.pdf

^{13}C NMR (D_2O): δ 191.954, 185.877, 176.077, 171.763, 167.231, 138.373, 98.269, 93.131, 90.571, 82.305 (HCHO), 63.495

Spectrum Name: 070331D_glycolaldehyde_H13CHO_ref=hcho_82.305_nt=256_t=77.pdf

^{13}C NMR (D_2O): δ 171.767, 167.231, 161.391, 103.225, 98.262, 96.538, 92.998, 91.636, 90.655, 82.305 (HCHO), 69.137, 63.480, 11.138

Spectrum Name: 070331D_glycolaldehyde_H13CHO_040207_1400-2000.pdf

^{13}C NMR (D_2O): δ 171.767, 167.223, 103.213, 100.650, 100.192, 99.250, 98.788, 98.266, 96.034, 94.806, 93.165, 85.521, 82.553, 82.305 (HCHO), 82.007, 81.412, 79.661, 76.816,

76.560, 76.263, 74.161, 71.803, 71.551, 71.288, 65.459, 64.147, 63.728, 63.438, 63.090, 62.820, 62.537, 59.200, 58.643, 58.395, 56.171, 53.218, 40.542, 36.186, 26.676, 26.298

Spectrum Name: 070331D_glycolaldehyde_H13CHO_040507_1045am.pdf

^{13}C NMR (D_2O): δ 219.652, 171.748, 167.212, 167.067, 129.401, 116.748, 110.198, 103.217, 98.262, 82.305 (HCHO), 76.813, 76.545, 71.563, 71.139, 65.387, 63.865, 63.209, 26.195.

Spectrum Name: 070331D_glycolaldehyde_H13CHO_040907_nt=256.pdf

^{13}C NMR (D_2O): δ 171.622, 171.428, 166.877, 150.149, 102.874, 110.683, 109.863, 106.410, 102.874, 97.911, 95.622, 93.227, 88.024, 85.323, 84.270, 82.546, 82.305 (HCHO), 81.954, 71.594, 71.407, 70.930, 63.777, 63.114, 62.454.

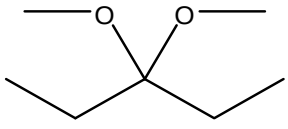
Spectrum Name: 070331D_glycolaldehyde_H13CHO_041007_nt=4096_0600-0900.pdf

^{13}C NMR (D_2O): δ 171.763, 167.216, 103.217, 102.393, 102.096, 100.257, 99.227, 98.266, 96.053, 82.305 (HCHO), 82.007, 76.816, 76.556, 76.266, 73.920, 73.657, 72.375, 71.796, 71.559, 71.300, 70.258, 67.065, 66.604, 65.372, 65.306, 64.132, 63.026, 61.855, 58.883, 58.650, 58.391, 40.546

Spectrum Name: 070331D_glycolaldehyde_H13CHO_nt=2048_040107_start_1500-1630.pdf

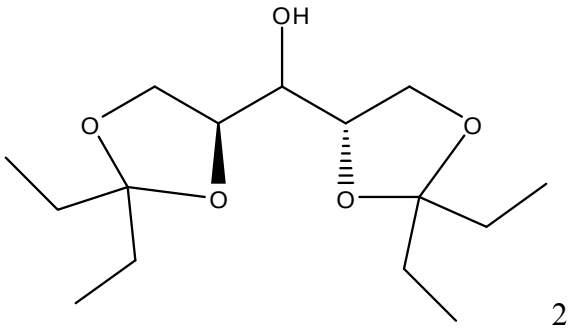
^{13}C NMR (D_2O): δ 171.767, 167.227, 103.217, 98.262, 94.859, 82.305 (HCHO), 82.007, 76.819, 76.556, 76.266, 71.551, 65.513, 63.506, 62.827

3-Pentulose Synthetic Procedure



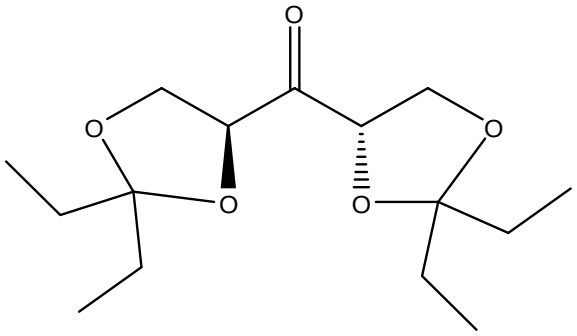
1

3,3-Dimethoxypentane (1) To a stirred soln. of the 3-pentanone (2 mol), trimethyl orthoformate (5 mol), and MeOH (700 ml), TsOH (500 mg) was added at r.t. After 4 days, the mixture was poured onto ice, worked up with Et_2O, and washed with sat. $NaHCO_3$ soln. and brine, then distilled. 115 g (63%). B.p. 120 ± 124_. ^{1}H-NMR: 0.82 (t, 6 H); 1.60 (q, 4 H); 3.15 (s, 6 H). ^{13}C NMR (D_2O): δ 123.3(3'), 51.2(OCH_3), 31.3(2',4'), 8.1(1',5')



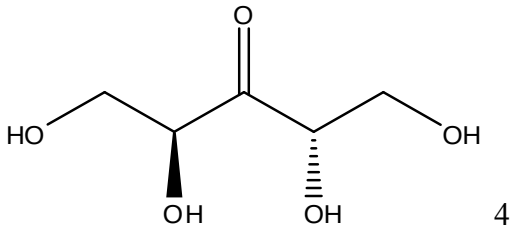
2

(2*S*,4*S*)-1,2:4,5-Di-*O*-(3,3-pentylidene)arabitol (2). To a refluxing suspension of L-arabitol (5.0 g, 32.84 mmol) in 3,3-dimethoxypentane (19.6 g, .148 mol) and THF (50 mL) was added CSA (2.3 g, 9.9 mmol) and the reaction was stirred at reflux for 5 min. Triethylamine (10 mL) was added to the refluxing reaction, and the mixture was concentrated in vacuo and loaded directly onto a silica gel column (hexane/ethyl acetate 8:2) yielding a colorless oil **7** (7.3 g, 77%). ^{13}C NMR (D_2O): δ 123.1(3'), 76.1(2,4), 69.2(3), 66.4(1,5), 31.2(2',4'), 7.9(1',5')



3

(2*S*,4*S*)-1,2:4,5-Bis(3,3-pentylidenedioxy)-3-pentanone (3). A suspension of SO_3 pyridine (5.1 g, 32.4 mmol) in CH_2Cl_2 (20 mL) was dissolved in DMSO (40 mL) and Et3N (5 mL, 36.11 mmol). This solution was immediately added dropwise to a stirred solution of **2** (3.0 g, 10.4 mmol) in CH_2Cl_2 (20 mL) and DMSO (35 mL) at -5 °C.The reaction mixture was stirred at 0 °C for 6 h. The reaction mixture was poured into a solution of saturated aqueous NH_4Cl:water:Et_2O:pentane (1:1:1:1, 100 mL), and the aqueous phase was extracted with an Et_2O:pentane mixture (1:1, 3 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4. After the solvent was removed in vacuo the obtained pale yellow oil was purified by column chromatography (hexane/ethyl acetate 9:1) to yield a colorless oil (483 mg, 99%). ^{13}C NMR (D_2O): δ 212.4(3), 124.1(3'), 75.2(2,4), 65.7(1,5), 32.1(2',4'), 8.2(1',5')



4

(2S,4*S*)-1,2,4,5-tetrahydroxy-3-pentanone (4). To a solution of **11** (1.09g, 3.8 mmol) in methanol (15 mL) and H_2O (0.3 mL) was added CSA (111 mg, 0.48 mmol). The mixture was stirred at room temperature for 48 h. Solid $NaHCO_3$ (120 mg) was added, and the reaction was stirred for 15 min. The suspension was directly loaded onto a silica gel column

(CH_2Cl_2/methanol 95:5). Elution with an 8:2 mixture of CH_2Cl_2/methanol yielded **4** as a colorless oil (513 mg, 90%).

^{13}C NMR (D_2O): δ 212.2 (3), 76.2(2,4), 63.3(1,5)

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BIOGRAPHICAL SKETCH

Heshan Illangkoon was born and raised in Montreal, Canada. His parents Herath & Nandani Illangkoon both immigrated from Sri Lanka in the 1970's. There he attended the French parochial school école Georges P. Vanier. Shortly before his eleventh birthday his parents moved to West Palm Beach, Florida. There he attended Pahokee elementary school (6th grade), Pahokee Jr./Sr. High School (7th grade), Wellington Landings Middle School (8th grade). In 1994, Heshan was chosen to attend Suncoast Community High School in Riviera Beach, Florida, ranked the 3rd best high school by U.S. News and World report. In 1998, he entered the University of Florida and performed undergraduate research with Dr. Steven A. Benner and graduated in December 2002. He continued working for the Benner Laboratories and in September 2003 entered the University of Florida chemistry graduate program. During his stint he was deeply involved with issues of shared governance and University management and was elected to the student body government as a graduate senator, and re-elected two additional times. He has served as chairman of the allocations standing committee and as chairman of the graduate and professional student caucus. He was also appointed as the vice-chair of the senate graduate issues committee and liaison to the faculty senate. He was also nominated to the college of liberal arts and sciences finance committee and the presidency of the graduate student council, both of which he humbly declined. In the Spring of 2009, Heshan, a co-founder of the of the progress party, was the party's vice-presidential candidate for University of Florida student government elections. To date, Heshan holds the title of being the longest serving Senator in University of Florida Student Government history. An avid photographer, he is also the official photographer for the Graham Center for Public Service. After graduating Heshan plans to travel to Tanzania and will be involved with an NGO. In the future, Heshan plans to pursue a law degree, a career with NASA, and hopes to culminate as a statesman and public servant, ideally, a U.S. Senator or Ambassador.