Formaldehyde as a Catalyst: Investigations on the Role of Formaldehyde as a Potential Prebiotic Catalyst & Desymmetrization Agent

By

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Abstract

Life, as we know it, has emerged from the association of simple building blocks (e.g. HCN, NH_3, aldehydes, etc). The reactions required to form the complex subunits of life face a great entropic barrier due to the intermolecular nature of their reactivity. Intermolecular reactions are slow at low concentrations, and therefore, the assembly of complex subunits requires the presence of a concentration mechanism. Formaldehyde, which was present in concentrations as high as 0.02 M, may have been used as a concentration mechanism on early Earth. By tethering two molecules together, formaldehyde allows catalysis via temporary intramolecularity. Moreover, formaldehyde has been shown to act as a hydrolase / hydratase mimic, allowing important rate accelerations in hydration and hydrolysis reactions which are of fundamental importance to prebiotic chemistry. Herein, the efficiency of formaldehyde as a catalyst, operating via temporary intramolecularity is demonstrated for a hydroamination reaction that occurs in dilute aqueous conditions. First, using soluble N-methylallylamine and N-methylhydroxylamine, formaldehyde allowed catalytic turnover at prebiotically relevant formaldehyde concentrations (0.02 M) for a model hydroamination reaction. The efficiency of formaldehyde was compared to other prebiotic aldehydes, demonstrating that although other prebiotic aldehydes are capable of inducing temporary intramolecularity, they were inferior.

A second small molecule which may have played a role in the origin of life is D-glyceraldehyde. Since life's molecules are homochiral, there is a need to explain how this homochirality arose. There have been many breakthroughs by the scientific community when it comes to addressing this challenge, however there is still no general consensus on the origins of homochirality from a prebiotic perspective. Herein, we demonstrate that D-glyceraldehyde is capable of templating a challenging intermolecular reaction while also transmitting some of its chirality to the product. Though the enantiomeric excess produced was generally low (usually around 20 %), there is a significance behind these results due to prebiotically relevant amplification procedures.

Lastly, formaldehyde is examined as a possible desymmetrizing agent; coupled with Brønsted acids, the possibility of formaldehyde to induce desymmetrization of alpha-amino or alpha-hydroxy diesters to produce azlactones, and oxalactones, respectively will be established. Moreover, the use of a chiral Brønsted acid would introduce the ability to achieve this transformation in an enantioselective manner. The resulting azlactones / oxalactones are valuable for two reasons: 1) the lactones are present in bioactive molecules, and 2) the lactones can be hydrolyzed to produce chiral alpha-amino / alpha-hydroxy acids. Therefore, we began a systematic study of the conditions required to allow this transformation to occur. This study indicates that the desymmetrization of an alpha-amino diester is possible, producing moderate yields of

the resulting azlactone. The desymmetrization of alpha-hydroxy diesters however proved more challenging, and no conversion was observed. Further investigation is required to the increase efficiency of the desymmetrizations, and experimentation with chiral Brønsted acids is required in order to discover enantioselective transformations.

Acknowledgements

Thank you to everyone in the Beauchemin lab for a wonderful two years. André, you are a great supervisor; you're both patient and knowledgeable and I enjoyed working with you very much. Thank you to Josh and Ryan for being great friends and excellent role models. Claudia, thank you for the help that you provided towards my paper, and thank you for being very positive and happy every single day – the lab really needed someone like you.

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List of Abbreviations

AIC - 5-amino-1*H*-imidazole-4-carbonitrile
ANN – 2-aminonicotinonitrile
Bn – Benzyl
Bu – Butyl
Cat. – Catalytic
CDI – 1,1'-carbonyldiimidazole
CPL – Circularly Polarized Light
D – Dextrorotary
d – Deuterium
δ – Chemical shift in parts per million
DMSO – Dimethyl sulfoxide
DPP – Diphenyl phosphate
ee – Enantiomeric Excess
Equiv. – Equivalent
g – Gram
h – Hours
HMGA – 3-hydroxy-3-methylglutaric acid
Hz – Hertz
IR – Infrared
J – Coupling constant
L – Levorotatory
L – Liter
m-CPBA – meta-Chloroperoxybenzoic acid
Me – Methyl
Min – Minute
Mmol – Millimole
mL – Milliliter
MS – Molecular sieves
NHC – *N*-heterocyclic carbene
NMR – Nuclear Magnetic Resonance Spectroscopy
ppm – Parts per million
R – Carbon based substituent
Rf – Retention Factor
rt – Room
TFA – Trifluoroacetic acid
THF – tetrahydrofuran

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Chapter 1 – Challenges in Prebiotic Chemistry

1.1 Introduction

Scientists have always been puzzled by the origins of life. Since the key Miller-Urey experiment in 1953,[1] there has been an increased interest in the search for how biological life arose. Scientists have pondered how complex molecules such as amino acids, nucleotides, and lipids have formed from simpler precursors such as formaldehyde, formamide, urea, ammonia, hydrogen cyanide, cyanate, etc (Figure 1.1). Though these complex molecules could have been delivered from extraterrestrial sources, it is commonly believed that they were formed from simpler precursors on

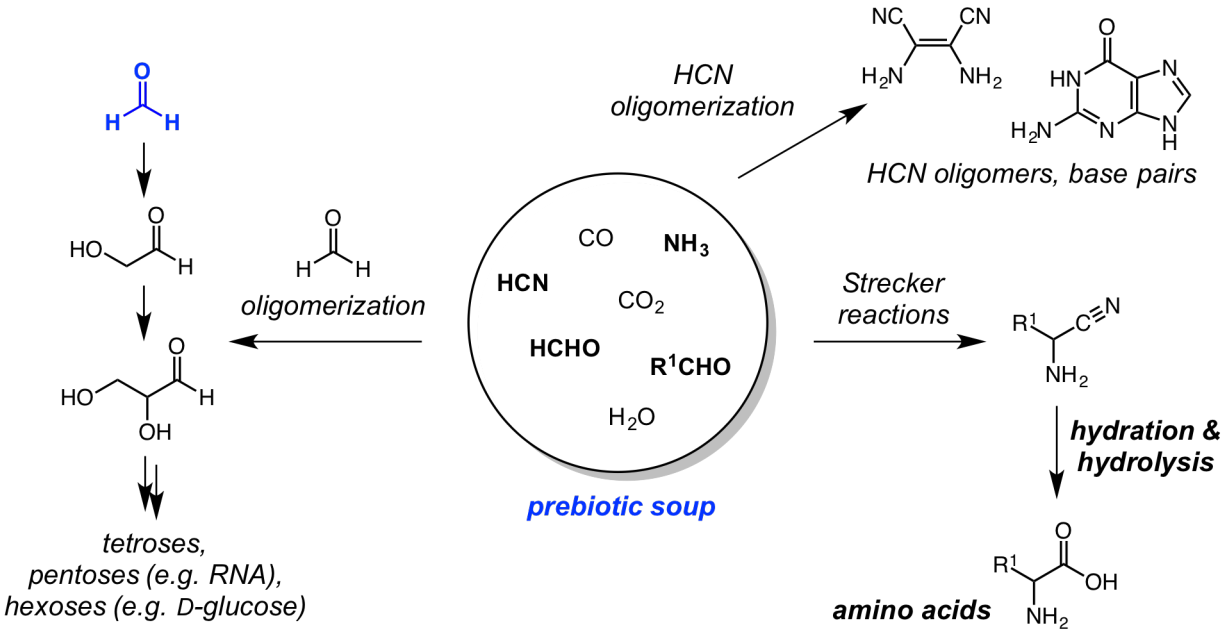


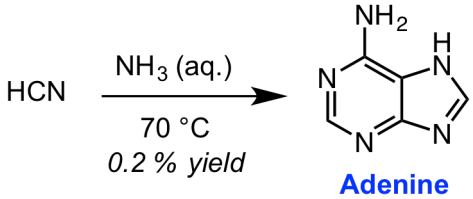
Figure 1.1 Formation of complex molecules from simpler precursors

earth.[2] Studying the origin of life from a chemical perspective is complex as there are a series of challenges that need to be addressed. Indeed, understanding the formation of

[1] Miller, S. L. *Science* **1953**, *117*, 528.
[2] Miller, S. L.; Orgel, L. E. *The Origins of Life on Earth,* Prentice-Hall, Englewood Cliffs, N.J., **1974.**

complex molecules without the aid of enzymes and other biological catalysts means overcoming various issues such as the identification of plausible pathways among a operating despite low substrate concentrations,[3] and low reaction rates.[4] These issues are problematic since all reactions required to form these complex molecules are intermolecular by nature, and there is an entropic barrier which needs to be overcome. Furthermore, reactions that proceed faster would have dictated chemical evolution, implying that there is a need for multiple solutions to these low concentrations and low rates.

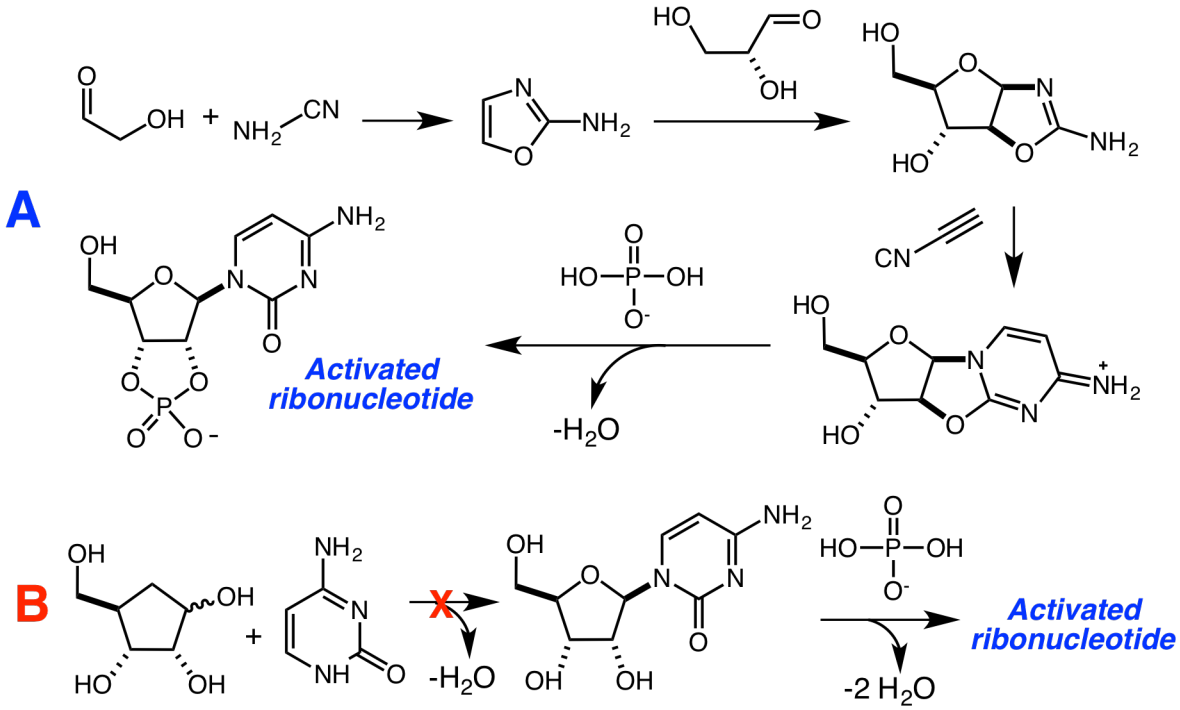
There are many examples of proposed prebiotic reactions in the literature that have been validated experimentally in a laboratory setting. Arguably, the most famous example of this would be the Urey-Miller experiment, where natural amino acids such as glycine, and alanine were generated from the continuous electric spark discharge of a prebiotic soup of molecules (NH_3, CH_4, H_2; Figure 1.1).[1] Less famous, and yet, equally important, would be Oró's synthesis of adenine – a molecule that is critical for the generation of self-replicating biological systems.[5] In this synthesis, HCN oligomerizes at



Scheme 1.1 Oró's prebiotic synthesis of adenine

[3] Jamshidi, M. P.; MacDonald, M. J.; Beauchemin, A. M. *Orig. Life Evol. Biosph.* **2017**, *accepted*.
[4] Ruiz-Mirazo, K.; Briones, C.; de la Escorusa, A. *Chem. Rev.* **2014**, *114*, 285.
[5] (a) Oró, J. *Nature* **1961**, *4794*, 1193. (b) Oró, J.; Kimball, A. P. *Arch. Biochem. Biophys.* **1961**, *94*, 217. (c) Oró, J.; Kimball, A. P. *Arch. Biochem. Biophys*. **1961**, *96*, 293.

70 °C over to ultimately produce adenine in small concentrations (Scheme 1.1). Later syntheses also describe adenine forming in higher yields, however these syntheses begin with advanced HCN oligomer intermediates (i.e. 4-aminoimidazole-5-carboxamidine).[5a] A third example of an innovative prebiotic synthesis comes from Sutherland *et al.*, who have solved a long-standing challenge in prebiotic chemistry – the synthesis of ribonucleotides (Scheme 1.2). RNA, the polymeric form of ribonucleotides is an informational polymer that must have arisen via chemical reactions in early Earth; attempts of synthesizing ribonucleotides in laboratory settings, however, had all failed



Scheme 1.2 Sutherland's *et al.* synthesis of activated ribonucleotides

(Scheme 1.2 B).[6] Sulterland *et al.* were able to demonstrate an alternative, prebiotically relevant, pathway to the generation of activated ribonucleotides, therefore solving a 50

[6] Joyce, G. F. *Nature* **2002**, *418*, 214.

year old puzzle. Previous, more intuitive syntheses would always fail at the initial condensation reaction between ribose and the nucleotide; Sutherland *et al*. bypass this step, by instead proceeding via an oxazole intermediate (Scheme 1.2 A). These syntheses however, all commence with high substrate concentrations, and from a prebiotic standpoint, this is not realistic. The low concentration issue needs to be addressed so that these prebiotic syntheses, and other future prebiotic syntheses may be more influential. For life to emerge from a prebiotic soup, these building blocks must react with each other at prebiotically relevant concentrations, and develop into self-replicating machinery (RNA) that would eventually code for enzymes and other proteins.

Another issue, and perhaps one of the most difficult questions to answer from an origin of life perspective, is the origin of homochirality. Life's molecules are chiral, and so, there must have been a mechanism to introduce and amplify this chirality. Without biological catalysts to template these intermolecular reactions, and without favorable conditions (i.e. low concentrations), scientists must ask themselves a question: how do highly ordered molecules arise from simple organic building blocks? In response to this question, scientists have faced the issue by proposing a myriad of theories with experimental evidence to assert these theories.

1.2 The Low Concentration Issue

One of the first problems which must be addressed is the issue of low concentration. Large bodies of water such as those that would have been found on

primitive earth would offer a dilute setting for life to initiate. The intermolecular reactions required to form more complex systems would be very slow at low concentrations, and so, this problem must be addressed before any prebiotic syntheses may be considered. There are now several accepted theories within the scientific community that address this issue.

1.2.1 Evaporating Pools of Water

Earth's early environments are typically described as being much warmer than they are today.[7] Such conditions would offer the possibility of small bodies of water with concentrated pools organic molecules (Figure 1.2). This theory was first suggested by Lohrmann and Orgel,[8] and since then, there have been many prebiotic syntheses using temperatures of up to 100 °C. Although this may seem strange, given the conditions of

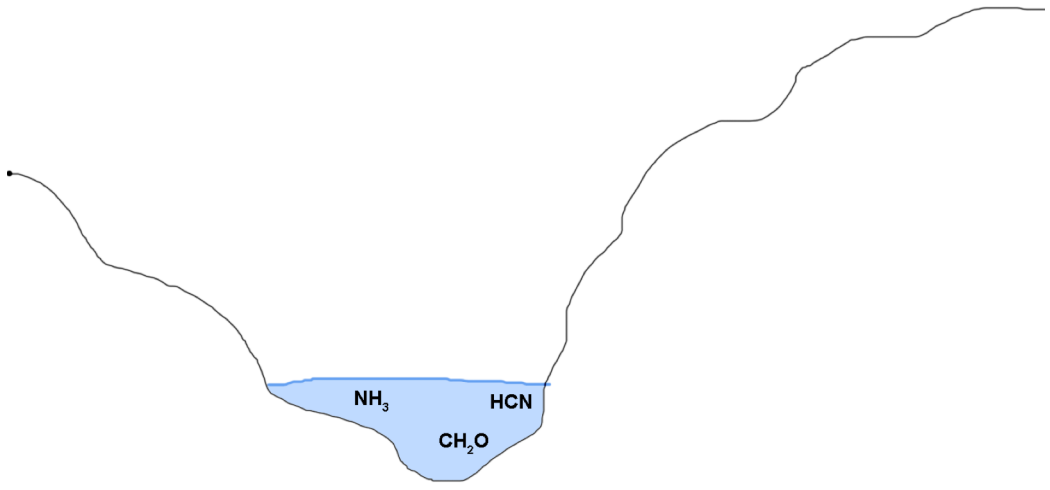


Figure 1.2 Diagram representing dry down conditions

[7] Cleaves II, H. J.; Michalkova, S. A.; Hill, F. C.; Leszczynski, J.; Sahai, N.; Hazen, R. *Chem. Soc. Rev.* **2012**, *41*, 5502.
[8] Lohrmann, R.; Orgel, L. E. *Science* **1971**, *171*, 490.

Earth today, there is evidence suggesting that Earth could have been extremely hot due to high amounts of atmospheric CO_2.[9] Therefore, experiments claiming prebiotic origins while using high temperatures remain feasible and valid.

Since the first use of dry down conditions by Lohrmann and Orgel, there have been other proposed prebiotic syntheses involving dry down conditions (Table 1.1).[10]

Table 1.1 Prebiotic syntheses involving dry down conditions

Target	Reference
Nucleosides	Fuller *et al.* 1972a Fuller *et al.* 1972b Burton *et al.* 1974
Nucleotides	Nelson *et al.* 2001 Schwartz *et al.* 1972 Schwartz and Chittenden 1977
Polynucleotides	Usher and McHale 1976 Usher 1977 Usher and Yee 1979
Polyphosphates	Bishop *et al.* 1972 Lohrmann and Orgel 1971 Lohrmann and Orgel 1973 Ostberg and Orgel 1972 Ostberg *et al.* 1973 Keefe and Miller 1995
Peptides	Lohrmann *et al.* 1975

[9] Knauth, L. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2005**, *219*, 53.

[10] (a) Fuller, W. D.; Sanchez, R. A.; Orgel L. E. *J. Mol. Biol.* **1992**, *67*, 25. (b) Fuller, W. D.; Sanchez, R. A.; Orgel L. E. *J. Mol. Evol.* **1992**, *1,* 249. (c) Burton, F. G.; Lohrmann, R.; Orgel, L. E. *J. Mol. Evol.* **1974**, *3,* 141. (d) Nelson, K. E.; Robertson, M. P.; Levey, M.; Miller, S. L. *Orig. Life Evol. Biosph.* **2001**, *31*, 221. (e) Schwartz, A. W. *Biochim. Biophys. Acta* **1972**, *281*, 477. (f) Schwartz, A. W.; Chittenden, G. J. F. *Biosystems,* **1977**, *9*, 87. (g) Usher, D. A.; McHale, A. H. *Science* **1976**, *192*, 43. (h) Usher, D. A. *Science*, **1977**, *196*, 311. (i) Usher, D. A.; Yee, D. *J. Mol. Evol.* **1979**, *13*, 287. (j) Bishop, M. J.; Lohrmann, R.; Orgel, L. E. *Nature* **1972**, *237*, 162. (k) Lohrmann, R.; Orgel, L. E. *Nature* **1973**, *244*, 418. (l) Ostberg, R.; Orgel. L. E. *J. Mol. Evol.* **1972**, *1*, 241 (m) Ostberg, R.; Orgel, L. E.; Lorhman, R. *J. Mol. Evol.* **1973**, *2*, 231. (n) Keefe, A. D.; Miller, S. L. *J. Mol. Evol.* **1995**, *41*, 693. (o) Lohrmann, R.; Ranganathan, R.; Sawai, H.; Orgel, L. E. *J. Mol. Evol.* **1975**, *5*, 57. (p) Sawai, H.; Lohrmann, R.; Orgel, L. E. *J. Mol. Evol.* **1975**, *6*, 165. (q) Oró, J.; Stephen-Sherwood, E. *Origins of Life* **1976**, *1*, 241. (r) Flores, J. J.; Leckie, J. O. *Nature*, **1973**, *244,* 435. (s) Yanagawa, H.; Nishizawa, M.; Kojima, K. *Origins of Life* **1984**, *14*, 267. (t) Deamer, D. W.; Oró, J. *Biosystems* **1980**, *12*, 167. (u) Eichberg, J.; Sherwood, E.; Epps, D. E.; Oró, J. *J. Mol. Evol.* **1977**, *10*, 221.

	Sawai *et al.* 1975 Oró and Stephen-Sherwood 1976 Flores and Leckie 1973 Yanagawa *et al.* 1984
Lipids	Deamer and Oró 1980 Eichberg *et al.* 1977

Dry down conditions have indeed been shown to be an effective mode of accelerating the formation for many of life's precursors at low concentrations. The combination of increased heat and concentrations of organic building blocks allow for a measurable yield which would not have been possible otherwise. However, the dry down conditions do not encompass syntheses which involve volatile organic precursors such as HCN, which would have evaporated unless the solutions were very basic (unless little evaporation occured). HCN has been shown to be essential for the prebiotic synthesis of pyrimidine and purine derivitves,[7] and so, there is the need for alternative solutions to the low concentration issue.

1.2.2 Eutectic Mixtures

A second theory that has been proposed as a solution for the low concentration issue is the formation of eutectic mixtures. When dilute solutions of organic molecules reach the interface of their liquid and solid phase, there is the formation of

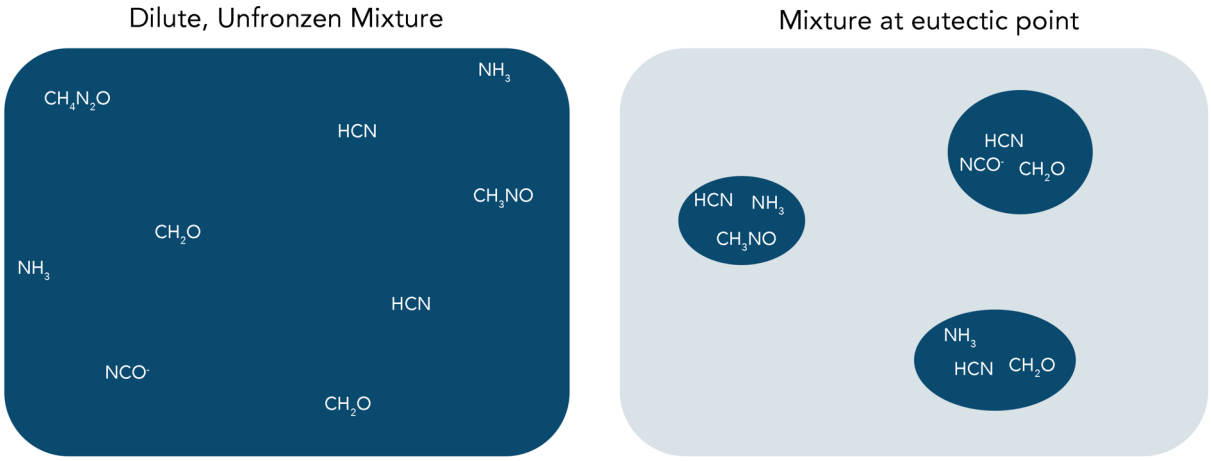
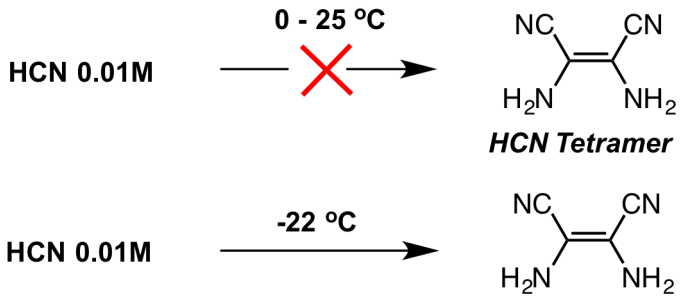


Figure 1.3 Representation of concentration under eutectic conditions

supersaturated microfluid inclusions where intermolecular reactions can proceed more rapidly (Figure 1.3).[7] These eutectic solutions often include very high concentrations of organic matter; for example, a eutectic solution of HCN contains 74.5 mol. % HCN.[14b] Leslie Orgel first proposed freezing as a solution to the low concentration issue,[11f] suggesting that life may have originated at cold temperatures. This claim was supported



Scheme 1.3 Formation of HCN tetramer in eutectic solutions

by experimental evidence which has shown that the HCN tetramer only forms under dilute solutions of HCN at the eutectic point (Scheme 1.3).

Over the years, eutectic mixtures have been exploited as a method for the prebiotic syntheses of nucleotides,[10d, 11] polynucleotides,[12] polypeptides,[13] and a variety of other complex organic molecules.[14] Earth's early atmospheres were low yielding for organic molecules,[11e] and therefore concentration by forming frozen solutions would circumvent this issue.

1.2.3 Clay Templating

One of the more common proposals when it comes to the low concentration issue is clay templating. Mineral surfaces present an opportunity for heterogeneous catalysis, which would allow for the favorable formation of complex organic species.[15] Various intermolecular forces (Vaan der Waal, dipole-dipole, complexation) attract organic species to the clay surface allowing for a localized increase in concentration. A good

[11] (a) Schwartz, A. W.; Joosten, H.; Voet, A. B. *BioSystems* **1982**, *15*, 191. (b) Levy, M.; Miller, S. L.; Brinton, K.; Bada, J. L. *Icarus* **2000**, *145*, 609. (c) Levy, M.; Miller, S. L.; Oró, J. *J. Mol. Evol.* **1999**, *49*, 165. (d) Miyakawa, S.; Cleaves, H. J.; Miller, S. L. *Orig. Life Evol. Biosph.* **2002**, *32*, 209. (e) Cleaves II, H. J.; Nelson, K. E.; Miller, S. L. *Naturwissenschaften* **2006**, *93*, 228. (f) Sanchez, R.; Ferris, J.; Orgel, L. E. *Science* **1966**, *153*, 72. (g) Menor-Salván, C.; Marín-Yaseli, M. R. *Chem. Eur. J.* **2013**, *19*, 6488. (h) Menor-Salván, C.; Ruiz-Bermejo, M.; Osuna-Esteban, S.; Veintemillas-Verdaguer, S. *Orig. Life. Evol. Biosph.* **2009**, *39*, 250. (i) Orgel, L. E. *Orig. Life Evol. Biosph.* **2004**, *34*, 361. (j) Menor-Salván, C.; Ruiz-Bermejo, D. M.; Guzmán, M. I.; Osuna-Esteban, S.; Veintemillas-Verdaguer, S. *Chem. Eur. J.* **2009**, *15*, 4411.

[12] (a) Monnard, P.; Kanavarioti, A.; Deamer, D. W. *J. Am. Chem. Soc.* **2003**, *125*, 13734. (b) Kanavarioti, A.; Monnard, P.; Deamer, D. W. *Astrobiology*, **2001**, *1*, 271. (c) Stribling, R.; Miller, S. L. *J. Mol. Evol.* **1991**, *32*, 289.

[13] (a) Agten, S. M.; Suylen, D. P.; Hackeng, T. M. *Bioconjugate Chem.* **2016**, *27*, 42. (b) Liu, R.; Orgel, L. E. *J. Am. Chem. Soc.* **1997**, *119,* 4791.

[14] (a) Neish, C. D.; Somogyi, Á.; Imanaka, H.; Lunine, J. I.; Smith, M. A. *Astrobiology* **2008**, *8*, 273. (b) Sanchez, R. A.; Ferris, J. P. Orgel, L. E. *J. Mol. Biol.* **1967**, *30*, 223.

[15] (a) For a recent review, see: Cleaves II, H. J.; Scott, A. M.; Hill, F. C.; Leszczynski, J.; Sahai, N.; Hazen, R. *Chem. Soc. Rev.* **2012**, *41*, 5502. (b) Paecht-Horowitz, M.; Berger, J.; Katchalsky, A. *Nature* **1970**, *228,* 636. (c) Lahav, N.; White, D.; Chang, S. *Science* **1978**, *201*, 67.

example of this clay catalysis comes from Lahav *et al.*,[15c] who demonstrated that diglycine and even larger oligomers were formed in the presence of clay after one week, whereas there were only traces of diglycine found after 35 days in the absence of clay (Figure 1.4). Furthermore, montmorillonite (a type of clay) has been demonstrated to promote the polymerization of activated adenosine and uridine derivatives, producing 25-50 unit oligonucleotides (the length considered necessary for primitive biochemical functions).[16]

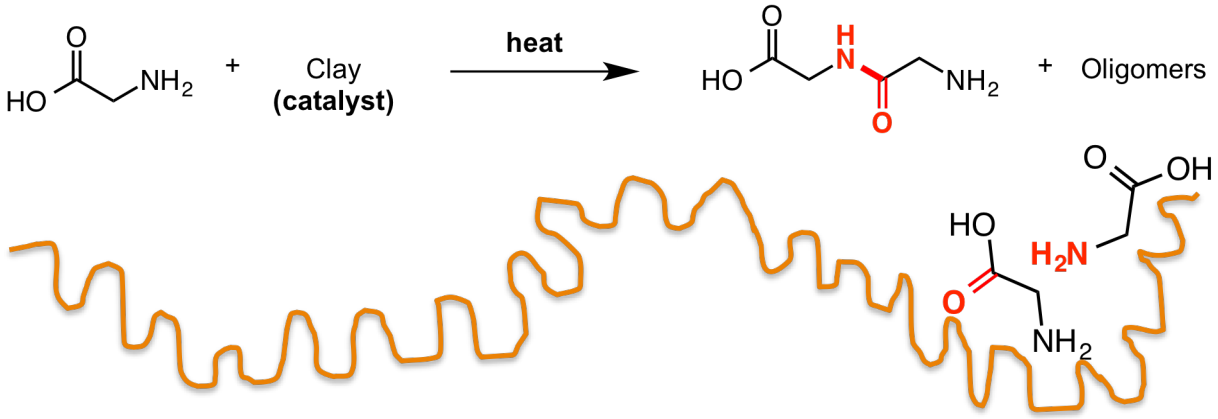


Figure 1.4 Polypeptide formation catalyzed by clay

Clay catalysis has been shown to be an effective way of accelerating dilute reactions under plausible prebiotic conditions to form complex organic species. There are, however, a few other methods of concentration which may have worked in unison with the methods described so far.

[16] (a) Huang, W.; Ferris, J. P. *J. Am. Chem. Soc.* **2006**, *128*, 8914. (b) Joshi, P. C.; Aldersley, M. F.; Delano, J. W.; Ferris, J. P. *J. Am. Soc. Chem.* **2009**, *131*, 13369.

1.2.4 Other Modes of Concentration

In addition to the methods described so far, there have been a few other proposals for how the concentration of organic molecules may have been locally increased. These methods include molecular crowding,[17] aerosols,[18] membranes / vesicles,[19] and small molecule templating.[20] Although these methods are experimentally sound, they are not commonly used to describe prebiotic syntheses and so they will not be explained in this dissertation.

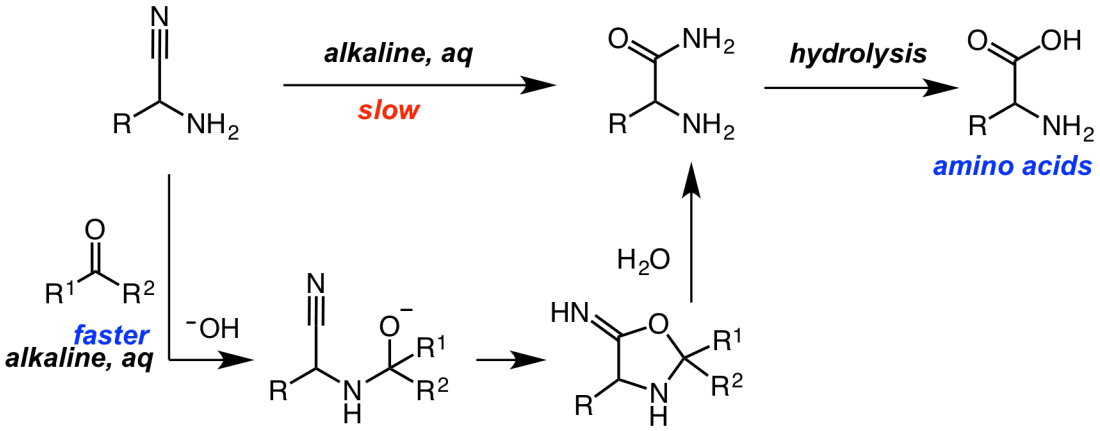
1.3 The Issue of Low Rates

Without the aid of biological catalysts to accelerate challenging intermolecular reactions, there is a question of how complex organic molecules are formed, regardless of the concentrated environments described in section 1.2. Although clay templating offers rate acceleration for some of the essential prebiotic reactions such as peptide,[15c, 21] nucleotide oligomers,[16] and lipid membrane formation,[22] there remains the need for additional explanations of how difficult intermolecular such as hydrolyses and hydrations

[17] (a) Mayer, C.; Schreiber, U, Dávila, M. J. *Orig. Life Evol. Biosph.* **2015**, *45*, 139. (b) Hansma, H. G. *Orig. Life Evol. Biosph.* **2014**, *44*, 307.
[18] (a) Demou, E.; Donaldson, D. J. *J. Phys. Chem. A* **2002**, *106*, 982. (b) Dobson, C. M.; Ellison, G. B.; Tuck, A. F.; Vaida, V. *Proc. Natl. Acad. Sci.* **2000**, *97*, 11864. (c) Donaldson, D. J.; Tervahattu, H.; Tuck, A. F.; Vaida, V. *Orig. Life Evol. Biosph.* **2004**, *34*, 57. (d) Ellison, G. B.; Tuck, A. F.; Vaida, V. *J. Geophys. Res.* **1999**, *104*, 11633. (e) Lerman, L. *Orig. Life Evol. Biosph.* **1996**, 369. (f) Ruiz-Bermejo, M.; Menor-Salván, C.; Osuna-Esteban, S.; Veintemillas-Verdaguer, S. *Orig. Life Evol. Biosph.* **2007**, *37*, 123. (g) Tuck, A. *Surv. Geophys.* **2002**, *23*, 379. (h) Shah, D. O. The Origin of Membranes and Related Surface Phenomena. In *Exobiology*; Ponnamperuma, C., Ed.; North-Holland: Amsterdam, 1972; Vol. 23; p 235-265.
[19] Mayer, C.; Schreiber, U.; Dávila, M. J. *Orig. Life Evol. Biosph.* **2015**, *45*, 139.
[20] (a) Shwartz, A. W.; Goverde, M. *J. Mol. Evol.* **1982**, *18*, 351. (b) Hulshof, J.; Ponnamperuma, C. *Orig. Life Evol. Biosph.* **1976**, *3*, 197.
[21] Leyton, P.; Zárate, R. A.; Fuentes, S.; Paipa, C.; Gómez-Jeria, J. S.; Leyton, Y. *BioSystems* **2011**, *104*, 118.
[22] (a) Hanczyc, M. M.; Fujikawa, S. M.; Szostak, J. W. *Science* **2003**, *302*, 618.

took place. Hydrolyses and hydrations are of fundamental importance to the prebiotic synthesis since the hydration and hydrolysis of alpha-amino nitriles is the generally accepted pathway for forming alpha-amino acids.[23] These reactions proceed effortlessly in the presence of hydrolases and hydratases, respectively, but on a young Earth, without biological catalysts many of these reactions would have been difficult to achieve.[24]

Commeyras, Pascal, and Taillades demonstrated the remarkable efficiency of carbonyl compounds in promoting the hydrolysis and hydration of alpha-amino nitriles (ca. 10^5 rate acceleration) to form natural and unnatural amino acids (Scheme 1.4).[25] The



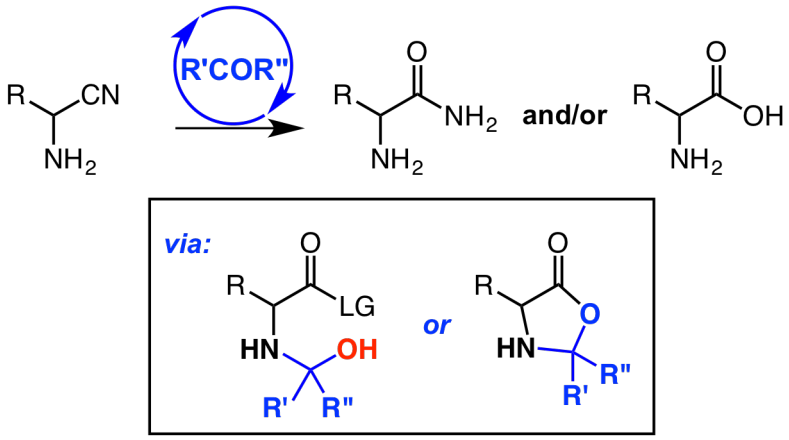
Scheme 1.4 Carbonyl promoted hydration of alpha-amino nitriles

abundance of aldehydes and ketones in prebiotic chemistry makes it possible that hydrolysis and hydration reactions were accelerated by carbonyl compounds. Indeed,

[23] *Origins and Synthesis of Amino Acids*; Hughes, A. B., Ed.; Amino Acids, Peptides and Proteins in Organic Chemistry, Vol. *1*; Wiley–VCH: Weinheim, Germany, 2009.
[24] Pascal, R. *Eur. J. Org. Chem.* **2003**, *2003*, 1813.
[25] (a) Pascal, R.; Taillades, J.; Commeyras, A. *Tetrahedron* **1978**, *34*, 2275. (b) Pascal, R.; Taillades, J.; Commeyras, A. *Bull. Soc. Chim. Fr. II* **1978**, *3-4*, 177. (c) Pascal, R.; Taillades, J.; Commeyras, A. *Tetrahedron* **1980**, *36*, 2999.

the potential role of prebiotic carbonyl compounds in promoting the synthesis of alpha-amino acids,[26] and HCN oligomers,[27] has been explored by the scientific community.

The demonstrated uses of carbonyl compounds by the pioneers described earlier have thus far been stoichiometric examples; our group has demonstrated that carbonyl compounds can be used in catalytic amounts as hydratase / hydrolase mimics for the hydration / hydrolysis of alpha-amino nitriles (Scheme 1.5).[28] These studies showed that formaldehyde, the most abundant prebiotic aldehyde,[29] was actually the most efficient carbonyl compound when it comes to the hydration of alpha-amino nitriles. A recent report by Wagner *et al.* has demonstrated that homochiral sugars are capable of achieving the hydration of alpha-amino nitriles with the formation of a slight enantiomeric excess, though the yields were poor.[30]



Scheme 1.5 Hydration / hydrolysis of alpha-amino nitriles using carbonyls as catalysts

[26] (a) Taillades, J.; Beuzelin, I.; Garrel, L.; Tabacik, V.; Bied, C.; Commeyras, A. *Orig. Life Evol. Biosph*. **1998**, *28*, 61. (b) Eschenmoser, A. *Chem. Biodiv.* **2007**, *4*, 554.

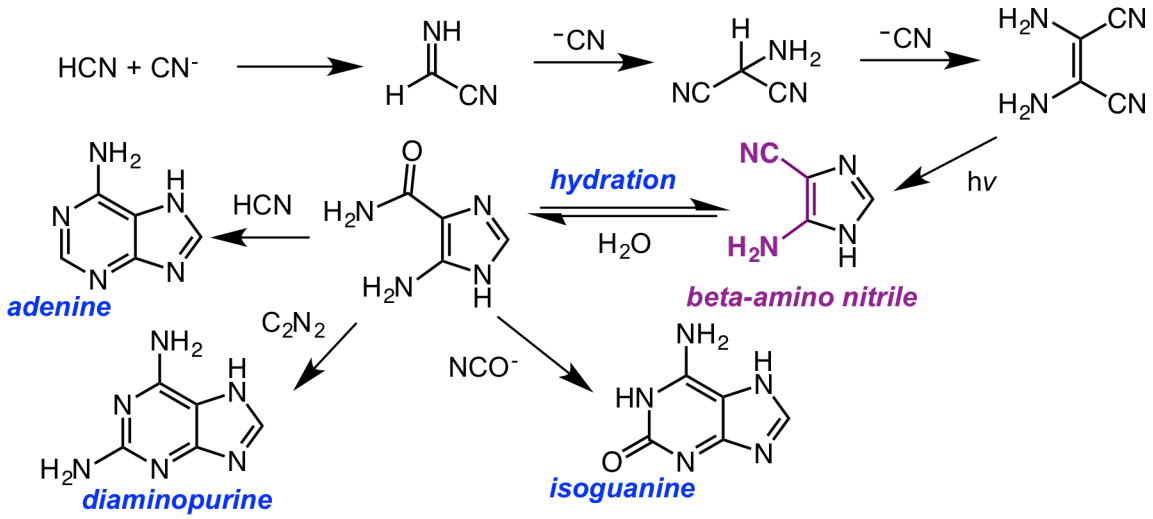
[27] (a) Thanassi, J. W. *J. Org. Chem*. **1975**, *40*, 2678. (b) Koch, K.; Schweizer, W. B.; Eschenmoser, A. *Chem. Biodiv.* **2007**, *4*, 541.

[28] Chitale, S.; Derasp, J. S.; Hussain, B.; Tanveer, K.; Beauchemin, A. M. *Chem. Commun.* **2016**, *52*, 13147.

[29] Cleaves II, H. J. *Precambian Res.* **2008**, *164,* 111.

[30] Wagner, A. J.; Zubarev, D. Y.; Aspuru-Guzik, A.; Blackmond, D. G. *ACS Cent. Sci.* **2017**, Doi: 10.1021/acscentsci.7b00085.

In contrast to the detailed research on the hydration of alpha-amino nitriles, the hydration of beta-amino nitriles using carbonyl compounds has not yet been explored. This process could be of prebiotic relevance since oligomerization of HCN monomers to produce beta-amino nitriles are common prebiotic reactions,[31] and hydration of these oligomers would produce precursors of adenine and other key purine intermediates (Scheme 1.6).[32]



Scheme 1.6 Oligomerization of HCN, and hydration of beta-amino nitrile to produce purines

In summary, clay templating could have offered a solution to the low reaction rates that would have plagued difficult intermolecular reactions such as peptide bond and lipid membrane formation. Other reactions such as hydration and hydrolysis which are easily carried out by biological catalysts need alternative modes of acceleration. Carbonyl compounds have been established as competent catalysts and promoters for

[31] (a) Ruiz-Bermejo, M.; Zorzano, M. P.; Osuna-Esteban, S. *Life* **2013**, *3*, 421. (b) Ferris, J. P.; Joshi, P. C.; Edelson, E. H.; Lawless, J. G. *J. Mol. Evol.* **1978,** *11,* 293.
[32] Ferris, J. P.; Orgel, L. E. *J. Am. Chem. Soc.* **1966**, *88*, 1074.

the hydration of alpha-amino esters, but the hydration of beta-amino nitriles with prebiotic aldehydes have not yet been reported.

1.4 Homochirality

Life's molecules are homochiral, meaning that the molecules contain the same configuration around a chiral center. There is a need to explain how this homochirality arose in order to better understand prebiotic reactivity leading to complex molecules. Addressing the origin of homochirality has been, and still remains, one of the greatest challenges in prebiotic chemistry. The origin of homochirality is made particularly puzzling by the fact that most prebiotic routes towards amino acids and nucleotides start from an achiral origin (i.e. formaldehyde, cyanoacetalene, formamide, etc.).[4] The source of this homochirality is likely abiotic in origin since proteins composed of a mixture of D- and L-amino acids would not form well-defined quaternary structures capable amplifying enantiomeric excess (ee).[33] This summary will therefore focus on abiotic routes for the amplification of ee. Homochirality has been proposed to arise from either the environment itself or from a statistical / natural occurring inequality between enantiomeric forms of molecules.

1.4.1 Homochirality from the Environment

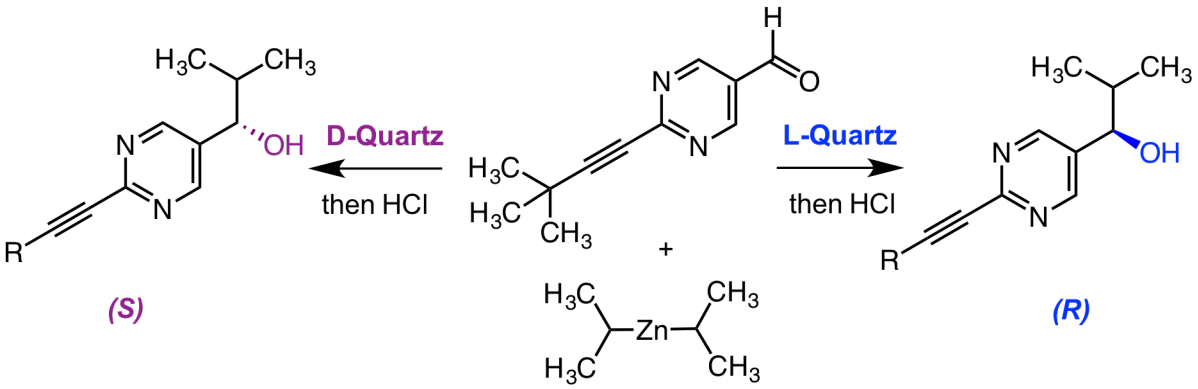
[33] (a) Avetisov, V.; Goldanski, V. *Proc. Natl. Acad. Sci. U.S.A* **1996**, *93*, 11435. (b) Idelson, M.; Blout, R. *J. Am. Chem. Soc.* **1958**, *80*, 2387.

The dawn of homochirality has been extensively studied for many years, and most explanations thus far have focused on the onset of an initial asymmetric deviance followed by amplification of this asymmetry.[34] One way for this initial introduction of ee to have occurred is from the environment itself. Naturally occurring enantiomorphic quartz crystals,[35] degradation of a single enantiomer by circularly polarized light,[36] and the Earth's rotation have all been proposed as environmental sources of ee.[37]

Single crystals of quartz (SiO_2 polymers) are found in naturally occurring dextrorotatory and levorotatory forms.[38] This ordered morphology could act as an asymmetric template by preferentially adsorbing one enantiomer over the other. For example, Bonner has shown that L-quartz preferentially adsorbs up to 20% of S-alanine over its enantiomer.[39] Perhaps the most striking example of this comes from Soai *et al.* who have demonstrated that catalytic amounts of D- or L-quartz can generate up to 97% ee for the formation of a (S)- and (R)-pyrimidyl alkanols, respectively (Scheme 1.7).[40] The aldehyde binds to the acidic quartz on one face, allowing for the alkyl zinc to preferentially react with one face of the aldehyde. This allowed for a minute asymmetric

[34] Weissbuch, I.; Leiserowitz, L.; Lahav, M. *Top. Curr. Chem.* **2005**, *259*, 123.
[35] Klabunovskii, E. I. *Astrobiology* **2001***, 1*, 127.
[36] Meierhenrich, U. J.; Nahon, L.; Alcaraz, C.; Bredehöft, J. H.; Hoffmann, S. V.; Barbier, B.; Brack, A. *Angew. Chem. Int. Ed.* **2005**, *44*, 5630.
[37] Kovacs, K. L.; Keszthelyi, L.; Goldanskii, V. J. *Orig. Life* **1981**, *11,* 93.
[38] Frondel, C. *Am. Mineralogist* **1978**, *63*, 17.
[39] Bonner, W. A. The Quest for Chirality. In *American Institute of Physics Conference Proceedings;* Cline, D. B. Ed.; American Institute of Physics Press: Woodbury, NY, 1996; Vol. 379; p 17.
[40] (a) Soai, K.; Osanai, S.; Kadowaki, K.; Yonekubo, S.; Shibata, T.; Sato, I. *J. Am. Chem. Soc.* **1999**, *121*, 11235. (b) Soai, K.; Shibata, T.; Morioka, H.; Choji, K. *Nature* **1995**, *378*, 767.

induction which was amplified via asymmetric autocatalysis to near homochirality.[41] Despite these findings, many have disregarded quartz in the origin of homochirality because the D- and L- forms are found in equal abundance in nature.[38,42] Recent reports have claimed that this asymmetric autocatalysis does not always require a homochiral face to initiate [Yun & Gellman have demonstrated this occurrence on an achiral Cu(111) face].[43] These findings may re-open the discussion on an inorganic mineral initiated origin of homochirality.



Scheme 1.7 Soai's amplification of homochirality

A second way which the environment may induce initial asymmetry upon molecules is through circularly polarized light (CPL).[36] Amino acids such as leucine (2.5% ee),[44] glutamic acid (0.22% ee),[45] alanine (0.06% ee),[45] and tryptophan (3% ee),[46] have been asymmetrically photolyzed by CPL; although this lead to only small values of ee,

[41] Soai, K.; Kawasaki, T.; Matsumoto, A. *Acc. Chem. Res.* **2014**, *47*, 3643.
[42] Klabunovskii, E. I.; Thiemann, W. *Orig. Life Evol. Biosph.* **2000**, *30*, 431.
[43] (a) Yun, Y.; Gellman, A. J. *Nature* **2015**, *7*, 520. (b) Karakolos, S.; Hong, J.; Zaera, F. *Angew. Chem. Int. Ed.* **2016**, *128*, 6333.
[44] Flores, J. J.; Bonner, W. A. Massey, G. A. *J. Am. Soc. Chem.* **1977**, *99*, 3622.
[45] Norden, B. *Nature* **1977**, *266,* 567.
[46] Greenberg, J. M.; Kouchi, A.; Niessen, W.; Irth, H.; Van Pradijs, J.; de Groot, M.; Hersmen, W. *J. Biol. Phys.* **1994**, *20*, 61.

these results are significant because of amplification procedures which have been demonstrated to be valid.[40] Meteoritic samples have shown to contain amino acids with enantiomeric excess,[47] indicating that an initial source for the arrival for homochirality may have been extraterrestrial. Since extraterrestrial sources of CPL are fairly common,[48] experiments demonstrating this selective degradation are scientifically sound.

There have been many examples of homochirality originating from environmental factors such as enantiomorphic quartz crystals, circularly polarized light, and even the Earth's rotation. These sources could have been the initiator of homochirality in life's molecules, however, they do not encompass all of the ee found in all prebiotic molecules (i.e. not all amino acids have been enantioenriched via CPL) and therefore there is the need for other sources of homochirality.

1.4.2 Homochirality from Statistical / Naturally Occurring Inequality

The dawn of homochirality has been extensively studied for many years, and most explanations focus on the onset of an initial asymmetric deviance followed by amplification of this asymmetry. It is interesting to note that the prevailing form of amino acids and sugars (i.e. L-amino acids, and D-sugars) are favored by electroweak interactions, and this minute stabilization could have been the initial source of

[47] (a) Cronin, J. R.; Pizzarello, S. *Science* **1997**, *275*, 951. (b) Engel, M. H.; Macko, S. A. *Nature* **1997**, *389*, 265.
[48] (a) Mason, S. F. *Nature* **1997**, *389*, 804. (b) Cerf, C.; Jorissen, A. *Space Sci. Rev.* **2000**, *92*, 603. (c) Bailey, J.; Chrysostomou, A.; Hough, J. H.; Gledhill, T. M.; McCall, A.; Clark, S.; Ménard, F.; Tamura, M. *Science* **1998**, *281*, 672.

asymmetry.[49] However, this theory has been mostly discounted since these electroweak interactions only introduce very minute amounts of ee, and experimental attempts of amplifying this ee have failed.[50]

What is more likely to have contributed to homochirality, however, is probability and statistics; it is statistically improbable to achieve a 50 / 50 mixture of enantiomers from a chemical reaction since the number of molecules are finite.[50b,51] This non-zero value have ee has been successfully amplified, especially in the presence of phase transitions where microscopic bifurcation would destabilize the racemic mixture towards one configuration.[43]

Amplification procedures typically rely on the enantiomer in excess catalyzing its own formation, and inhibiting the growth of the opposite enantiomer.[52] This amplification is achieved by initiating from a non-equilibrium position, and applying a driving force (i.e. crystallization, polymerization, chemical reactions or supramolecular organizations). As long as the system stays in disequilibrium, the system will remain stable in a homochiral orientation. For example, in a system on the brink of crystallization, a small chiral imbalance could result in homochirality as the crystals that are growing

[49] (a) Mason, S. F.; Tranter, G. E.; *Proc. R. Soc. London, A* **1985**, *397*, 45. (b) Kondepudi, D. K.; Nelson, G. W. *Nature* **1985**, *314*, 438.
[50] (a) Bonner, W. A. *Chirality* **2000**, *12*, 114. (b) Avalos, M.; Babiano, R.; Cintas, P.; Jiménez, J. L.; Palacios, J. C. *Tetrahedron: Asymmetry* **2000**, *11*, 2845.
[51] Cintas, P. *ChemPhysChem* **2001**, *2*, 409.
[52] (a) Blackmond, D. G. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, No. a002147.

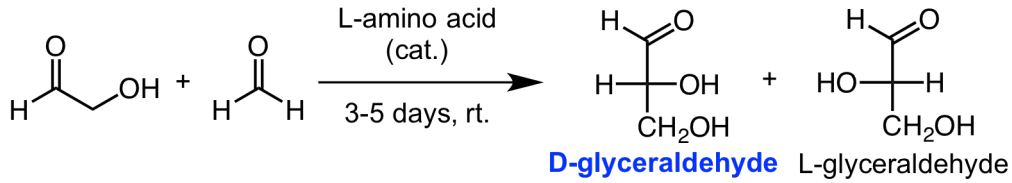
would interact better with the same enantiomer in the solid / liquid interface.[53] This process, named 'chiral amnesia', has also been demonstrated to work for the deracemization of select amino acids,[54] however this process is limited in scope, and is therefore not a general solution to the issue of homochirality.

Despite the advancements made towards answering the question of homochirality in prebiotic chemistry, there are still a great deal of limitations and so the question remains unanswered. These limitations include: reactions that are not prebiotically plausible (e.g. Soai's amplification of chirality is based on pyrimidyl alkanols),[40] and limited scope (CPL and chiral amnesia only work for select amino acids).[44,45,46,54]

[53] (a) Kondepudi, D. K.; Kaufman, R. J.; Singh, N. *Science* **1990**, *250,* 975. (b) Viedma, C. *Astrobiology* **2007**, *7,* 312. (c) Viedma, C. *Phys. Rev. Lett.* **2005**, *94*, 5504.

[54] (a) Kaptein, B.; Noorduin, W. L.; Meekes, H.; van Enckevort, W. J. P.; Kellogg, R. M.; Vlieg, E. *Angew. Chem. Int. Ed.* **2008**, *47*, 7226. (b) Noorduin, W. L.; Meekes, H.; van Enckevort, W. J. P.; Millemaggi, A.; Leeman, M.; Kaptein, B.; Kellogg, R. M.; Vlieg, E. *Angew. Chem. Int. Ed.* **2008**, *47*, 6445. (c) Noorduin, W. L.; Izumi, T.; Millemaggi, A.; Leeman, M.; Meekes, H.; van Enckevort, W. J. P.; Kellogg, R. M.; Kaptein, B.; Vlieg, E.; Blackmond, D. G. *J. Am. Chem. Soc.* **2008***, 130*, 1158. (d) Viedma, C.; Noorduin, W. L.; Ortiz, J. E.; de Torres, T.; Cintas, P. *Chem. Commun.* **2011**, *47,* 671. (e) Viedma, C.; Ortiz, J. E.; de Torres, T.; Izumi, T.; Blackmond, D. G. *J. Am. Soc. Chem.* **2008**, *130*, 15274. (f) Viedma, C.; Verkuijl, B. J. V.; Ortiz, J. E.; de Torres, T.; Kellogg, R. M.; Blackmond, D. G. *Chem. Eur. J.* **2010**, *16*, 4932. (g) Noorduin, W. L.; van der Asdonk, P.; Meekes, H.; van Enckevort, W. L..; Kaptein, B.; Leeman, M.; Kellogg, R. M.; Vlieg, E. *Angew. Chem. Int. Ed.* **2009**, *48*, 3278.

Homochiral sugars such as D-glyceraldehdye can be produced from the aldol reaction of formaldehyde and glycolaldehyde when catalytic amounts of L-amino acids such as alanine, are present (Scheme 1.8).[55] Catalytic amounts of L-amino acids produced a slight enantiomeric excess of D-glyceraldehyde, which was further amplified by taking advantage of the selective solubilities of the D and L forms (up to 92% ee). As mentioned above, alanine has been successfully enantioenriched using prebiotic methods, so it would be feasible to assume that D-glyceraldehyde could have played a role in prebiotic chemistry. Moreover, as previously mentioned, it has been demonstrated that homochiral sugars can produce ee in the hydration of Strecker adducts to produce amino acid precursors;[30] this, coupled with the fact that aldehydes have ability to act as powerful tethering catalysts,[3,24] implies that homochiral sugars should be investigated more thoroughly for their role in producing ee in prebiotic chemistry contexts.



Amino acid	Ratio, D/L
L-serine	50.3/49.7
L-alanine	50.8/49.2
L-phenylalanine	52.2/47.8
L-valine	52.2/47.8
L-leucine	54.4/45.6
L-glutamic acid	60.7/39.3
L-proline	28.9/71.1

Amplification → 92/8

Scheme 1.8 Formation of D-glyceraldehyde catalyzed by L-amino acids

[55] Breslow, R.; Cheng, Z.-L. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 5723.

Chapter 2 – The Role of Aldehydes in Prebiotic Chemistry

2.1 Introduction: Prebiotic Aldehydes as Tethering Catalysts

As mentioned in section 1.2, the concentration of organic molecules on primitive Earth would not have been high.[3] This is an issue which must be considered since the reactions required to form complex molecules are intermolecular, and intermolecular reactions are inherently slow at low concentrations. Current approaches to addressing this issue include physical methods to increase concentration (i.e. freezing, evaporating) or templating methods (clay induced pseudo-intramolecularity). Unexplored, however, are examples where small molecules, either in catalytic or stoichiometric amounts, tether two molecules in order to address low concentrations through temporary intramolecularity (Figure 2.1). Tethering two molecules to produce intramolecularity is

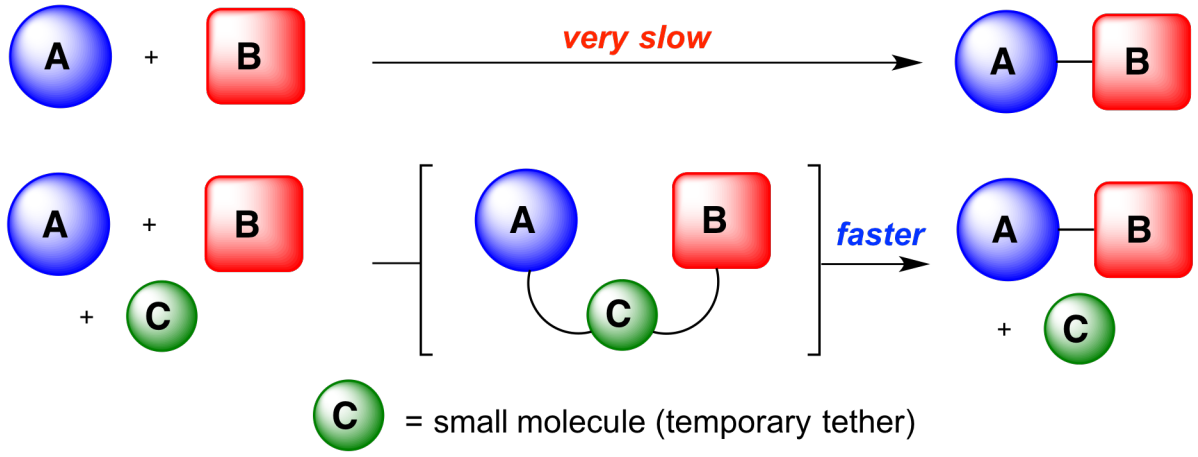


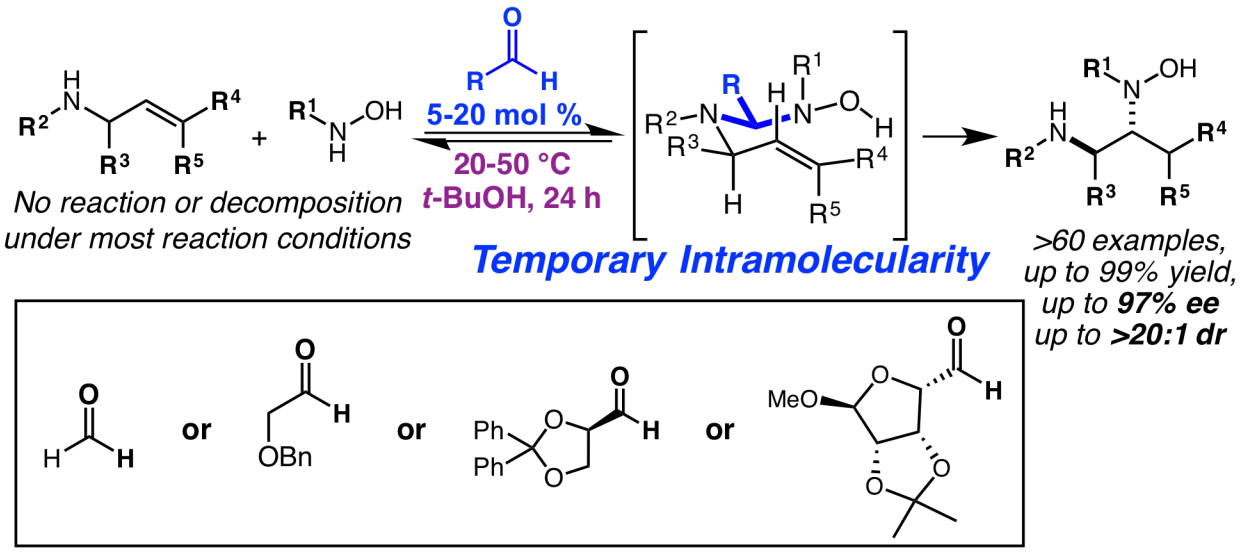
Figure 2.1 Small molecule inducing temporary intramolecularity

well established in synthetic organic chemistry as a method of rate acceleration in challenging intermolecular reactions.[56] These strategies, however, typically require

[56] (a) Diederich, F.; Stang, P. J. *Templated Organic Synthesis*; Wiley-VCH: Chichester, 2000. (b) Bols, M.; Skrydstrup, T. *Chem. Rev*. **1995**, *95*, 1253. (c) Fensterbank, L.; Malacria, M.; Sieburt, S. *Synthesis* **1997**, 813. (d) Gauthier Jr.; D. R., Zandi, K. S.; Shea, K. J. *Tetrahedron* **1998**, *54*, 2289.

stoichiometric amounts of the tethering unit; furthermore, the tether requires one or two steps to form, and one subsequent step is required to cleave the tether once the "temporary intramolecularity" has been achieved and used to allow an intermolecular reaction to proceed.

Recently, our group has demonstrated that aldehydes can act as powerful tethering catalysts for a difficult hydroamination reaction.[57] Subsequent studies with homochiral aldehydes demonstrated that this transformation can be done asymmetrically as well (Scheme 2.1).[58] Interestingly, the simpler aldehydes proved to be the most effective ones, with formaldehyde demonstrating remarkable tethering efficiency.[59]



Scheme 2.1 Catalysis through temporary intramolecularity using aldehydes

[57] MacDonald, M. J.; Schipper, D. J.; Ng, P. J.; Moran, J.; Beauchemin, A. M. *J. Am. Chem. Soc.* **2011**, *133*, 20100.
[58] (a) MacDonald, M. J.; Hesp, C. R.; Schipper, D. J.; Pesant, M.; Beauchemin, A. M. *Chem. Eur. J.* **2013**, *19*, 2597. (b) Hesp, C. R.; MacDonald, M. J.; Zahedi, M. M.; Bilodeau, D. A.; Zhao, S.; Pesant, M.; Beauchemin, A. M. *Org. Lett.* **2015**, *17*, 5136.
[59] Guimond, N.; MacDonald, M. J.; Lemieux, V.; Beauchemin, A. M. *J. Am. Chem. Soc.* **2012**, *134*, 16571.

Formaldehyde is well-established as a powerful tool to crosslink molecules; this is demonstrated by its widespread use in academia as well as industry as a crosslinking reagent. Examples of this include: immobilization of cells,[60] promoting the association of biomolecules,[61] and polymer formation (i.e. urea-formaldehyde resin).[62] In industry, it has been used as a crosslinking reagent to produce textiles, plywood, adhesives, insulation, etc. (ca. 32 million tons in 2006).[63] A prebiotically relevant example comes from Shwartz, and Goverde who have demonstrated that formaldehyde and related compounds are capable of accelerating the oligomerization of HCN.[64]

This crosslinking ability, coupled with the fact that formaldehyde was present in concentrations has high as 0.02 M in prebiotic oceans,[26a,29] hint towards the notion that formaldehyde could have played more of a role than that of a prebiotic reagent. It is possible for formaldehyde to have coupled challenging intermolecular reactions, thus offering supplementary modes of concentration via temporary intramolecularity. To provide experimental support for this hypothesis, our hydroamination reaction was used as a model, and the reaction conditions were altered significantly in order to conform with that of prebiotic environments. Even if such hydroamination reactions are non

[60] (a) Karmakar, S.; Harcourt, E. M.; Hewings, D. S.; Lovejoy, A. F.; Kurtz, D. M.; Ehrenschwender, T.; Barandun, L. J.; Roost, C.; Alizadeh, A. A.; Kool, E. T. *Nat. Chem.* **2015**, *7*, 752. (b) Sutherland, B. W.; Toews, J.; Kast, J. *J. Mass. Spectrom.* **2008**, *43*, 699.
[61] (a) Carey, M. F.; Peterson, C. L.; Smale, S. T. *Cold Spring Harb. Protoc.* **2009**, *4*, 1. (b) Poorey, K.; Viswanathan, R.; Carver, M. N.; Karpova, T. S.; Cirimotich, S. M.; McNally, J. G.; Bekiranov, S.; Auble, D. T. *Science* **2013**, *342*, 369.
[62] Dunky, M. *Int. J. Adhes. Adhes.* **1998**, *18*, 95.
[63] Salthammer, T.; Mentese, S.; Marutzky, R. *Chem. Rev.* **2010**, *110*, 2536.
[64] Schwartz, A. W.; Goverde, M. *J. Mol. Evol.* **1982**, *18*, 351.

prebiotic, it offers the possibility of using this catalytic reaction to probe the ability of formaldehyde to operate as a catalyst in water. From a prebiotic context, using model reactions to represent concepts is precendented due to the complicated nature of prebiotic reactions. A good example of this from the literature is Soai's autocatalytic amplification of homochirality, where a prebiotically relevant ingredient (enantiopure quartz minerals, for example) is used to induce chirality in a non-prebiotic system (pyrimidyl alkanols).[41]

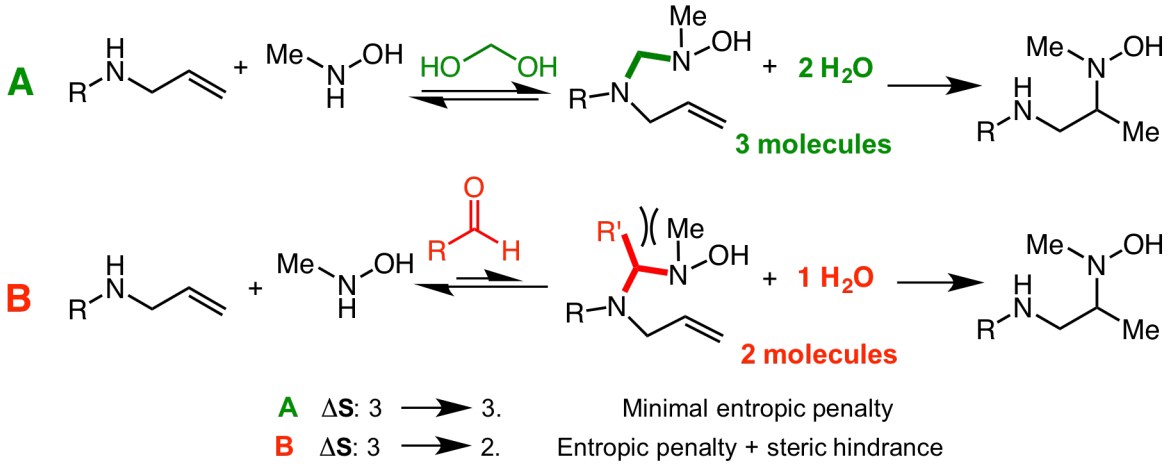
If formaldehyde is indeed capable of tethering molecules in water, and accelerating challenging intermolecular reactions, other prebiotic aldehydes should be tested. Specifically, D-glyceraldehyde should be investigated to probe the feasibility of asymmetric tethering in a prebiotic context. As mentioned in section 1.4.2, D-glyceraldehyde can be synthesized with up to 92 % ee using prebiotic methods (induction of chirality from a an enantiopure amino acid followed by amplification via selective solubilities of the enantiomers of glyceraldehyde).[55] Though there has been a great deal of progress in exploring the origin of homochirality, there is still no general consensus on where this chirality originated from.[44,45,46,54] Since life cannot exist without this chirality, there is a need to unearth its origin; it is possible for homochiral aldehydes, either in the form of catalytic tethering agents or even stoichiometric templates, to have participated in the induction of asymmetry, and therefore this should be investigated further in an experimental setting.

Carbonyl compounds such as formaldehyde have also been shown to enhance the rate of hydration / hydrolysis of alpha-amino nitriles (ca. 10^5 rate enhancement).[24] Pascal, Taillades, and Commeyras were pioneers in this field, demonstrating that stoichiometric amounts of carbonyl compounds were able to act as hydratase / hydrolase mimics.[25] These are important findings since the hydration and subsequent hydrolysis of alpha-amino nitriles is the currently accepted route to alpha-amino acids, and in a prebiotic world without biological catalysts, these hydrations would be slow. Recently, our group has demonstrated that this hydration reaction can be achieve under catalytic amounts carbonyl groups, and that formaldehyde was the most efficient aldehyde for this process.[28] As mentioned in section 1.3, the hydration of beta-amino nitriles with carbonyl compounds has not yet been explored. The hydration of beta-amino nitriles is an important process that needs to be studied since the hydration of beta-amino nitriles would produce purine intermediates. Reactions which proceed faster would have dictated chemical evolution, and so the role of carbonyl compounds in the hydration of beta-amino nitriles should be explored.

2.2 Results and Discussion

2.2.1 Formaldehyde as a Tethering Catalyst in Water

To find conditions in which aldehydes could be tested for their tethering ability meant a significant departure from our previous hydroamination conditions. Whereas before the goal was to obtain high yields within 24 hours in organic solvents (1 M), while using heat, now the goal was to achieve these hydroamination reactions at low concentrations, in water, ideally at room temperature. There is an issue, however, when it comes to using formaldehyde in water since it is present as a hydrate. Upon aminal formation, there is the release of two molecules of water, and in aqueous solutions, this could impede the formation of the aminal intermediate due to LeChâtellier's principle (Scheme 2.2 A). When considering this dilemma through an entropic perspective,



Scheme 2.2 Temporary Intramolecularity: Formation of Mixed Aminal With Formaldehyde Hydrate (A), vs Normal Aldehydes (B)

however, it is evident that aminal formation is not disfavored: two reagents and one catalyst react to form the mixed aminal and two molecules of water (3 → 3). Stable aldehydes which are not present as a hydrate (Scheme 2.2 B) would face an entropic

penalty when forming the mixed aminal (3 → 2). It is therefore possible that formaldehyde could act as a tethering catalyst in water, and so investigations began using water soluble reagents and formaldehyde as a focus point.

A very important first step was ensuring that all of the molecules that would be used are water soluble. For this, *N*-methyl hydroxylamine hydrochloride was used as the water-soluble hydroxylamine; allylamine and *N*-methylallylamine were chosen as the water soluble allylamines, and dimethylsulfone was chosen as the internal standard that would be used to monitor the reaction progress through NMR yields (Figure 2.2).

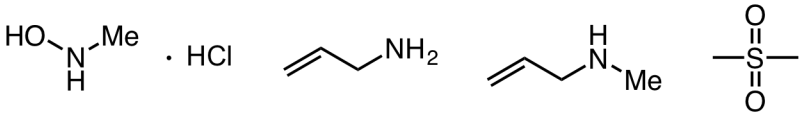
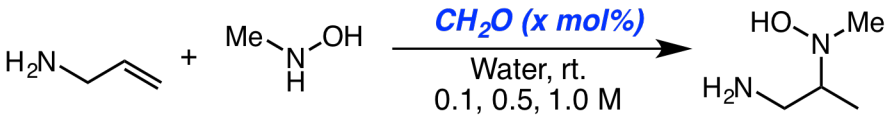


Figure 2.2 Water-soluble reagents chosen for this study

The next thing that needed to be addressed was the concentration of the reaction. For this, a few different molarities were screened, and it appeared that a concentration of 0.1 M was suitable since it demonstrated a steady increase in product yield over a period of 8 days (Table 2.1). This would be ideal for the comparison of different aldehydes to formaldehyde. Though it was possible to monitor reaction progress at concentrations lower than 0.1 M, this was decided against since the reaction progress was too slow, and trace amounts of oxygen in the reaction vessels would lead to oxidation and therefore product degradation. Moreover, concentrations of formaldehyde on primitive Earth were placed at 0.02 M,[26a,29] making a reaction concentration of 0.1 M an ideal starting point if formaldehyde is to act as a tethering

catalyst at 20 mol %. From this early screen, it was evident that formaldehyde could act as a tethering catalyst in water, however there were complications that needed to be addressed, which are described below.

Table 2.1 The effect of concentration on the hydroamination reaction



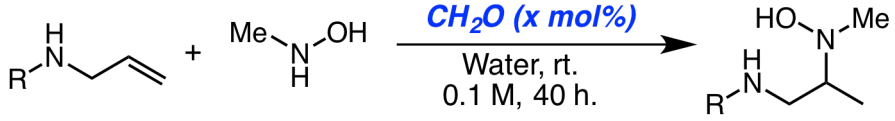
CH_2O (x mol%)	Conc.	Reaction Time	NMR Yield (%)
20%	1.0 M	1 day	54
		3 days	62
		8 days	59
20%	0.5 M	1 day	39
		3 days	56
		8 days	55
20%	0.1 M	1 day	13
		3 days	31
		8 days	51
0%	1.0 M	1 day	<1
		3 days	<1
		8 days	2
0%	0.5 M	1 day	<1
		3 days	1
		8 days	1
0%	0.1 M	1 day	<1
		3 days	<1
		8 days	<1

The reaction yields were determined using dimethyl sulfone as an internal standard (^{1}H NMR). Reactions contained: allylamine (2.5 equiv.), dimethyl sulfone (1 equiv.), and *N*-methylhydroxylamine (1 equiv.). Reactions were done in rigorously degassed distilled water, under an atmosphere of argon.

A major issue that needed to be addressed was the reproducibility issues [e.g. in one case, the reaction would proceed normally as it did in Table 2.1 for 0.1 M concentrations, but in another case the yields would decrease after a few days (Table

2.2)]. What was noticed is that the hydroamination product would decompose after long periods of time if there was oxygen present; furthermore, oxidation of the *N*-methyl group on either reagent would lead to the formation of formaldehyde which explains the increase in background reactivity in some cases. To prevent this, the water had to be deoxygenated via four successive freeze-pump-thaw cycles, and that the allylamine substrates were distilled prior to use. Once these steps were taken, reproducibility was achieved successfully each time.

Table 2.2 Early complications noticed in the hydroamination reaction



R	CH_2O	NMR Yield (%)
Me	10%	29
Me	0%	16
H	10%	0
H	0%	0

Conditions: see Table 2.1

With the reproducibility issues addressed, it became clear that formaldehyde was indeed capable of acting as a tethering catalyst in water (Figure 2.3). Formaldehyde provided a 6-fold increase in the amount of measured product when compared to the background. For the case of *N*-methylallylamine a maximum of 66 ± 2 % was observed after 8 days in the presence formaldehyde, whereas the background, with no formaldehyde, produced 13 ± 3 % of the hydroamination product (the background has been demonstrated by Zhao *et al*. to be a result of a strong hydrogen bond, which allows

for the uncatalyzed hydroamination to occur).[65] A similar result was observed for allylamine, where the catalyzed trials produced an average yield of 45 ± 10 %, compared to 6 ± 6 % for the background after 8 days. With the hypothesis that formaldehyde is capable of acting as a tethering catalyst in water validated, it was time to measure the ability of other key prebiotic carbohydrates to act as tethering catalysts.

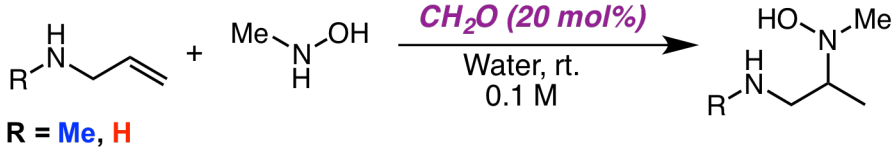
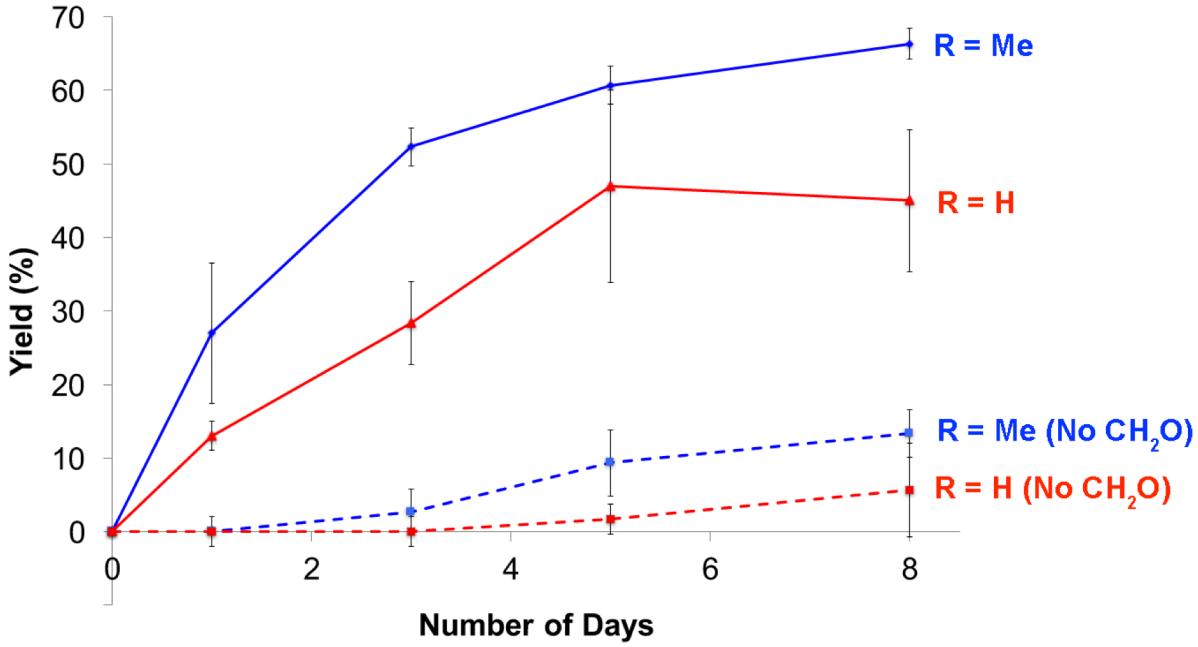


Figure 2.3 Hydroamination yields over a period of 8 days

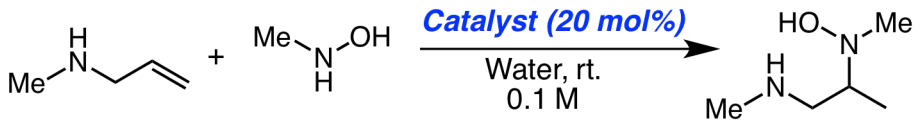


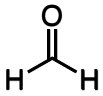
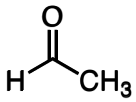
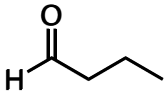
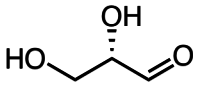
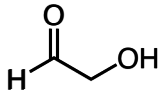
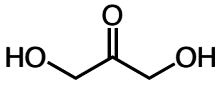
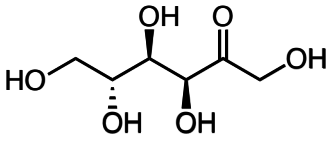
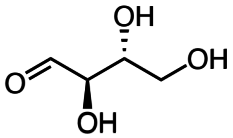
A variety of prebiotically relevant carbohydrates were chosen for this test, the results of which are summarized in Table 2.3, and Figure 2.4. What was immediately clear, is that formaldehyde is a superior tethering catalyst when compared to other

[65] Zhao, S.; Bilodeau, E.; Lemieux, V.; Beauchemin, A. M. *Org. Lett.* **2012**, *14*, 5082.

prebiotic carbohydrates. More stable aldehydes suffer from unfavorable tether formation

Table 2.3 Carbohydrates screened as tethering catalysts for hydroamination



Catalyst	Reaction Time	NMR Yield (%)
	1 day	16
	3 days	52
	5 days	61
	8 days	67
	1 day	4
	3 days	16
	5 days	33
	8 days	41
	1 day	4
	3 days	18
	5 days	34
	8 days	41
	1 day	3
	3 days	14
	5 days	23
	8 days	32
	1 day	5
	3 days	8
	5 days	9
	8 days	6
	1 day	1
	3 days	6
	5 days	7
	8 days	5
	1 day	<1
	3 days	3
	5 days	8
	8 days	18
	1 day	1
	3 days	1
	5 days	9
	8 days	20
No Catalyst	1 day	2
	3 days	7
	5 days	14
	8 days	21

Conditions: see Table 2.1

(Scheme 2.2 B). Moreover, all aldehydes, except formaldehyde, contain a carbon chain

which would contribute to destabilizing steric interactions in the temporary tether that is formed. Despite this, butyraldehyde and acetaldehyde acted as modest tethering catalysts, supporting that they are capable of producing temporary intramolecularity over long periods of time. D-Glyceraldehyde also acted as a modest tethering catalyst, providing 32 % of the hydroamination product after 8 days. What was strange was that

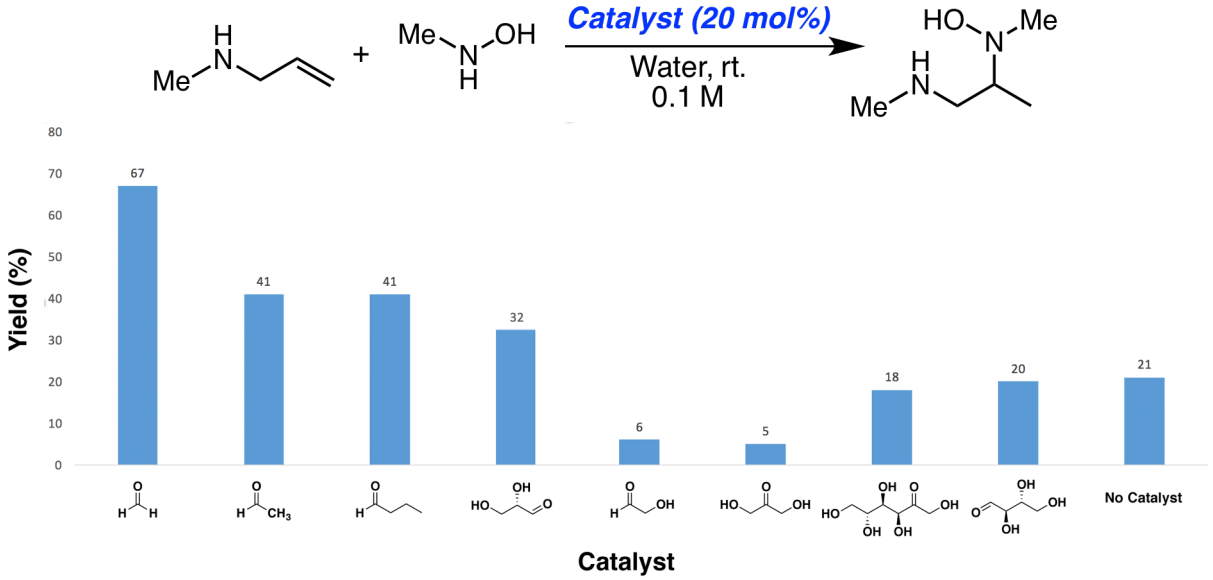


Figure 2.4 Carbohydrates screened as tethering catalysts for hydroamination

1,3-dihydroxyacetone and glycolaldehyde dimer actually inhibited the reaction. This could have been due to a variety of factors such as more stable starting materials (carbohydrate dimers),[66] or stabilization of a symmetrical aminal (i.e. 1 molecule of glycolaldehyde tethering two molecules of hydroxylamine, stabilization by an internal hydrogen bond), which has been shown to be a resting state in related studies.[59] Other carbohydrates demonstrated similar yields as the control experiment, indicating that

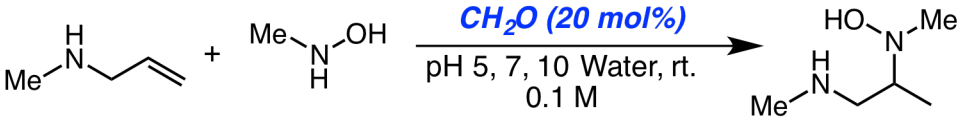
[66] (a) Fratzke, A. R. J. PhD. Dissertation, Iowa State University, 1985. (b) Kua, J.; Galloway, M. M.; Millage, K. D.; Avila, J. E, De Haan, D. O. *J. Phys. Chem.* **2013**, *117*, 2997.

they did not participate as a tethering catalyst. Aldehydes, such as glyoxylate and pyruvate, were not tested due to past results from our hydroamination reaction.[57] These result provide calibration on the ability of select prebiotically relevant carbohydrates to act as tethering catalysts at low concentrations in water, and they show that formaldehyde is a superior tethering catalyst.

Next, the effect of pH on the tethering capabilities of formaldehyde were evaluated (Table 2.4). Estimates place the pH of early oceans to have been from 5-11; most likely however, the pH is estimated to be closer to the current day value of pH = 8.[29] When studying the effect of pH, it was noticed that the reaction did not proceed for either the catalyzed reaction (20 mol. % CH_2O), or the uncatalyzed reaction under acidic conditions (pH = 5). This is logical since under acidic conditions, *N*-methylallylamine would be protonated and therefore unable to form the mixed aminal intermediate which is required to form the expected hydroamination product. Under buffered neutral conditions, the reaction proceeded normally, providing 65 % of the hydroamination product after 8 days. Interestingly, the background (no CH_2O) was suppressed when compared to unbuffered conditions which suggests that the hydrogen bonding required for this background reactivity is disrupted.[65] At pH = 10, there was no change in reactivity, meaning that both the catalyzed and the background reaction provided similar yields to the unbuffered conditions (59, and 9 %, respectively). This is understandable

seeing as how the pH of an unbuffered reaction was measured to be 10 throughout the course of the 8 days.

Table 2.4. The effect of pH on hydroamination



pH	Reaction Time	NMR Yield (%)	NMR Yield No CH_2O (%)
5	1 day	0	0
	3 days	0	0
	5 days	0	0
	8 days	0	0
7	1 day	21	0
	3 days	45	0
	5 days	65	1
	8 days	65	2
10	1 day	18	1
	3 days	41	1
	5 days	58	4
	8 days	59	9

Conditions: see Table 2.1

Overall, these results demonstrate that the hydrate of formaldehyde is capable of accelerating a difficult intermolecular reaction in water by tethering two molecules and therefore providing temporary intramolecularity. Though the turnover is only modest, the concept is validated, showing that small molecules could accelerate slow intermolecular prebiotic reactions, giving a complementary strategy to other non-covalent templating approaches that have been reported (e.g. the ligation of short oligonucleotides).[67] Moreover, the results give calibration on the ability of key

[67] (a) Cafferty, B. J.; Musetti, C.; Kim, K.; Horowitz, E. D.; Krishnamurthy, R.; Hud, N. V. *Chem. Commun.* **2016**, *52*, 5436. (b) Jain, S. S.; Anet, F. A. L.; Stahle, C. J.; Hud, N. V. *Angew. Chem. Int. Ed.* **2004**, *43*, 2004.

carbohydrates in providing temporary intramolecularity in water, and provide a strong foundation to explore the use of prebiotic reactions that could be accelerated through temporary intramolecularity.

2.2.3 D-Glyceraldehyde in Producing Enantiomeric Excess

With formaldehyde and other prebiotic carbohydrates' tethering capabilities established, we decided to probe the abilities of homochiral aldehydes in providing an asymmetric tethering environment. The main focus of this study was D-glyceraldehyde, since it is the simplest aldehyde with a chiral center. Furthermore, as mentioned in section 1.4.2, D-glyceraldehyde can be produced with 92 % ee using prebiotic modes of amplification.[55] Our group has also demonstrated that homochiral aldehydes, such as a D-glyceraldehyde derivative, can act as highly asymmetric tethering catalysts (up to 97 % ee observed) for a challenging intermolecular hydroamination reaction.[58a] Consequentially, we began to test the ability of D-glyceraldehyde to act as an asymmetric tethering catalyst using our hydroamination reaction as a model to investigate the abilities of homochiral carbohydrates to act as a new source of homochirality in prebiotic reactions.

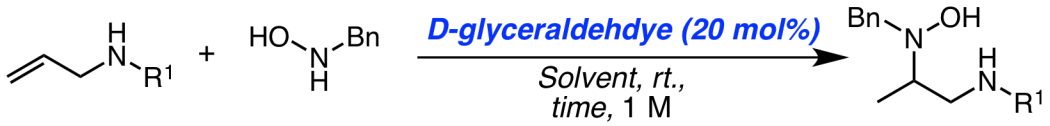
As shown in section 2.2.1, D-glyceradehyde was shown to act as a modest tethering agent in the hydroamination reaction of *N*-methylallylamine and *N*-methylhydroxylamine in water at low concentrations. To probe the ability of D-glyceraldehyde to provide enantiomeric excess in these reactions, it was required to use

bulkier substrates, and more favorable conditions (i.e. organic solvents, 1 M) in order to maximize the chances of obtaining homochirality before moving on to more challenging substrates (with smaller *N*-alkyl substituents), and conditions.

To begin, the hydroamination of diallylamine / *N,N*-allylbenzylamine with *N*-benzylhydroxylamine was studied using D-glyceraldehyde as the tethering catalyst. These substrates were relatively bulky and therefore it was thought that they would provide ideal results for an asymmetric tethering experiment. Since these larger substrates were thought to be insoluble in water, we began the investigation in organic solvents (Table 2.5). Product formation was observed in *tert*-butyl alcohol (28 %), however there was no ee observed for this system. A subsequent attempt in benzene, however, did indeed produce 20 % of enantiomeric excess, however the yield here was low (7 %). Since the ee here was the same as the catalyst loading, it was thought that increasing the loading to 100 mol. % could produce a higher level of asymmetry, and yields; unfortunately, this only boosted the yield (46 %), while diminishing the ee of the hydroamination product to 6 %. Chloroform provided similar results, with 25 % yield, and 5 % ee. Next, benzene was used again, although this time with a bulkier allylamine (*N*,*N*-allylbenzylamine) in hopes of increasing the ee, however this provided similar results to diallylamine (19 % ee). THF, and acetonitrile were tried as well, though they did not increase the enantiomeric excess (18, and 5 % ee, respectively). D-Erythrose, another prebiotic aldehyde (oligomer of formaldehyde), was also screened, and

demonstrated similar results to D-glyceraldehyde, providing 32 % yield and 6 % ee in THF (Table 2.5, last entry). We thought perhaps that these aldehydes were undergoing racemization before having a chance to complete the tethering required to induce

Table 2.5 Summary of hydroamination results with D-glyceraldehyde as a tethering catalyst



R^1	Solvent	Time	Isolated Yield (%)	ee (%)
Allyl	*t*-BuOH	24 h	28	0
Allyl	Benzene	24 h	7	20
Allyl	Benzene	24 h	46[a]	6[a]
Allyl	$CHCl_3$	24 h	25	5
Bn	Benzene	24 h	19	19
Allyl	THF	24 h	25	18
Allyl	MeCN	24 h	15	5
Allyl	THF	24 h	19[a]	7[a]
Allyl	THF	24 h	41[b]	7[b]
Allyl	THF	3 h	30[c]	3[c]
Allyl	THF	3 days	48	11
Allyl	H_2O	24 h	6	46
Allyl	H_2O/*t*-BuOH (1:1)	24 h	0	-
Bn	H_2O/dioxane (1:1)	24 h	0	-
Bn	H_2O	24 h	0[d]	-[d]
Bn	THF	24 h	32[e]	6[e]

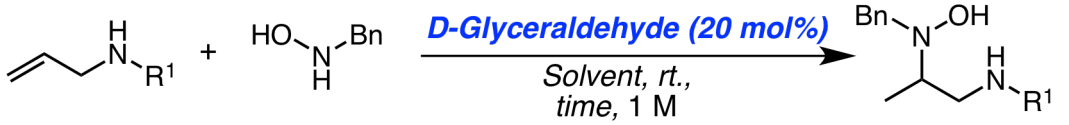
[a] 100 mol% of D-glyceraldehyde was used, [b] **Performed at 60 °C,** [c] **Performed at 80 °C,** [d] *N*-(*sec*-Butyl)hydroxylamine was used as the hydroxylamine, [e] 20 mol% D-Erythrose was used instead of D-glyceraldehyde. Reaction conditions: allylamine (2.5 equiv.), hydroxylamine (1 equiv.), performed under an atmosphere of argon in degassed solvents.

asymmetry. Consequentially, we attempted to drive the reaction forward in less time by adding heat. At 60, and 80 °C, there was actually a decrease in ee (7, and 3 %, respectively), indicating that racemization of the aldehyde occurs more rapidly at higher temperatures. Attempting to do the opposite (leaving the reaction for longer), actually decreased the ee, providing 11 % after 3 days. Interestingly, the highest level of ee

obtained was actually in water, where 46 % ee was observed, although with low yield (6 %). This low yield was thought to have come from low solubilities of the substrates, and therefore trials with co-solvents were done. The reaction, however, did not benefit from these co-solvents (*t*-BuOH, and dioxane), and therefore no hydroamination product was observed.

A subsequent attempt at confirming these yields in water using ^{1}H NMR yields demonstrated that very little of the hydroamination product forms, even after two days (Table 2.6). Curious as to the origin of these low yields, the solubility of various hydroxylamines and allylamines were tested by mixing equimolar amounts of substrate and dimethyl sulfone (internal standard) in water, and gauging how much of the substrate was in solution by comparing to the internal standard (Figure 2.5). These solubility tests demonstrate that a significant amount of the *N*-benzylhydroxylamine was not in solution. Attempts at using a more soluble hydroxylamine [*N*-(*sec*-butyl)hydroxylamine] in water did not alter the stark outlook.

Table 2.6 ^{1}H NMR yields of hydroamination in water using D-glyceraldehyde



R1	Solvent	Time (days)	NMR yield[a]
Bn	H_2O	1	7
Allyl	H_2O	1	4
Allyl	H_2O	2	10
Allyl	H_2O:tBuOH (1:1)	1	0

[a] determined by ^{1}H NMR using dimethyl sulfone as an internal standard. Reaction conditions: see Table 2.5.

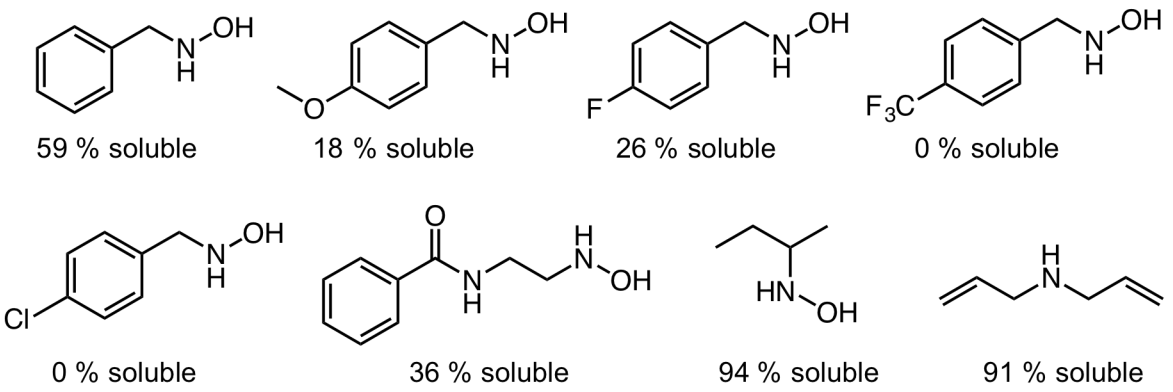


Figure 2.5 Solubility of hydroamination substrates in water

These results indicate that although D-glyceraldehyde does act as a modest tethering catalyst for a few challenging intermolecular reactions, it does not perform nearly as well as formaldehyde (in terms of turnover). It is interesting, nonetheless, that some of the asymmetry from D-glyceraldehyde can be transferred during the course of the reaction to the product. Since D-glyceraldehyde was most likely a prebiotic molecule,[55] and because even the smallest amounts of enantiomeric excess in prebiotic molecules have been amplified to near homochirality,[34,40,52,55] these results are significant.

2.2.4 Formaldehyde on the Hydration of Beta-Amino Nitriles

As mentioned in section 1.3, formaldehyde, and other carbonyl compounds are capable of acting as powerful hydratase and hydrolase mimics, for the hydration and hydrolysis of alpha-amino nitriles. This is an important discovery for prebiotic chemistry since the hydration, and subsequent hydrolysis of Strecker adducts is the accepted prebiotic route to alpha-amino acids.[23] It is also known from work in the pharmaceutical industry that such hydration and hydrolysis reactions require forcing conditions to

proceed. Conversely the hydration of beta-amino nitriles with carbonyl compounds have not yet been explored. Today, there are enzymes that can achieve these hydrations / hydrolyses effortlessly,[68] however in an era without biological catalysts these transformations are difficult and therefore very slow. The hydration of beta-amino nitriles is an important transformation from a prebiotic standpoint since the oligomerization of HCN monomers to produce beta-amino nitriles is common,[31] and the hydration of these would produce precursors of adenine and other key purine intermediates.[32] Faster reactions would dictate chemical evolution, and it is therefore important to explore the potential for formaldehyde and other key prebiotic carbonyls to catalyze the hydration of beta-amino nitriles.

Since the hydration of actual purine precursors is difficult to monitor by ^{1}H NMR (lack of suitable proton reporters for ^{1}H NMR analysis, difficult to synthesize, etc.), the hydration of a model beta-amino nitrile can offer great insights into the efficiency of this process. As a result, 2-aminonicotinonitirle (**ANN**) was chosen as a representative for 5-amino-1*H*-imadazole-4-carbonitrile (**AIC**) (Figure 2.7). **ANN** offers three characterizable protons, and both substrates are aromatic, and both substrates contain a basic site. Subsequent studies will therefore be done with **ANN**.

[68] Prasad, S.; Bhalla, T. C. *Biotechnology Advances* **2010**, *28*, 725.

The study commenced with similar conditions to that of our alpha-amino hydration (i.e. 1 M, in water with 20 mol % of formaldehyde and sodium hydroxide).[28] Immediately, it was noticed that the desired product was forming by ^{1}H NMR (22 %

5-amino-1*H*-imidazole-4-carbonitrile (**AIC**)

2-aminonicotinonitrile (**ANN**)

Figure 2.7 Beta-amino nitrile chosen to represent purine precursor

yield), where the background reactions (i.e. no formaldehyde, or no base) were not forming the amide (Table 2.7, entries 1 – 3). Unfortunately, attempts of isolating this product were not successful due to the formation of multiple dimers of **ANN** (up to 5

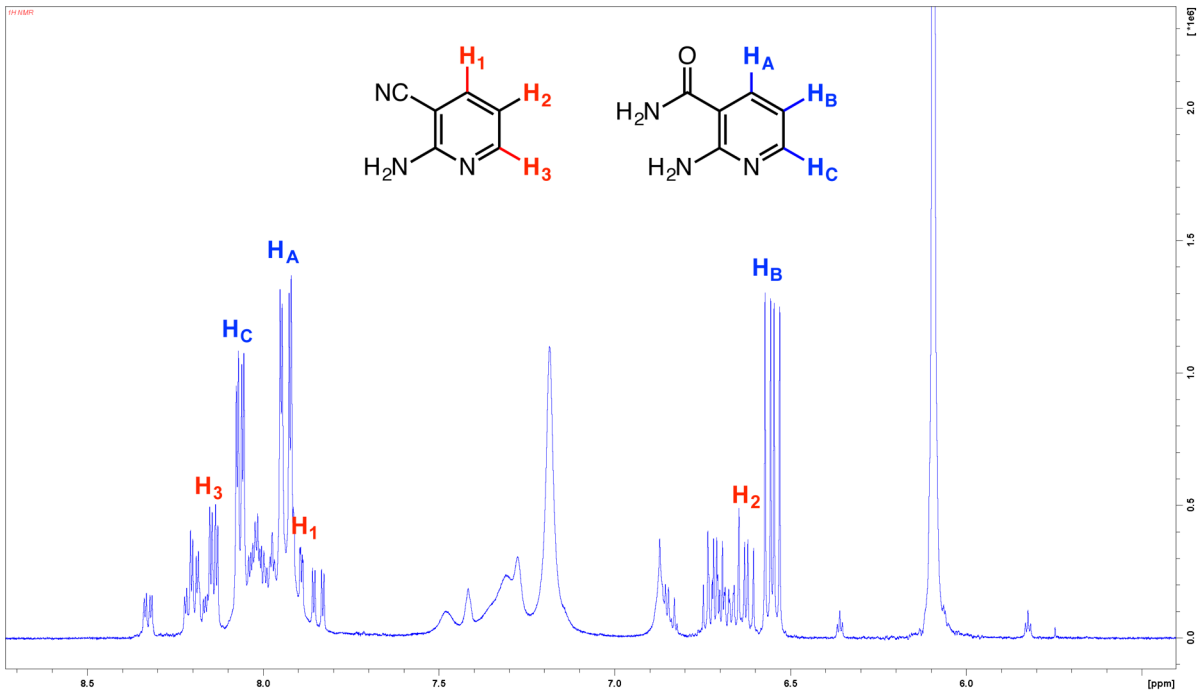


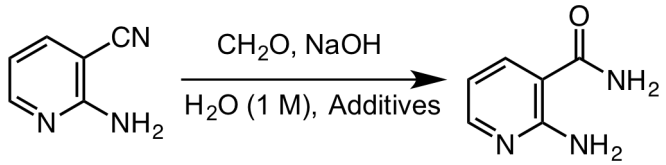
Figure 2.6 Diagnostic peaks used to quantify beta-amino amide

dimers in some cases), all with similar polarities and therefore retentions. The hydration

of the **ANN** substrate was therefore monitored and quantified by ^{1}H NMR (comparison to internal standards). Diagnostic peaks of the product displayed in Figure 2.6, and the product yields were determined by comparing to an internal standard. With this, a systematic study began to find conditions in which formaldehyde could efficiently catalyze this hydration. Fluctuating the amount of base and formaldehyde from 10 to 100 mol % (entries 3 – 9), only boosted the yield of this hydration to 32 % (entry F). Trying acidic conditions (entries 10, and 11), lead to the formation of a dimer species (2: 1, **ANN**: CH_2O), which traps the formaldehyde, leaving it unproductive and unable to participate in hydration. What was noticed is that there were issues with solubility (i.e. undissolved starting material floating in the reaction medium). The catalytic and stoichiometric use phase of a transfer agent (benzyltrimethylammonium chloride, entries 12, and 13) did not help increase the yield of this hydration product (16, and 14 % yield respectively). Dihydroxyacetone was tested instead of formaldehyde (entry 14), and this resulted in no detection of the amide. Glycolaldehyde (entry 15), produced 5 % of the amide by NMR. Since the hydration of alpha-amino nitriles would produce the alpha-amino acid if left for too long,[28] we had the concern that perhaps this system was producing small amounts of acid which was consuming the sodium hydroxide that was in the system. As a result, entry 16, with 1 equivalent of sodium carbonate was performed, but unfortunately, there was no increase in the desired product. Lastly, under the assumption that formaldehyde was trapped as a dimer species (i.e. two molecules of

product tethered with one molecule of aldehyde, or one molecule of product tethered with one molecule of **ANN**, etc.), we decided to try 120 mol. % of formaldehyde, to see if this would result in turnover. Unfortunately, this only diminished the yields (entry 17, 10 % yield), and so the search to find conditions in which formaldehyde could efficiently catalyze this hydration was terminated.

Table 2.7 Key results from the hydration of a beta-amino nitrile



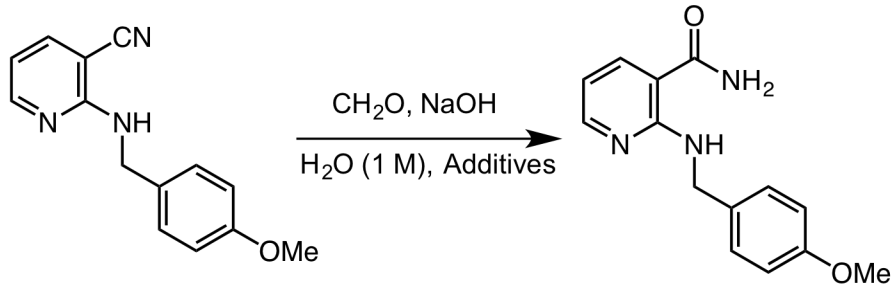
Entry	CH_2O (mol. %)	NaOH (mol. %)	Additive	Time (hours)	NMR Yield
1	-	20	-	3	0%
2	20	-	-	3	0%
3	20	20	-	3	22%
4	20	50	-	3	14%
5	10	10	-	3	11%
6	50	50	-	3	32%
7	20	100	-	3	15%
8	100	100	-	1.5	22%
9	100	20	-	1.5	23%
10	20	-	20% HCl	3	16%[a]
11	20	-	1.2 eq HCl	3	3%[a]
12	20	20	20 mol. % benzyltrimethylammonium chloride	3	16%
13	20	20	1 eq. benzyltrimethylammonium chloride	3	14%
14	-	20	20% dihydroxy acetone	4	0%
15	-	20	20% glycolaldehyde	4	5%
16	20	20	1 eq Na_2CO_3	3	16%
17	1.2 eq	20	-	24	10%

[a] Yield refers to dimer (2: 1, **ANN**: CH_2O). Order of addition: substrate, water, NaOH, then CH_2O. Reaction done under an atmosphere of argon.

Formaldehyde, and other simple carbohydrates were shown to be powerful catalysts when it comes to the hydration of alpha-amino nitriles. The versatility of this process was demonstrated by the fact that this hydration tolerated a wide range of

primary and secondary alpha-amino nitriles.[28] Consequentially, the hydration of a secondary beta-amino nitrile was also examined (Table 2.8). We also thought that this would lead to the formation of less dimers, therefore leaving more CH_2O available for

Table 2.8 Hydration of secondary beta-amino nitrile



Entry	Additives	CH_2O (mol. %)	NaOH (mol. %)	Time (hours)	Results
18	-	20	20	24	No conversion[a]
19	-	-	20	24	No conversion[a]
20	-	1 equiv.	20	1	Traces of amide
21	20 mol. % benzyltrimethylammonium chloride	20	20	2	Traces of amide
22	1: 5, *i*PrOH: H_2O	20	20	1	No Conversion
23	1: 1, Dioxane: H_2O	20	20	1	Traces of amide
24	1: 1, MeCN: H_2O	1.2 equiv.	1.2 equiv.	3	6% Amide[b]

[a] Starting material remained even after 24 hours, [b] Yield determined by NMR (comparing with 1,3,5-trimethoxybenzene). Reaction conditions: see Table 2.7.

catalysis. Under the standard conditions (20 mol % CH_2O, and NaOH), the hydration of this substrate proved more challenging than that of the primary alpha-amino nitrile [0 % of the hydrated product even after 24 hours (entry 18)]. Unsurprisingly, the background reaction (no CH_2O, entry 19) also showed no conversion; in fact, this system only began showing traces of conversion when 1.0 equivalents of CH_2O were used (entry 20). Here, a phase transfer agent (benzyltrimethylammonium chloride) appeared to have a moderate effect, increasing the amount of substrate in solution and therefore providing trace conversions of the amide (entry 21). Because of this result, a few co-solvent systems

(isopropyl alcohol, dioxane, and acetonitrile) were experimented with (entries 22 – 24); a 6 % NMR yield was noticed when 1.2 equivalents of CH_2O and NaOH were used in a 50/50 acetonitrile/water solution. Overall, the hydration of secondary beta-amino nitriles seems to be a challenging process due to low solubility of substrates.

It is unclear why the hydration of a beta-amino nitriles are so much more difficult than that of alpha-amino nitriles. Entropically speaking, these two systems should not be too different since the beta-amino nitrile, **ANN**, has a rigid backbone and therefore does not allow for rotation. Overall, formaldehyde proved to be unsuccessful as a catalyst for the hydration of beta-amino nitriles under these conditions.

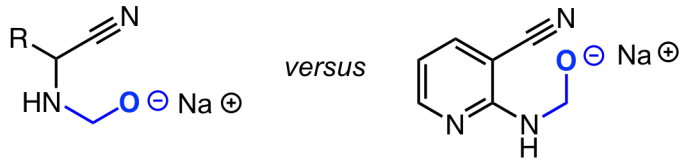
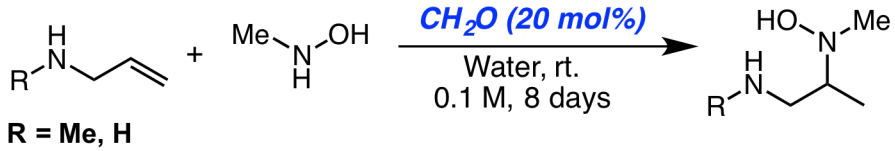


Figure 2.8 Intermediates in the hydration of alpha- and beta-amino nitriles

2.3 Conclusion: Prebiotic Aldehydes as Tethering Catalysts

The low concentration issue in prebiotic chemistry is a challenge which must be addressed so that prebiotic syntheses may be validated. Accepted methods for addressing this issue involve physical processes such as evaporating and freezing or templating processes such as clay templated intramolecularity. Unexplored, however, are processes where temporary intramolecularity is introduced by small molecules as a solution to the low concentration issue. To test this in an experimental setting, we used our model hydroamination reaction and altered the conditions to resemble prebiotic settings (i.e. low concentration, in water). This study showed, after 8 days, that

formaldehyde is indeed capable of acting as a tethering catalyst in water (Figure 2.9). The tethering ability of various other prebiotic aldehydes and carbohydrates were also tested. These carbohydrates did not perform as well as formaldehyde, which further supports the unique efficiency of formaldehyde as a tethering catalyst.

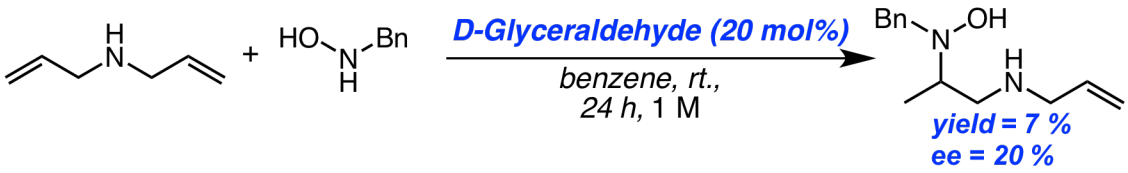


	R = Me (%)	R = H (%)
CH_2O	66 ± 2	45 ± 10
No CH_2O	13 ± 3	6 ± 6

Figure 2.9 Formaldehyde as a tether in a model hydroamination reaction

With formaldehyde demonstrating potential as a tethering catalyst, it is feasible to assume that chiral prebiotic aldehydes, such as D-glyceraldehyde, may have also performed as tethering catalysts. Aldehydes containing a stereocentre could have served a dual purpose – performing as a tethering catalyst, while also transferring their stereochemical information to the products. While there are experimentally supported theories that explain how some of these homochiral molecules are made, there is still no general consensus on the origin of homochirality. Since D-glyceraldehyde can be produced with up to 92 % ee using prebiotic modes of templating and amplification,[55] D-glyceraldehyde could have acted as a templating catalyst and consequentially, an enantiopure templating agent. To test this hypothesis, our model hydroamination reaction was used again, this time with bulkier substrates, and D-glyceraldehyde as the catalyst. The results of this study indicated that D-glyceraldehyde was indeed capable

of templating a challenging intermolecular reaction in a selective manner; in general, the ee was limited to 20 % (Scheme 2.3). From a synthetic chemistry perspective, these values of ee are considered low, however, from an origin of life perspective these values are significant because of preexisting modes of amplification.



Scheme 2.3 D-Glyceraldehyde providing enantiomeric excess in a hydroamination reaction

Lastly, the ability of aldehydes to accelerate the hydration of beta-amino nitriles was examined. The oligomerization of HCN to produce beta-amino nitriles is common, and hydration of these beta-amino nitriles would produce adenine and other purine intermediates. In an era without biological catalysts, these reactions would be challenging, and therefore slow. Consequentially, the hydration of a model beta-amino nitrile was studied using conditions similar to those developed for the hydration of our alpha-amino nitriles. The yield of the beta-aminoamide was generally low, with a maximum yield of 32 % being observed with 50 mol% CH_2O and NaOH. This outcome suggests that, it is unlikely that formaldehyde would have acted as a hydration catalyst for beta-amino nitriles under these conditions.

Chapter 3 – Towards the Desymmetrization of Alpha-amino, and Alpha-hydroxy Diesters

3.1 Introduction

The development of methods to obtain optically active organic (enantiopure) compounds is an area which is consistently expanding, however there is always the need for novel approaches towards the synthesis of these compounds. Racemic mixtures of enantiomers would provide unpredictable outcomes when it comes to their pharmacological effects (e.g. thalidomide), and therefore it is important to develop efficient synthetic routes that provide a single enantiomer. In particular, the synthesis of enantiopure tetra-substituted (quaternary) carbon centers (Figure 3.1) remains an exceptional challenge since this entails generating sterically crowded motifs. This is a challenge, however, that is critical to address since quaternary carbons offer increased structural diversity and they are very frequently found in natural products and drugs. As a result, there have been great advances towards the stereocontrolled synthesis of quaternary carbon stereocentres.[69,70]

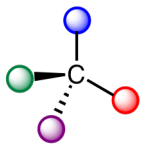


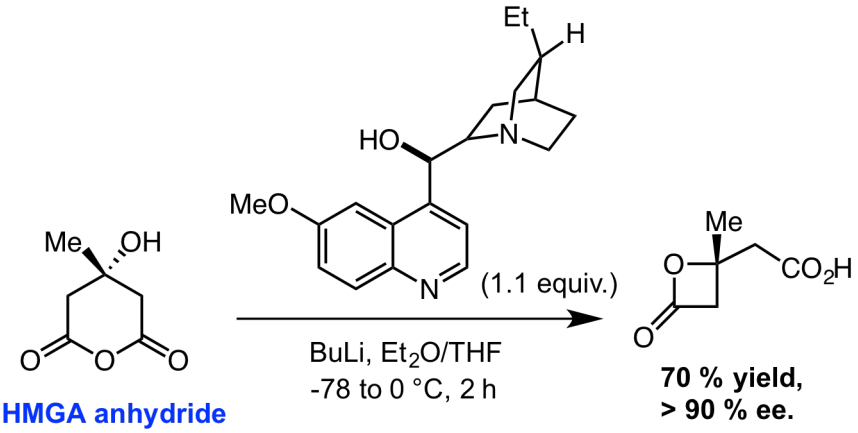
Figure 3.1 Representative diagram for a stereogenic, tetra-substituted carbon center

Many methods exist for obtaining enantioenriched organic species; the most attractive of these methods, however, operate via catalytic pathways. These methods fall into three general categories: 1) C-C bond forming reactions, 2) kinetic resolutions, and

[69] Wang, C.; Lu, Z. *Org. Chem. Front.* **2015**, *2*, 179.
[70] For a recent review, see: Zeng, X.-P.; Cao, Z.-Y.; Wang, Y.-H.; Zhou, J. *Chem. Rev.* **2016**, *116*, 7330.

3) desymmetrization reactions. Enantioselctive desymmetrization reactions are particularly attractive since they allow for formation of stereogenic centers from easily accessible symmetrical compounds.[71]

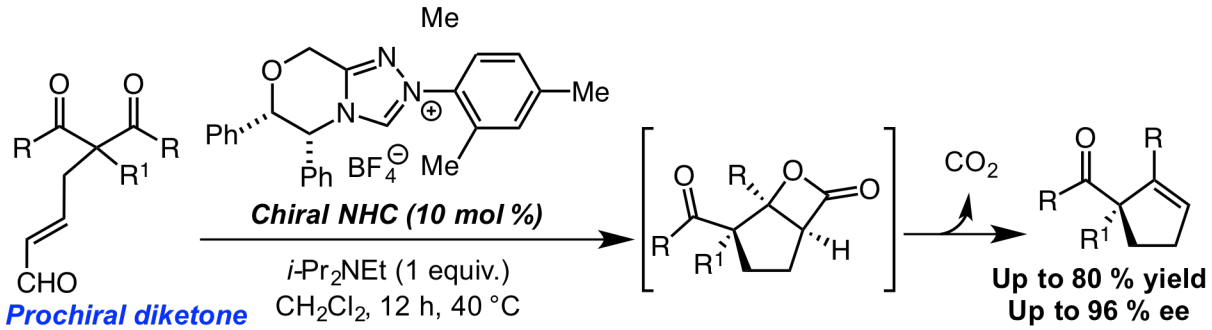
Enantioselective desymmetrization offers an attractive mode of activating symmetric (prochiral) substrates with chiral reagents or catalysts in order to produce enantioenriched products. Desymmetrization of prochiral substrates is attractive given that the maximum theoretical yield is 100 %, whereas the maximum yield in kinetic resolutions cannot exceed 50 %, unless a dynamic racemization process can be engineered to occur faster than the kinetic resolution. Another benefit of starting with symmetric compounds is that they are relatively simple to prepare. A good example of this desymmetrization comes from the preparation of beta-lactones from 3-hydroxy-3-methylglutaric acid (HMGA) anhydrides, where the symmetrical HMGA anhydride is treated with a chiral base, producing the enantioenriched beta-lactones (Scheme 3.1).[72]



Scheme 3.1 Enantioselective desymmetrization of HMGA anhydride

[71] Shintani, R.; Fu, G. C. *Angew. Chem. Int. Ed.* **2002**, *41*, 1057.
[72] Hashimoto, K.; Kitaguchi, J.-I.; Mizuno, Y.; Kobayashi, T.; Shirahama, H. *Tetrahedron Lett.* **1996**, *37*, 2275.

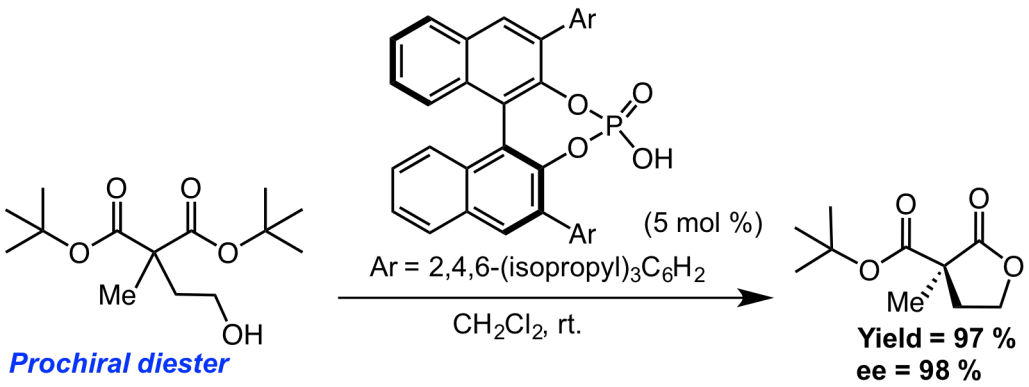
Catalytic enantioselective procedures to obtain desymmetrized products are much more enticing since the source of asymmetry in this would be more economical. Because of this advantage, there have been great strides towards proccesses where chiral catalysts are used to desymmetrize symmetrical compounds;[70] one such example comes from the NHC catalyzed desymmetrization of 1,3-diketones to produce alpha,alpha-disubstituted cyclopentenes (Scheme 3.2).[73] Here, the chiral NHC catalyst condenses on the vinylogous aldehyde, which is protonated to produce an enol. This enol then undergoes an enantioselective aldol reaction, triggering an acylation of the alkoxide intermediate to generate the beta-lactone intermediate which loses CO_2 to generate the desired product.



Scheme 3.2 Catalytic desymmetrization of prochiral diketone

One example of desymmetrization which stood out to our group comes from Petersen and coworkers who demonstrated the use of chiral phosphoric acids to form enantioenriched lactones from hydroxy-diesters (Scheme 3.3).[74] This transformation was intriguing to our group because we envisoned that this could possibly be done with

[73] Wadamoto, M.; Phillips, E. M.; Reynolds, T. E.; Scheidt, K. A. *J. Am. Chem. Soc.* **2007**, *129*, 10098.
[74] Wilent, J.; Petersen, K. S. *J. Org. Chem.* **2014**, *79*, 2303.



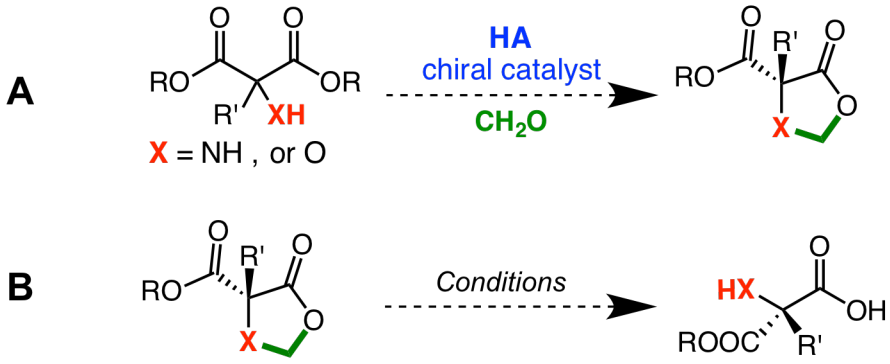
Scheme 3.3 Desymmetrization of prochiral diester with chiral phosphoric acid

formaldehyde as a linker / tethering from an alpha-hydroxy or alpha-amino diester to produce oxalactones or azlactones, respectively (Scheme 3.4 A). This is appealing in two regards: 1) both the azlactone, and the oxalactone are found in bioactive compounds (Figure 3.2),[75] and 2) the resulting lactones can be hydrolyzed to produce chiral alpha-hydroxy and alpha-amino acids (Scheme 3.4 B). Current routes to access the chiral azlactones require starting from chiral amino acids and then using formaldehyde to form the cyclized azlactone.[75d-e] Starting from a chiral source of starting materials is limiting however, and so there is the need for additional methods to access these chiral lactone derivatives. From a strategic perspective, this also offered the opportunity to explore the concurrent use of temporary intramolecularity with asymmetric Brønsted acid catalysis,[70] a dual-catalysis[76] strategy that could be applicable in other synthetic transformations.

[75] (a) Weaver, R.; Gilbert, I. H.; Mahmood, N.; Balzarini, J. *Bioorganic Med. Chem. Lett.* **1996**, *6*, 2405. (b) Massil, S. E.; Shi, G.-Y.; Klotz, I. M. *J. Pharm. Sci.* **1984**, *73*, 1851. (c) Whitten, J. P.; Baron, B. M. NMDA antagonist. US 5095009 A1, March 10, 1992. (d) Barbay, K.; Dyatkin, A. B.; Gong, Y.; He, W.; Miskowski, T. A. Pyridazinones as antagonists of alpha4 integrins. US 2005/192279 A1, September 1, 2005. (e) Yim, J. H.; Kim, C II.; Lee, S. G.; Kim, D.; Han, S. J.; Lee, H. K.; Kim, S. J.; Kim, T. K.; Kang, P.-S.; Park, H.; Park, H. J.; Koh, H. Y.; Park, M. R.; Park, Y.-K. Method for preparing Ramalin. US 2013/211133 A1, August 15, 2013.

[76] (a) Wasilke, J.-C.; Obrey, S. J.; Baker, T.; Bazan, G. C. *Chem. Rev.* **2005**, *105*, 1001. (b) Afewerki, S.; Córdova, A. *Chem. Rev.* **2016**, *116*, 13512.

With these goals in mind, we began to synthesize the starting alpha-hydroxy and alpha-amino diesters.



Scheme 3.4 Our group's interest in the desymmetrization of prochiral diesters

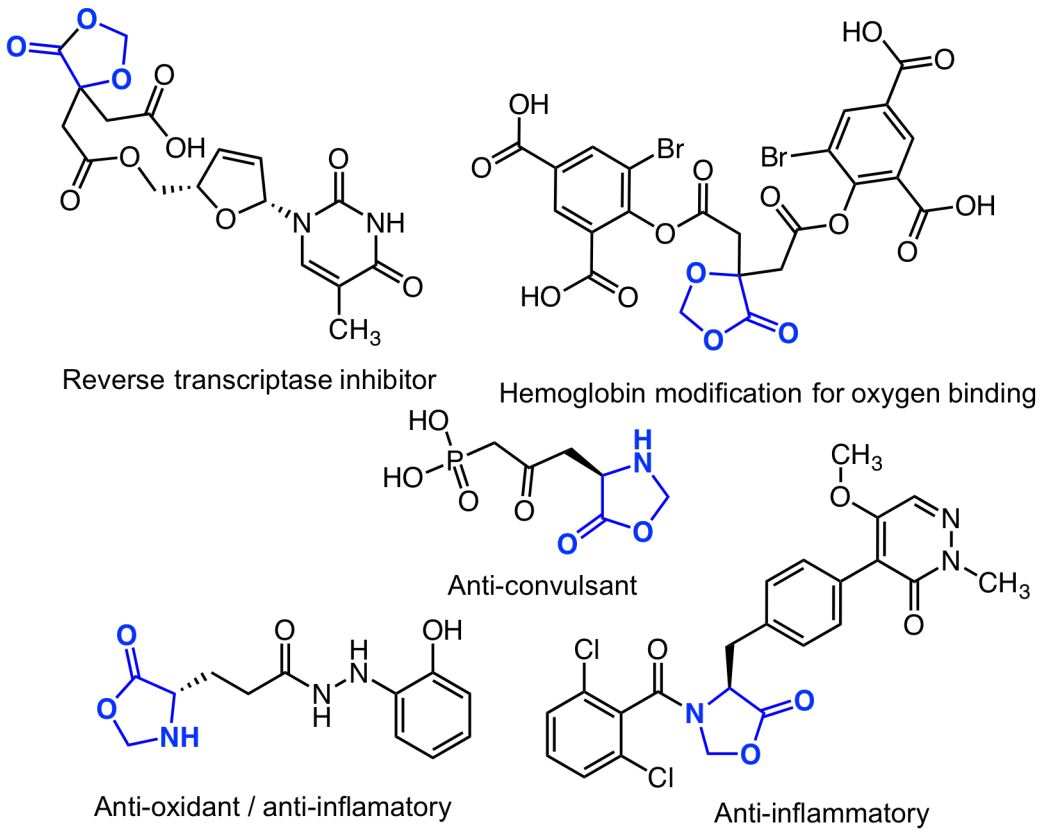
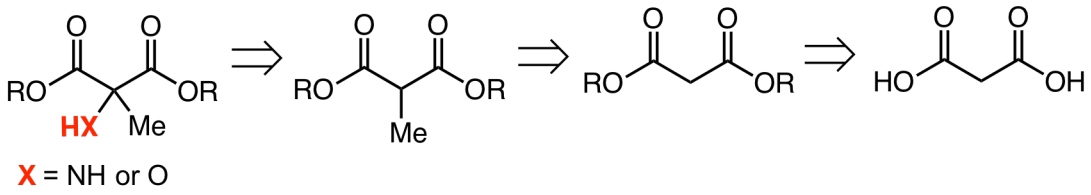


Figure 3.2 Examples of bioactive molecules with oxalactone or azlactone moieties.

3.2 Results and Discussion

3.2.1 Substrate Synthesis

To begin, it was required to develop a synthesis for the desired alpha-hydroxy and alpha-amino diesters (Scheme 3.5). The general idea was to begin form a malonic acid to form the diester, then methylate, and then finally form the hydroxy- or amino-diesters. At the end, alpha-hydroxylation and amination was required, and these were the most difficult steps; the hydroxylation was achieved in one step after exploring a few routes (see below), however the amination was more challenging since electrophilic amination procedures are not feasible (complex reagent synthesis).[77]

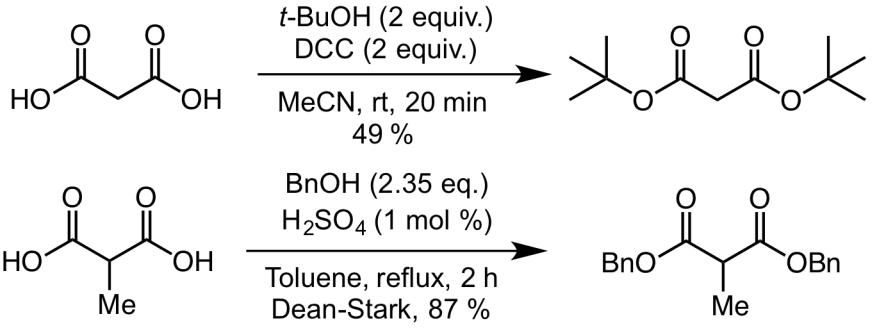


Scheme 3.5 Retrosynthetic plan for synthesis of alpha-hydroxy and alpha-amino

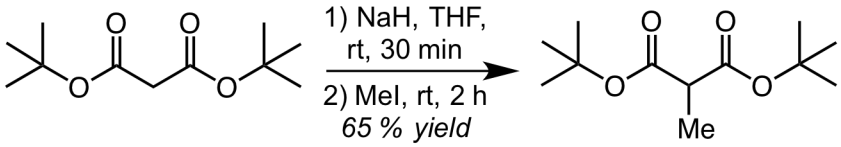
The diesters were produced routinely, either by DCC coupling in the case of di-*tert*-butyl malonate, or by esterification in the presence of a Dean-Stark apparatus for di-benzyl malonate (Scheme 3.7). Although it was feasible to synthesize di-*tert*-butyl malonate, it was ultimately decided that it is more cost effective purchase this, and start the synthesis from the diester. Methyl-malonic acid was used for the esterification to produce dibenzyl 2-methylmalonate since this provided a better overall yield. Next, the

[77] Smulik, J. A.; Vedejs, E. *Org.Lett*. **2003**, *5*, 4187.

methylation of di-*tert*-butyl malonate was achieved in a decent yield to produce di-*tert*-butyl 2-metylmalonate (Scheme 3.6).

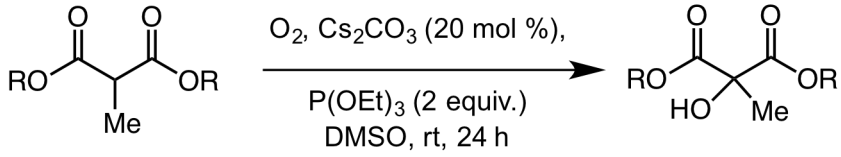


Scheme 3.7 Synthesis of diesters from malonic acid



Scheme 3.6 Methylation of diesters

With the methylated diesters in hand, it was time to hydroxylate / aminate the substrates. Hydroxylation at the alpha position of a diester proved to be challenging, and as a result, a few different literature procedures were scanned until we found one that worked reliably (Scheme 3.8).[78] Attempts of using m-CPBA,[79] or air / CsF,[80] or palladium on carbon / O_2 all failed, likely due to the bulky nature of these substrates.[81]

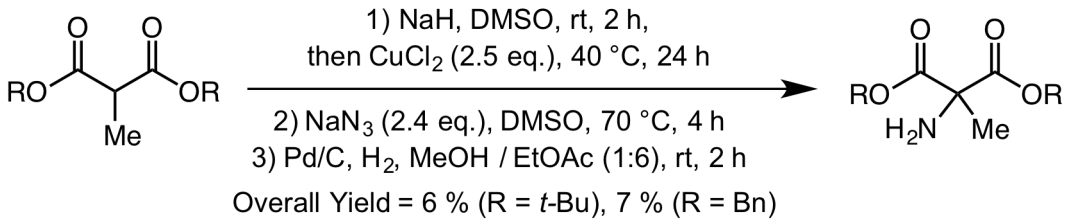


Yield = 42 % (R = *t*-Bu), 58 % (R = Bn)

Scheme 3.8 Synthesis of alpha-hydroxy diesters

[78] Liang, Y.-F.; Jiao, N. *Angew. Chem. Int. Ed.* **2014**, *53*, 548.
[79] Asahara, H.; Nishiwaki, N. *J. Org. Chem.* **2014**, *79*, 11735.
[80] Toshiko, W.; Tsutomu, I. *Tet. Lett.* **1999**, *40*, 7795.
[81] Monguchi, Y.; Takahashi, T.; Iida, Y.; Fujiwara, Y.; Inagaki, Y.; Maegawa, T.; Sajiki, H. *Synlett* **2008**, *15*, 2291.

The synthesis of the alpha-amino diesters required more than one step; they were synthesized by alpha-chlorination, and then subsequent azidation and reduction steps (Scheme 3.9). Although the yields were low, there was enough of the desired product synthesized to commence studying desymmetrization experiments with formaldehyde.

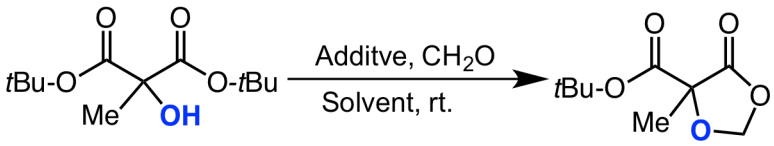


Scheme 3.9 Synthesis of alpha-amino diesters

3.2.2 Towards the Desymmetrization of Diesters using Formaldehyde

With the alpha-hydroxy and alpha-amino diesters synthesized, we began to test the ability of formaldehyde to condense on these functional diesters, and produce the cyclized oxalactone or azlactone species. Beginning with di-*tert*-butyl 2-hydroxy-2-methylmalonate, a substrate that had similar properties to the gamma-hydroxy diester

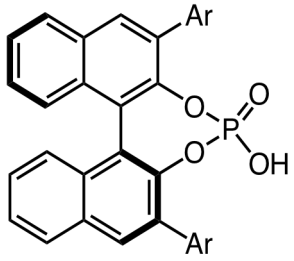
Table 3.1 Attempts of desymmetrizing di-*tert*-butyl 2-hydroxy-2-methylmalonate



Entry	CH_2O	Additive	Solvent	Temp	Time	Result
1	1 eq.	10 mol % DPP	CH_2Cl_2 (wet)	rt	24 h	No conversion
2	1 eq.	10 mol % DPP	CH_2Cl_2 (wet)	rt	24 h	No conversion
3	Para-CH_2O	10 mol % DPP	CH_2Cl_2	rt	24 h	No conversion
4	1 eq.	10 mol % **A**	CH_2Cl_2	rt	24 h	No conversion
5	1 eq.	10 mol % DPP	CH_2Cl_2	rt	3 days	No conversion
6	Para-CH_2O	10 mol % **A**	CH_2Cl_2	rt	1 day	No conversion
7	Para-CH_2O	10 mol % DPP	CH_2Cl_2	rt	3 days	No conversion
8	1.2 eq.	10 mol % DPP	CH_2Cl_2	rt	24 h	No conversion
9	2 eq.	10 mol % DPP	CH_2Cl_2	rt	24 h	No conversion
10	5 eq.	10 mol % DPP	CH_2Cl_2	rt	24 h	No conversion
11	2 eq.	10 mol % DPP	CH_2Cl_2	rt	3 days	No conversion
12	5 eq.	10 mol % DPP	CH_2Cl_2	rt	3 days	No conversion

DPP = Diphenyl phosphate
No conversion = pure starting material (i.e. no change at all)
Conditions: reaction flask was flame-dried reaction and purged with argon. Order of addition: substrate, solvent, acid, and then formaldehyde. Formaldehyde was from a 37 % stock solution in water with methanol as a stabilizing agent.

A

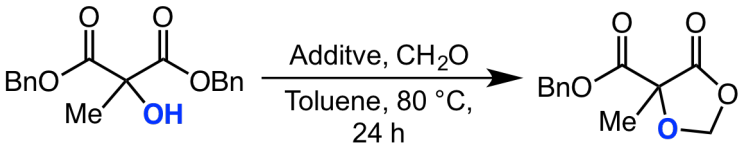


reported by the Petersen group,[74] a variety of conditions and Brønsted acids were investigated. From this early screening (entries **1 – 11**), we found that di-*tert*-butyl 2-hydroxy-2-methylmalonate remained very unreactive under these conditions (Table 3.1). Varying the time, from 1 day to 3 days, had no effect, neither did increasing the strength

of the Brønsted acid (acid **A**, experiments 4, and 6). The dormancy of this substrate may stem from the unreactive nature of alcohols, and also the great amount of steric hindrance produced by the flanking di-*tert*-butyl groups. To test if this inactivity was a result of the bulky leaving group, the substrate was switched to dibenzyl 2-hydroxy-2-methylmalonate.

Switching the substrate to dibenzyl 2-hydroxy-2-methylmalonate (Table 3.2) provided a similar outcome, with no conversion being observed (entries **1b – 8b**).

Table 3.2 Attempts of desymmetrizing dibenzyl 2-hydroxy-2-methylmalonate



Entry	CH_2O	Additive	Result
1	1 eq.	10 mol % DPP	No conversion
2	1 eq.	1 eq. DPP	No conversion
3	1 eq.	1 eq. Et_3N	No conversion
4	1 eq.	0.2 eq. Et_3N	No conversion
5	0.2 eq.	0.2 eq. Et_3N	No conversion
6	2 eq. $(CH_2O)_n$	0.2 eq. Et_3N	No conversion
7	2 eq. $(CH_2O)_n$	1 eq. Et_3N	No conversion
8	2 eq. $(CH_2O)_n$	2 eq. Et_3N	No conversion

DPP = diphenyl phosphate. Conditions: see Table 3.1

Notably, catalytic or stoichiometric triethylamine, instead of a Brønsted acid did not improve the reactivity of this desymmetrization. The formaldehyde stock solution which was used in entries **1 – 5** contained water and methanol, which could have potentially competed with the alpha-hydroxy diester in reacting with formaldehyde; to see if this was playing a role in hindering the reactivity, paraformaldehyde was used in the presence of triethylamine since paraformaldehyde is known to depolymerize in alkaline

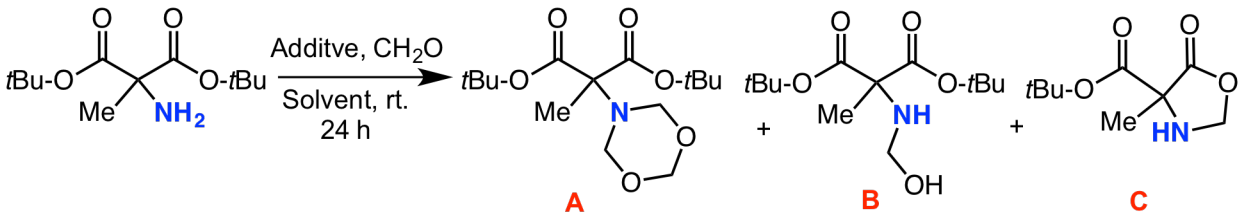
solutions.[82] Unfortunately, however, attempting to use formaldehyde from depolymerized paraformaldehyde had no effect on the reactivity of this alpha-hydroxy diester (entries **6 – 8**). This experiment, therefore provides support that the alpha-hydroxy diesters may not be nucleophilic enough to react with formaldehyde in a manner which would lead to desymmetrization. To circumvent this issue, we moved to a substrate that is more nucleophilic: di-*tert*-butyl 2-amino-2-methylmalonate.

Amines are much more nucleophilic than alcohols (less electronegative, therefore increased lone pair availability), as a result switching to an alpha-amino diester would give a greater possibility of generating the mixed aminal required to form a cyclized product. With this in mind, we began exploring the desymmetrization of an alpha-amino diester (Table 3.3). It was immediately clear that the alpha-amino diester was indeed more reactive towards formaldehyde than the alpha-hydroxy diesters. This increase in reactivity, however, was displayed in the form of formaldehyde adducts **A**, and **B**, rather

[82] Helander, K. G. *Biotechnic & Histochemistry* **1999**, *75*, 19.

than the desired product **C**. These adducts were first noticed under the standard

Table 3.3 Attempts of desymmetrizing di-*tert*-butyl 2-amino-2-methylmalonate



Entry	CH_2O	Additive	Solvent	Result (ratio of products)
1	1 eq.	10 mol % DPP	CH_2Cl_2	2 : 1 : 0
2	1 eq	10 mol % DPP	CH_2Cl_2	2 : 1 : 0[b]
3	-	10 mol % DPP	CH_2Cl_2	No conversion
4	2 eq.	10 mol % DPP	CH_2Cl_2	1 : 1 : 0
5	0.2 eq.	10 mol % DPP	CH_2Cl_2	1 : 1 : 0[a]
6	1 eq.	10 mol % TFA	CH_2Cl_2	4 : 1 : 0
7	1 eq.	10 mol % DPP	THF	1 : 5.5 : 0
8	1 eq.	10 mol % DPP, 10 mol % H_2O	THF	1 : 5 : 0
9	1 eq.	10 mol % DPP, 50 mol % H_2O	THF	1 : 3.5 : 0
10	1 eq.	10 mol % DPP, 2 eq H_2O	THF	1 : 4 : 0
11	1 eq.	10 mol % DPP, 14 eq H_2O	THF	1 : 17 : 0[a]
12	1 eq.	1 eq. DPP, 1 eq. H_2O	THF	1 : 0 : 0
13	1 eq.	10 mol % DPP	Toluene	1 : 27 : 0[a,d]

[a] only traces of each produced [b] Reaction done for 3 days [d] Reaction done at 80 °C. DPP = Diphenyl phosphate. Conditions: see Table 3.1

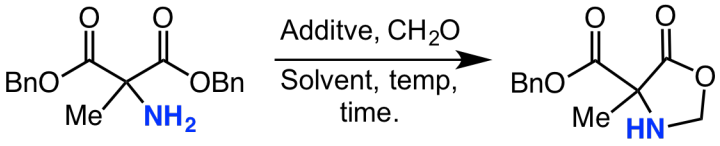
conditions (1 eq. CH_2O with 10 mol % DPP, entry **1**). Attempts of breaking cycle **A** open by stirring for a longer period of time (3 days) under the acidic conditions lead to identical results, with a 2 : 1 ratio of **A** and **B** (entry **2**). Entry **3** demonstrates that without formaldehyde, there is no reactivity, yielding pure starting material as a result. Altering the amount of formaldehyde from 1 equivalent to 2 or 0.2 equivalents provided a similar outcome as entry **1**, generating products **A** and **B** in a 1 : 1 ratio (entries **4**, and **5**, respectively). Using a stronger acid to perhaps break cyclic derivative, **A**, and generate more of **B**, did not work, this in fact generated a greater amount of **A** (entry **6**). Interestingly, by switching solvents to THF, the formation of **A** with respect to **B** was

diminished, providing a 1 : 5.5 ratio (entry 7). It was believed that in the presence of water and acid, the cyclic derivative, A, would rapidly break open to generate more of B (which could subsequently produce C); although this was true (entries 8 – 11), water also suppressed the formation of these products. Attempting to drive this reaction forward by increasing the loading of diphenyl phosphate was unsuccessful, and this instead generated more of A (entry 12). Lastly, heating did generate more of B, however this also again diminished the overall conversion greatly, producing only traces of the mixed aminal (entry 13). Overall, the di-*tert*-butyl 2-amino-2-methylmalonate remained intact under a variety of conditions; this may be a result of the fact that the *tert*-butyl groups are very bulky, and any attack on the pi* of the malonate ester would have been sterically impeded. This lead us to begin experimenting with a fourth and final substrate: dibenzyl 2-amino-2-methylmalonate.

The last substrate tested was dibenzyl 2-amino-2-methylmalonate, which, again, demonstrated no reactivity at room temperature in THF under a variety of conditions (Table 3.4; acidic or basic, entries 1 – 3). When switching solvents, and increasing the temperature of the reaction to 80 °C, it was noticed, for the first time, that the desired azlactone was forming in yields as high as 22 % (entries 4 – 7). These results were reproducible, and the background, without CH_2O, shows no traces of this product (entry 8). The yield of the azlactone was increased by decreasing the reaction time (entry 9), indicating that the reaction reverts to the starting material over long periods of time.

Entry **10** demonstrates that this reaction does not work well with catalytic amounts of DPP, indicating that further optimization is required. Interestingly, the yield was boosted

Table 3.4 Attempts of desymmetrizing dibenzyl 2-amino-2-methylmalonate



Entry	CH_2O	Additive	Solvent	Temp	Time	% Yield (%)
1	1.1 eq.	10 mol % DPP	THF	rt	24 h	0
2	1 eq.	1 eq. Et_3N	THF	rt	24 h	0
3	1 eq.	0.2 eq. Et_3N	THF	rt	24 h	0
4	1 eq.	10 mol % DPP	Toluene	80 °C	24 h	3
5	1 eq.	20 mol % DPP	Toluene	80 °C	24 h	9
6	1 eq.	50 mol % DPP	Toluene	80 °C	24 h	22
7	1 eq.	1 eq. DPP	Toluene	80 °C	24 h	11
8	0 eq.	1 eq. DPP	Toluene	80 °C	24 h	0
9	1 eq.	1 eq. DPP	Toluene	80 °C	4 h	20
10	1 eq.	10 mol % DPP	Toluene	80 °C	4 h	1
11	1 eq.	1 eq. DPP	Toluene	80 °C	2 h	53
12	1 eq.	1 eq. DPP	Toluene	150 °C	2 h	0

DPP = Diphenyl phosphate. Conditions: see Table 3.1

to 53 % by stopping the reaction at 2 hours, further supporting the fact that the azlactone is formed early on in the reaction (entry **11**). Attempting to further increase this yield by increasing the heat to 150 °C did not work, and resulted in decomposition of the starting material instead (entry **12**). Overall, the azlactone was successfully produced, with the highest yield being 53 % when using 1 equivalent of CH_2O and DPP, at 80 °C in toluene. Further optimization is required to increase this yield, and decrease the loading of the Brønsted acid used; enantiopure Brønsted acids should then be tested to see if this reaction can be done in an enantiospecific manner.

3.3 Conclusion: Towards the Desymmetrization of Diesters using Formaldehyde

The desymmetrization of prochiral substrates to achieve enantiopure organic molecules with quaternary stereocentres remains an ongoing challenge in synthetic organic chemistry. We were interested using formaldehyde and Brønsted acids to desymmetrize alpha-hydroxy and alpha-amino diesters since this would generate oxalactones and azlactones, respectively, which are present in compounds with biological activity (see section 3.1). Employing chiral Brønsted acids would provide the opportunity to generate these lactones in an enantiospecific manner, which is valuable since these lactones are typically synthesized from molecules that are already enantiopure (alpha-hydroxy and alpha-amino acids), and this is not convenient. Moreover, the oxalactones and azlactones could potentially be hydrolyzed to generate enantiopure alpha-hydroxy and alpha-amino acids.

The study began by experimenting with the desymmetrization of two alpha-hydroxy diesters (di-*tert*-butyl 2-hydroxy-2-methylmalonate and dibenzyl 2-hydroxy-2-methylmalonate). After many trials, it was ultimately decided that the alpha-hydroxy diesters were not nucleophilic enough to perform the desired transformation. With this in mind, we moved towards the more nucleophilic alpha-amino diesters. Though di-*tert*-butyl 2-amino-2-methylmalonate did not display any reactivity (likely due to a very bulky reaction center), the less bulky dibenzyl 2-amino-2-methylmalonate substrate did

produce the desired azlactone (up to 53 % yield). Further optimization is required however since this modest yield was only generated with stoichiometric amounts of Brønsted acid (diphenyl phosphate). Moreover, experimentation with chiral Brønsted acids needs to be done as well to see if the azlactone can be synthesized in an enantiopure form.

Appendix I. Claims to Original Research

Claims to Original Research

1) Development of conditions for the hydroamination of allylic amines, and hydroxylamines in water, at low concentrations using formaldehyde as a catalyst, including comparative studies with various prebiotically relevant carbohydrates.

2) Development of conditions for the enantioselective hydroamination of allylic amines, and hydroxylamines using D-glyceraldehyde as a templating agent / catalyst.

3) Development of conditions for the hydration of beta-amino nitriles using formaldehyde under prebiotically relevant conditions.

4) Development of conditions for the desymmetrization of alpha-amino diesters to produce azlactones.

Publications from This Work

On the Ability of Formaldehyde to Act as a Tethering Catalyst in Water Jamshidi, M. P.; MacDonald, M. J.; Beauchemin, A. M. *Orig. Life Evol. Biosph.* **2017**; doi: 10.1007/s11084-017-9538-1.

Presentations from This Work

M. P. Jamshidi, M. J. MacDonald, A. M. Beauchemin. *Accelerating Challenging Intermolecular Reactions in Water: A New Role For Fomaldehyde in Prebiotic Chemistry*. Québec-Ontario Mini-Symposium for Synthetic and Bioorganic Chemistry, Montréal, QC. November 6 – 8, 2015.

Appendix II. Experimental Section

General Information

All reactions were performed under an atmosphere of argon unless otherwise indicated. Reactions were purified by column chromatography with 40-63 μm silica gel. Thin layer chromatography (TLC) was performed on pre-coated (silica gel F_{254}) aluminum sheets. Visualization of the TLCs were done with either UV in the case of molecules with a pi system, elsewise, it was done with $KMnO_4$.
Infrarerd spectrum were recorded neat on Nicolet 6700 FT-IR by Thermo Scientific. ^1H NMR were recorded on Bruker Advance 400 MHz, or Bruker Advance 300 MHz at room temperature, and are reported in ppm using either deuterated water, deuterated chloroform, or deuterated dimethyl sulfuxide as solvents to suspend the samples. ^{13}C NMR were recorded at 100 MHz using the Brucker Advance 400 MHz instrument. High-resolution mass spectrometry was achieved with a Kratos Concept IIH mass spectrometer. High performance liquid chromatography (HPLC) was achieved with an Agilent 1200 series HPLC.
Hydroamination reactions in water were done in rigorously degassed, distilled water. Degassing was performed by freeze-pump-thaw cycles (3x).
Internal standards were added to the reaction upon completion, except for the case of the hydroamination reactions where they were added in the beginning.

Materials.

Solvents. Solvents used were dry (i.e. taken from solvent purification system directly before use), unless otherwise indicated.

Formaldehyde. Formaldehyde was purchased as a 37 wt. % stock solution in H_2O from Alfa Aeser, and used without any further purification. It is well known that formaldehyde is mostly present as a hydrate in the presence of water: $K_{eq} = 2 \times 10^3$.[83] Stock solutions were stable for weeks. It is also important to note that bottles of formalin form a white precipitate over time, which resulted in lower concentrations of formaldehyde in solution.
Deoxygenated water. The presence of traces of oxygen in water caused reproducibility issues, especially at long reaction times. While in all cases the reactions in the presence of formaldehyde led to faster formation of the product, removal of oxygen was necessary to unambiguously determine the catalytic activity of aldehydes. It should be noted that

[83] (a) Norkus, E.; Pauliukaitė, R.; Vaškelis, A.; Butkus, E.; Jusys, Z.; Krenevičienė, M. *J. Chem. Res.* **1998**, *6*, 320. (b) Schubert, W. M.; Brownawell, D. W. *J. Am. Chem. Soc.* **1982**, *104*, 3487.

hydroxylamines are somewhat sensitive to oxygen, and that oxidation of a N-Me substituent can lead to the formation of a formaldehyde precursor (N-CH_2-OH).

Other Aldehydes & Carbohydrates. All aldehydes and carbohydrates were used without further purification. Glycolaldehyde dimer, acetaldehyde, buteraldehyde and ribose were purchased from Sigma Aldrich. Erythrose was purchased as a 70 wt. % syrup in water from Alfa Aesar. Dihydroxy acetone dimer was purchased from Alfa Aesar. D-Glyceraldehyde was purchased as a 90 wt. % syrup from from Combi Blocks.

***N*-Methyl Hydroxylamine HCl.** Purchased from Sigma Aldrich and used without further purification. An excess of allylic amine (2.5 equiv.) was used in order to deprotonate the HCl salt, and form the active hydroxylamine in situ.

Allylic Amines. Allylamine was purchased from Sigma Aldrich and distilled immediately before use. *N*-Methyl allylamine was purchased from Combi Blocks and was also distilled prior to use. Note in the cases where distillation was not performed, reproducibility issues were observed.

Methylmalonic acid. Purchased from Combi Blocks and used without further purification.

Malonic acid. Purchased from Combi Blocks and used without further purification.

Benzyl alcohol. Purchased from Sigma Aldrich and used without further purification.

***tert*-Butyl alcohol.** Purchased from Sigma Aldrich and used without further purifaction.

Palladium on carbon. Purchased from Strem Chemicals as a 10 % mixture of palladium on carbon, and used without further purification.

Cesium carbonate. Purchased from Sigma Aldrich and used without further prufication.

Formaldehyde as a Tethering Catalyst in Water

General Procedure for Aldehyde Screen

N-Methyl hydroxylamine•HCl (0.201 g, 2.50 mmol), dimethyl sulfone (0.235 g, 2.50 mmol), and *N*-methyl allylamine (0.600 mL, 6.25 mmol) were added to a round-bottom flask, and taken up in 25.0 mL of deoxygenated water (forming a 0.1 M solution). The round-bottom flask was then continuously purged with argon so as to remove atmospheric oxygen. Five individual flasks were loaded with the 0.1 mmol of catalyst (0.05 mmol in the case of dimers), providing a 20 mol % catalyst loading. These flasks were then sealed with a rubber septum and purged with argon. 5 mL of the previously prepared solution of *N*-methyl allylamine, *N*-methyl hydroxylamine•HCl, and dimethyl sulfone was added to each of these sealed flasks, and the flasks were further sealed with parafilm. 0.5 mL aliquots were taken after 1 day, 3 days, 5 days, and 8 days by syringe, and were added to 100 μL of D_2O; the reaction vessels were purged with argon after each aliquot was taken. ^1H NMR analysis allowed the calculation of product yield by comparing with the internal standard (dimethyl sulfone).

General Procedure for Testing Concentration Effect

Three different aqueous solutions of *N*-methyl hydroxylamine HCl (1 equiv.), dimethyl sulfone (1 equiv.), and allylamine (2.5 equiv.) were prepared with concentrations of 0.1 M, 0.5 M and 1.0 M. The water used to make these solutions was rigorously degassed as indicated above. Six individual flasks were sealed with a rubber septum, and 3 of them were loaded with 20 mol. % of formaldehyde. The flasks were purged with argon, and 5 mL of the solutions prepared above were added to each flask, producing 3 reaction vessels with 3 different concentrations, along with their respective blanks (i.e. no catalyst). 0.5 mL aliquots were taken after 1 day, 3 days, and 8 days by syringe, and were added to 0.1 mL of D_2O; the reaction vessels were again purged with argon after each aliquot was taken. ^1H NMR analysis allowed the calculation of product yield by comparing with the internal standard (dimethyl sulfone).

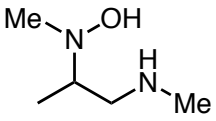
General Procedure for Testing the Effect of pH on the Reaction

Solutions with a 0.2 M concentration of phosphate buffers with pHs of 5, 7, and 10 were prepared by adding the appropriate amounts of monosodium phosphate and disodium phosphate. These solutions were then rigorously degassed as indicated above. Six flame-dried flasks charged with a stir bar were loaded with *N*-methyl hydroxylamine•HCl

(0.0417 g, 0.500 mmol), and dimethyl sulfone (0.0470 g, 0.500 mmol). These flasks were then sealed with a rubber septum and parafilm; while purging these flasks with argon, 5.00 mL of the previously prepared phosphate buffer solutions were added (two with pH 5, two with pH 7, and two with pH 10). Next, *N*-methylallylamine (0.120 mL, 1.25 mmol) was added to all 6 of the flasks, followed by the addition of formaldehyde (0.00745 mL, 0.100 mmol) to half of these flasks in such a manner that each pH has both a catalyzed and a background reaction. The reaction vessels were then further sealed with parafilm and allowed to stir for a period of 8 days. Aliquots (0.5 mL each) were taken after 1 day, 3 days, and 8 days by syringe, and were added to 0.1 mL of D_2O; the reaction vessels were again purged with argon after each aliquot was taken. ^{1}H NMR analysis allowed the calculation of product yield by comparing with the internal standard (dimethyl sulfone).

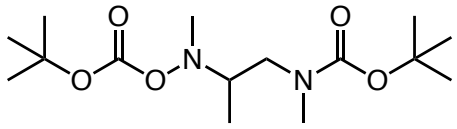
Isolation of Product

2-(Hydroxy(methyl)amino)-*N*-methylpropan-1-amine



N-Methylhydroxylamine•HCl (0.184 g, 2.20 mmol) was added to a round-bottom flask containing 22.0 mL of deoxygenated water (forming a 0.1 M solution). To this solution, *N*-methylallylamine (0.528 mL, 5.50 mmol) was added, followed by the addition of formaldehyde (0.0328 mL, 0.440 mmol). 2 mL of this solution was set aside for NMR analysis. The mixture was stirred at room temperature for 5 days, to afford the crude product 2-(hydroxy(methyl)amino)-*N*-methylpropan-1-amine in 69% yield (determined by NMR, using dimethyl sulfone as an internal standard). Due to the product's solubility in water and high polarity, the product needed to be derivatized prior to isolation, however, a crude ^{1}H NMR spectrum was recorded. ^{1}H-NMR (10% D_2O, H_2O): δ 2.60-2.96 (m, 3H), 2.55 (s, 3H), 2.53 (br s, 1H), 2.37 (s, 3H), 2.22 (br s, 1H), 0.99 (s, 3H).

tert-Butyl (2-(((*tert*-butoxycarbonyl)oxy)(methyl)amino)propyl)(methyl)carbamate



Isolated for characterization as follows: 20 mL of the unpurified reaction mixture of 2-(hydroxy(methyl)amino)-*N*-methylpropan-1-amine was isolated by forming an azeotrope with *tert*-butanol (95 wt. %) and concentrating under reduced pressure. The resulting white solid was dissolved in 2.5 mL of acetonitrile. To this solution, 4-dimethylaminopyridine (0.277 g, 3.78 mmol) was added, followed by di-*tert*-butyl dicarbonate (0.824 g, 3.78 mmol). The mixture was stirred at room temperature for 24 hours. The reaction mixture was then diluted with 3 mL of ethyl acetate, and extracted with 3 mL of water (3x). The organic phase was then washed twice with 2 mL of 1 M HCl. The organic layer was then washed with 3 mL of brine (4x), dried over sodium sulfate, filtered, and concentrated under

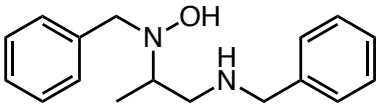
reduced pressure. The product was purified by column chromatography (1 % NH_4OH/1 % MeOH/CH_2Cl_2; TLC R_f = 0.20) to provide 0.236 g of pure *tert*-butyl (2-(((*tert*-butoxycarbonyl)oxy)(methyl)amino)propyl)(methyl)carbamate (34 % overall isolated yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.27- 3.13 (m, 3H), 2.82 (s, 3H), 2.70 (s, 3H), 1.44 (s, 9H), 1.42 (s, 9H), 1.00 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.5 (C), 152.9 (C), 82.5 (C), 79.0 (C), 61.4 (CH), 51.3 (CH_2), 41.5 (CH_3), 35.4 (CH_3), 28.6 (CH_3), 28.0 (CH_3), 12.6 (CH_3); IR (neat) cm^{-1} 1759, 1699, 1391, 1366, 1265, 1252, 1155, 706, 704; HRMS (ESI): Exact mass calcd for $C_{15}H_{30}N_2O_5Na$ [M+Na]$^+$: 341.2100. Found: 341.2052.

D-Glyceraldehyde in Producing Enantiomeric Excess

General Procedure (A) for Preparation of Racemic Hydroamination Products

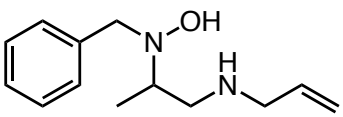
Hydroxylamine (1.00 mmol) was added to a flame dried vial charged with a stir bar. To this, benzene (1.00 mL) was added followed by allylamine (1.50 mmol). The flask was sealed and purged with argon and then formaldehyde in the form of a 0.37% aqueous solution (0.0184 mL, 0.200 mmol) was added. The reaction was stirred at room temperature for 24 hours, and then the solvent was removed in *vacuo*. The product was purified by column chromatography.

(*R & S*) - *N*-Benzyl-2-(benzyl(hydroxy)amino)propan-1-amine



Prepared according to general procedure (**A**) using *N*-benzylhydroxylamine (0.123 g, 1.00 mmol), and *N*-benzylallylamine (0.146 mL, 1.50 mmol). The product was purified by column chromatography [1: 5: 94, Et_3N, MeOH, CH_2Cl_2 (Rf = 0.1)] to obtain 0.224 g (83%) of the desired product as a pale oil. Spectral data were consistent with literature.[84]

(*R & S*)-*N*-(2-(Benzyl(hydroxy)amino)propyl)prop-2-en-1-amine



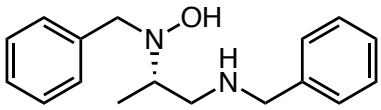
Prepared according to general procedure (**A**) using *N*-benzylhydroxylamine (0.123 g, 1.00 mmol), and diallylamine (0.185 mL, 1.50 mmol). The product was purified by column chromatography [1: 5: 94, Et_3N, MeOH, CH_2Cl_2 (Rf = 0.1)] to obtain 0.181 g (82%) of the desired product as a pale yellow oil. Spectral data were consistent with literature.[84]

General Procedure (B) for Optimization of D-glyceraldehyde Tethered Reactions

[84] MacDonald, M. J.; Hesp, C. R.; Schipper, D. J.; Pesant, M.; Beauchemin, A. M. *Chem. Eur. J.* **2013**, *19*, 2597.

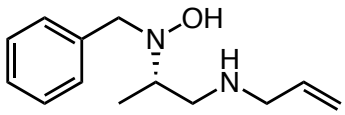
A flame dried vial charged with a stir bar was loaded with D-glyceraldehyde (20 mol%), solvent (2.00 mL), and hydroxylamine (1 equiv.). Next, allylamine (1.5 equiv.) was added and the vial was sealed and purged with argon. The reaction was allowed to stir overnight (24 hours) and then the solvent was removed in *vacuo*. The product was then purified by column chromatography.

(*S*)-*N*-Benzyl-2-(benzyl(hydroxy)amino)propan-1-amine



Prepared according to general procedure (**B**) using *N*-benzylhydroxylamine (0.246 g, 2.00 mmol), and *N*-benzyl-*N*-allylamine (0.442 g, 3.00 mmol). Purified by column chromatography [1: 5: 94, Et_3N, MeOH, CH_2Cl_2 (Rf = 0.1)] to produce 0.054 g (10 %) of the desired product as a white solid. Spectral data were consistent with literature.[84]

(*S*)-*N*-(2-(Benzyl(hydroxy)amino)propyl)prop-2-en-1-amine

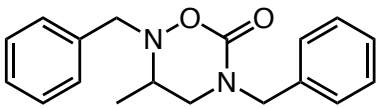


Prepared according to the general procedure (**B**) using *N*-benzylhydroxylamine (0.246 g, 2.00 mmol), and diallylamine (0.370 mL, 3.00 mmol) . Purified by column chromatography [1: 5: 94, Et_3N, MeOH, CH_2Cl_2 (Rf = 0.1)] to obtain 0.019 g (4 %) of the desired product. Spectral data were consistent with literature.[84]

General Procedure (C) for Determining Enantiomeric Excess of Hydroamination Products

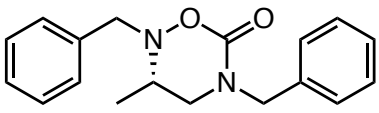
The hydroamination products (1 eqiuv.) above were dissolved in CH_2Cl_2 (0.5 M) in a flame dried vial loaded with a stir bar, and to this solution 1,1'-carbonyldiimidazole [CDI (1.5 equiv.)] was added. The vial was sealed and purged with argon and allowed to stir at room temperature for 3 hours. The solvent was then removed in *vacuo,* and the crude product was purified with column chromatography.

(*R* & *S*) - 2,5-Dibenzyl-3-methyl-1,2,5-oxadiazinan-6-one



Prepared according to general procedure (**C**), purified by column chromatography [1: 20, Et_2O: CH_2Cl_2 (Rf = 0.70)] to obtain 0.027 g (11 %) of the desired product. Spectral data were consistent with literature.[84] Chiral HPLC: ChiralPak AD-H, *i*-PrOH: hexane = 10: 90, 1.0 mL/min, 210 nm, t_{major} = 36.6 min, t_{minor} = 28.3 min.

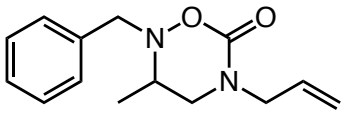
(*S*)-2,5-Dibenzyl-3-methyl-1,2,5-oxadiazinan-6-one



Prepared according to general procedure (**C**), purified by column chromatography [1: 20, Et_2O: CH_2Cl_2 (Rf = 0.70)] to obtain 0.045 g (32 %) of the desired product. Spectral data

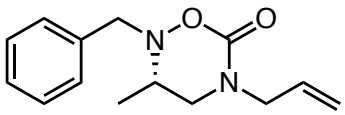
were consistent with literature.[84] Chiral HPLC: ChiralPak AD-H, *i*-PrOH: hexane = 10: 90, 1.0 mL/min, 210 nm, t_{major} = 36.6 min, t_{minor} = 28.3 min.

(*R* & *S*)-5-Allyl-2-benzyl-3-methyl-1,2,5-oxadiazinan-6-one



Prepared according to general procedure (**C**) using the hydroamination product (0.109 g, 0.500 mmol), and CDI (0.120 g, 0.750 mmol). Purified by column chromatography [1: 1, ethyl acetate: petroleum ether (Rf = 0.32)] to obtain 0.0663 g (54%) of the desired product. Spectral data were consistent with literature.[84] Chiral HPLC: ChiralPak AD-H, *i*-PrOH: hexane = 10: 90, 1.0 mL/min, 210 nm, t_{major} = 17.5 min, t_{minor} = 14.2 min.

(*S*)-5-Allyl-2-benzyl-3-methyl-1,2,5-oxadiazinan-6-one



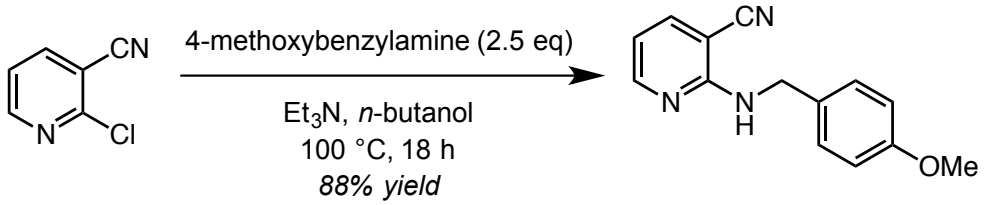
Prepared according to general procedure (**C**), purified by column chromatography [1: 1, ethyl acetate: petroleum ether (Rf = 0.32)] to obtain 0.010 g (20 %) of the desired product as a pale yellow oil. Spectral data were consistent with literature.[84] Chiral HPLC: ChiralPak AD-H, *i*-PrOH: hexane = 10: 90, 1.0 mL/min, 210 nm, t_{major} = 17.5 min, t_{minor} = 14.2 min.

Formaldehyde on the Hydration of Beta-Amino Nitriles

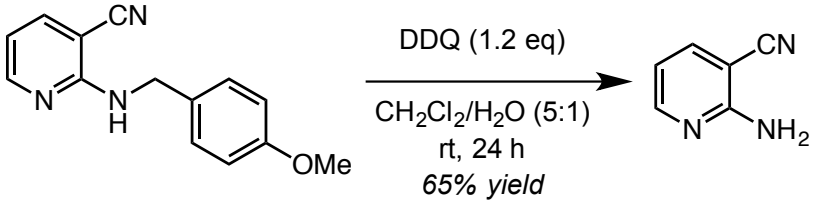
General procedure (D) for the Hydration of Aminonitriles

Aminonitrile (1 equiv) was added to a flame dried flask charged with a stir bar, followed by the addition of water (1.0 M). Next, NaOH was added as a 5 M solution in water (0.20 equiv. – 1.0 equiv.), followed by formaldehyde (0.20 equiv. – 1.0 equiv.). The reaction was stirred for a period of time under an atmosphere of argon, and then the water was removed by forming an azeotrope with *t*-BuOH (94 wt. %), and evaporating the mixture under reduced pressure. The products were not isolable (complex mixtures with similar Rf values), and therefore, yields of the aminoamides were determined by ^{1}H NMR (comparing with 1,3,5-trimethoxybenzne as an internal standard).

Preparation of Substrates



2-((4-Methoxybenzyl)amino)nicotinonitrile: A flame dried round-bottom flask charged with a stir bar was loaded with 2-chloronicotinonitrile (8.31 g, 60.0 mmol), and *n*-butanol (600 mL). To this solution, 4-methoxybenzylamine (19.8 mL, 150 mmol) was added slowly, followed by triethylamine (20.9 mL, 150 mmol). The solution was purged with argon and allowed to stir overnight (18 hours) at 100 °C. Once complete, the solution was cooled to room temperature and the *n*-butanol, and triethylamine were removed in *vacuo*. The product was recrystallized by dissolving the crude white solid in a minimal amount of boiling methanol. A minimal amount of hexanes were also added to this solution and, and the flask was then placed in the fridge overnight for recrystallization. The white crystals were then rinsed with a cold solution of 5% methanol / hexanes, and placed under vacuum to dry to afford 11.9 g (88%) of the desired product. Melting point: 121.5 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.29 (dd, *J* = 1.9, 5.0 Hz, 1H), 7.64 (dd, *J* = 1.9, 7.6 Hz, 1H), 7.26 (m, 2H), 6.86 (m, 2H), 6.60 (dd, *J* = 5.0, 7.6 Hz, 1H), 5.39 (br s, 2H), 4.62 (d, J = 5.5 Hz, 2H), 3.78 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 159.1, 158.1, 141.4, 130.3, 129.2, 116.7, 114.2, 112.2, 91.6, 55.3, 44.9; IR (neat) cm^{-1} 2218, 1592, 1507, 1461, 1423, 1409, 1361, 1339, 1300, 1244, 1171, 1102, 1053, 1028, 978; HRMS (EI): Exact mass calcd for $C_{14}H_{13}N_3O$ $[M]^+$: 239.1059. Found: 239.1069.



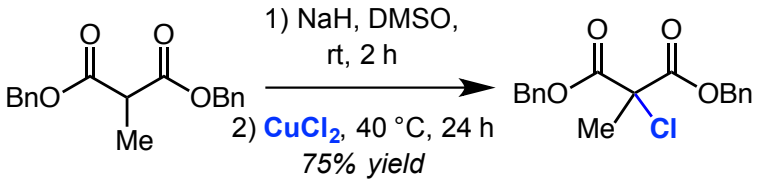
2-Aminonicotinonitrile: 2-((4-Methoxybenzyl)amino)nicotinonitrile (0.500 g, 0.222 mmol) was added to a round-bottom flask and purged with argon. CH_2Cl_2 (12.5 mL) and distilled water (2.50 mL) were added to the reaction flask, followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.605 g, 2.66 mmol). The reaction was stirred overnight (24 h) at room temperature and then quenched with saturated aqueous sodium bicarbonate (10 mL). The mixture was extracted with CH_2Cl_2 (15 mL x 3), and brine (10 mL), and the combined organic layers were dried with Na_2SO_4. The solvent was then removed in *vacuo* and the crude solid was purified by column chromatography [1: 3, ethyl acetate: hexane (dry loaded, Rf = 0.50 in 1: 1 ethyl acetate: hexanes)], to afford 0.152 g of 2-aminonicotinonitrile as a beige powder. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.23 (dd, 1H, *J* = 1.9, 5.0 Hz), 7.68 (dd, 1H, *J* = 1.9, 7.7 Hz), 6.69 (dd, 1H, *J* = 5.0, 7.7 Hz), 5.23 (br s, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 159.0, 152.5, 141.7, 116.3, 113.5, 91.7; IR (neat) cm^{-1} 3418, 3319, 2214, 1636, 1589, 1560, 1515, 1486, 1453, 1345, 1246, 1188, 1130. Low res mass calcd for $C_6H_5N_3$ $[M]^+$: 119.11. Found: 119.00.

Towards the Desymmetrization of Alpha-amino, and Alpha-hydroxy Diesters

General Procedure (E) for Optimization of Desymmetrization Conditions

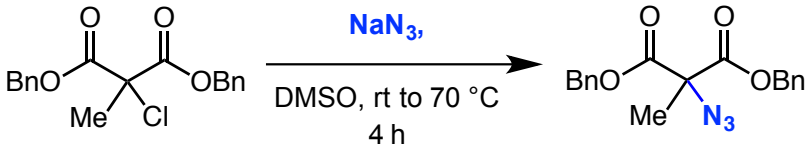
Amino/hydroxy esters were added to a flame dried flask charged with a stir bar, followed by the addition of dry solvent (1.0 M), diphenyl phosphate (0.20 equiv. – 1.0 equiv.), and formaldehyde (0.20 equiv. – 1.0 equiv.). The reaction mixture was stirred for a period of time and then 1,3,5-trimethoxybenzne (1.0 equiv.) was added as an internal standard for analysis by NMR.

Preparation of Substrates

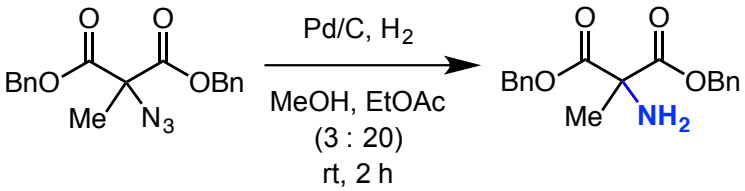


Dibenzyl 2-chloro-2-methylmalonate (synthesized according to modified literature procedure):[85] A round-bottom flask was flame dried and cooled under continuous purging of argon. To this flask, sodium hydride (2.06 g, 29.3 mmol) and DMSO (265 mL) were added and the solution was stirred under argon flow. To this stirred solution, dibenzyl 2-methylmalonate (8.04 g, 26.6 mmol) was added dropwise over a period of 30 minutes while purging with argon. Once the addition of dibenzyl 2-methylmalonate was complete, the flask was sealed, and allowed to stir at room temperature for 2 hours so as to complete deprotonation of the alpha proton. Then, anhydrous copper (II) chloride (10.7 g, 79.8 mmol) was added and the flask was purged again with argon and sealed. The reaction was then stirred at 40 °C for 24 hours. Once the reaction was complete it was cooled to 0 °C and quenched with saturated aqueous ammonium chloride (100 mL) and extracted with ethyl acetate (200 mL x 3), brine (100 mL), and finally water (100 mL). The combined organic layers were then dried with Na_2SO_4, filtered and the the solvent was removed in *vacuo*. The crude oil was purified *via* column chromatography [1: 20 ethyl acetate: hexane, Rf = 0.42 (1: 10, ethyl acetate: hexanes)] to afford 6.61 g (75%) of dibenzyl 2-chloro-2-methylmalonate. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.23-7.31 (m, 10H), 5.17 (s, 4H), 1.93 (s, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 167.0, 134.7, 128.6, 128.5, 128.1, 68.5, 66.1, 25.7. IR (neat) cm^{-1} 3034, 1743, 1498, 1455, 1380, 1262, 1215, 1114, 1070, 946, 905. Exact mass calcd for $C_{18}H_{17}ClO_4Na$ $[M+Na]^+$: 355.0708. Found: 355.1275.

[85] Shi, X.; Dai, L. *J. Org. Chem.* **1993**, *58*, 4596.

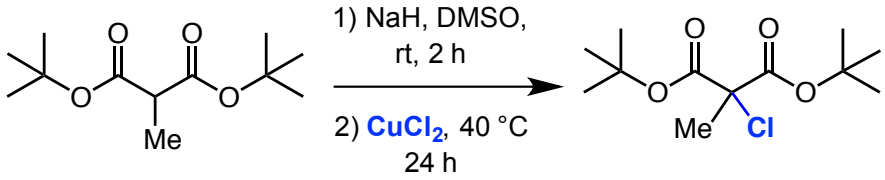


Dibenzyl 2-azido-2-methylmalonate (synthesized according to modified literature procedure):[86] To a stirred solution of sodium azide (3.10 g, 47.7 mmol) in DMSO (200 mL), dibenzyl 2-chloro-2-methylamalonate (6.61 g, 19.9 mmol) was added dropwise. The mixture was then sealed under an atmosphere of argon and allowed to stir at room temperature for 2 hours. The reaction mixture was then heated at 70 °C for 4 hours and cooled to room temperature. Once cooled, the mixture was extracted with diethyl ether (200 mL x 3), and brine (100 mL x 2). The combined organic layers were then dried with Na_2SO_4, filtered and the the solvent was removed in *vacuo*. Since this product was close in Rf to the starting material, it was used without further purification.

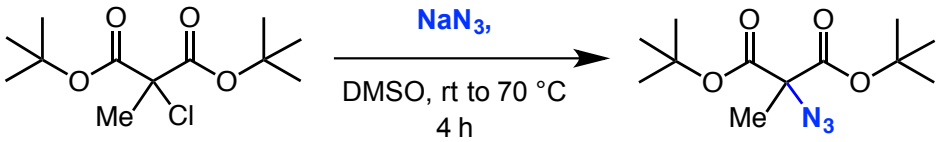


Dibenzyl 2-amino-2-methymalonate (synthesized according to modified literature procedure):[87] A round-bottom flask was flame dried and cooled under a continuous flow of argon. The flask was then charged with a magnetic stir bar and then loaded with 25% palladium on activated carbon (25 weight %, 1.31 g). The flask was then sealed and evacuated with a high vacuum pump and re-filled with argon four times so as to remove any oxygen from the flask. Once all of the oxygen had been removed, a 15% (v/v) solution of methanol in ethyl acetate (20 mL) was added via syringe to the reaction flask in order to soak the palladium on carbon. Once the palladium on carbon had been soaked, the remainder of the methanol / ethyl acetate (280 mL) solution was added slowly to the reaction flask. Then, while stirring the reaction mixture, dibenzyl 2-azido-2-methylmalonate (5.23 g, 15.4 mmol) was added dropwise, and the reaction was allowed to stir at room temperature for 2 hours. The reaction was then filtered through celite and the solvent was removed in *vacuo*. The crude oil was then purified by column chromatography (3: 10, ethyl acetate: hexane, Rf = 0.15) to afford 0.345 g (7%) of the desired product as a light yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.22-7.31 (m, 10H), 5.12 (s, 4H), 2.03 (br s, 2H), 1.57 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.8, 135.2, 128.6, 128.4, 128.1, 67.5, 62.6, 22.9; IR (neat) cm^{-1} 3033, 1731, 1587, 1498, 1454, 1375, 1329, 1175, 1105, 1050, 1029, 952, 906; Exact mass calcd for $C_{18}H_{20}NO_4$ $[M+H]^+$: 314.1387. Found: 314.1392.

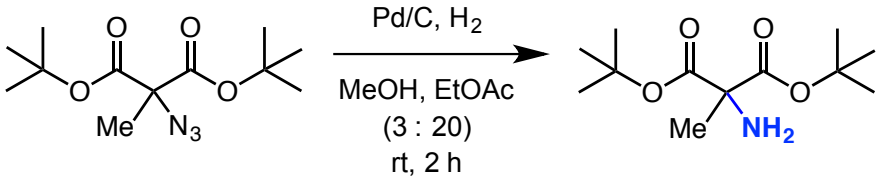
[86] Shibatomi, K.; Soga, Y.; Narayama, A.; Fujisawa, I.; Iwasa, S. *J. Am. Chem. Soc.* **2012**, *134*, 9836.
[87] Karmakar, R.; Suneja, A.; Singh, V. K. *Org. Lett.* **2016**, *18*, 2636.



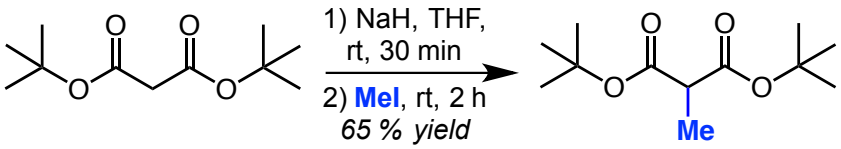
Di-*tert*-butyl 2-chloro-2-methylmalonate (synthesized according to a modified procedure):[85] A round-bottom flask was flame dried and cooled under continuous purging of argon. To this flask, sodium hydride (0.408 g, 10.2 mmol) and DMSO (100 mL) were added and the solution was stirred under argon flow. To this stirred solution, di-*tert*-butyl 2-methylmalonate (2.35 g, 10.2 mmol) was added dropwise over a period of 30 minutes while purging with argon. Once the addition of di-*tert*-butyl 2-methylmalonate was complete, the flask was sealed, and allowed to stir at room temperature for 2 hours so as to complete deprotonation of the alpha proton. Then, anhydrous copper (II) chloride (3.44 g, 25.5 mmol) was added and the flask was purged again with argon and sealed. The reaction was then stirred at 40 °C for 24 hours. Once the reaction was complete it was cooled to 0 °C and quenched with saturated aqueous ammonium chloride (30 mL) and extracted with ethyl acetate (50 mL x 3), brine (30 mL), and finally water (30 mL). The combined organic layers were then dried with Na_2SO_4, filtered and the the solvent was removed in *vacuo*. The product was not separable from the starting material (similar polarities), and was thus used without further purification as a crude oil.



Di-*tert*-butyl 2-azido-2-methylmalonate (synthesized according to modified literature procedure):[86] To a stirred solution of sodium azide (0.773 g, 11.9 mmol) in DMSO (70 mL), di-*tert*-butyl 2-chloro-2-methylmalonate [1.31 g,~4.95 mmol (crude solution of 2-chloro-2-methylmalonate)] was added dropwise. The mixture was then sealed under an atmosphere of argon and allowed to stir at room temperature for 2 hours. The reaction mixture was then heated at 70 °C for 4 hours and cooled to room temperature. Once cooled, the mixture was extracted with diethyl ether (40 mL x 3), and brine (30 mL x 2). The combined organic layers were then dried with Na_2SO_4, filtered and the the solvent was removed in *vacuo*. Since this product was close in Rf to the starting material, it was used without further purification as a crude oil.



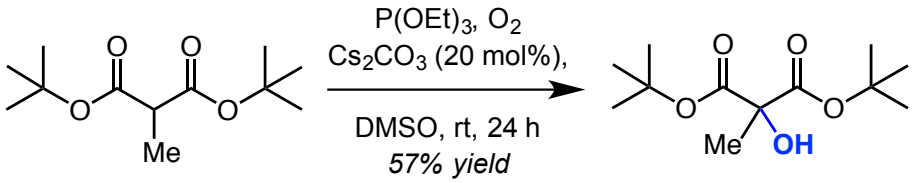
Di-*tert*-butyl 2-amino-2-methymalonate (synthesized according to modified literature procedure):[87] A round-bottom flask was flame dried and cooled under a continuous flow of argon. The flask was then charged with a magnetic stir bar and then loaded with 25% palladium on activated carbon (25 weight %, 0.128 g). The flask was then sealed and evacuated with a high vacuum pump and re-filled with argon four times so as to remove any oxygen from the flask. Once all of the oxygen had been removed, a 15% (v/v) solution of methanol in ethyl acetate (20 mL) was added via syringe to the reaction flask in order to soak the palladium on carbon. Once the palladium on carbon had been soaked, the remainder of the methanol / ethyl acetate (30 mL) solution was added slowly to the reaction flask. The flask was then put under vacuum, and re-filled with hydrogen (3x), and then the solution was bubbled with hydrogen for 5 minutes. Then, while stirring the reaction under an atmosphere of hydrogen gas, di-*tert*-butyl 2-azido-2-methylmalonate [0.513 g, ~1.76 mmol (crude substrate used)] was added dropwise, and the reaction was allowed to stir at room temperature for 2 hours. The reaction was then filtered through celite and the solvent was removed in *vacuo*. The crude oil was then purified by column chromatography (1: 20, methanol: CH_2Cl_2, Rf = 0.15) to afford 0.254 g (6% overall yield from di-*tert*-butyl 2-methylmalonate) of the desired product as a white solid. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.87 (br s, 2H), 1.45 (s, 18H), 1.42 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.5, 81.8, 63.2, 27.8, 22.6; IR (neat) cm^{-1} 3392, 2978, 2936, 1728, 1591, 1478, 1455, 1393, 1368, 1337, 1250, 1154, 1111, 977, 844; Exact mass calcd for $C_{12}H_{24}NO_4$ $[M+H]^+$: 246.1700. Found: Not found.[88]



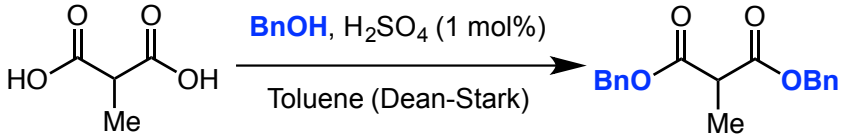
Di-*tert*-butyl 2-methylmalonate (synthesized according to modified literature procedure):[89] Di-*tert*-butyl malonate (3.50 g, 16.2 mmol) was added dropwise to a stirred solution of NaH (0.740 g, 16.2 mmol) in THF (19.0 mL). The solution was stirred for 30 minutes at room temperature. Next, methyl iodide (1.00 mL, 16.2 mmol), was added dropwise to this solution and the mixture was further stirred at room temperature for 2 hours. The solution was then quenched with NH_4Cl at 0 °C, and extracted 3x with ethyl acetate (20.0 mL). The combined organic layers were then washed with brine and then

[88] After many attempts of ESI and EI, no suitable fragmentation patterns were found, however, we feel confident, based on other characterization data (^{1}H NMR, ^{13}C NMR, IR) that we have the correct product.
[89] Qabaja, G.; Wilent, J. E.; Benavides, A. R.; Bullard, G. E.; Petersen, K. S. *Org. Lett.* **2013**, *15*, 1266.

dried with Na_2SO_4 and the solvent was removed in *vacuo*. The crude oil mixture was then purified by column chromatography with a 1 : 9 → 3 : 7 mixture of ethyl acetate : hexanes (Rf = 0.5 with 3:7 ethyl acetate : hexanes) to afford 2.42 g (65 %) of a clear oil. Spectral data were consistent with the literature.[87]

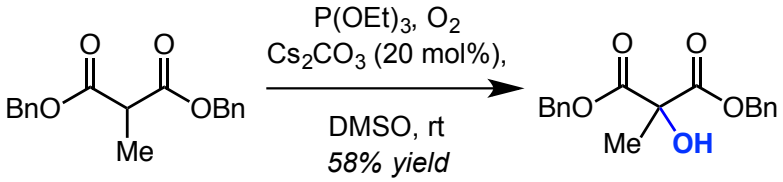


Di-*tert*-butyl 2-hydroxy-2-methylmalonate (synthesized according to modified literature procedure):[90] Di-*tert*-butyl 2-methylmalonate (8.50 g, 36.9 mmol), triethylphosphite (12.7 mL, 73.8 mmol), and catalytic cesium carbonate (2.40 g, 7.38 mmol) were added to DMSO (150 mL). The reaction flask was then put under vacuum, re-filled with oxygen (3x), and stirred overnight at room temperature. The solution was then diluted with ethyl acetate, and then washed with brine, and extracted with ethyl acetate (3x). The combined organic layers were then washed with Na_2SO_4 and then the solvent was removed in *vacuo*. The crude oil was then purified by column chromatography (1: 20 ethyl acetate: hexane, Rf = 0.15) to produce 5.19 g (57%) of the desired product. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.68 (br s, 1H), 1.52 (s, 3H), 1.47 (s, 18H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 170.3, 82.7, 27.8, 21.3; IR (neat) cm^{-1} 3475, 2979, 2939, 1723, 1458, 1369, 1287, 1254, 1152, 1109, 1041, 961, 917, 842; Exact mass calcd for $C_{12}H_{22}O_5Na$ [M+Na]$^+$: 269.1365. Found: 269.1365.



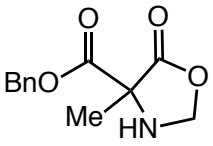
Dibenzyl 2-methylmalonate (synthesized according to literature procedure):[91] Methylmalonic acid (15.0 g, 127 mmol), and benzyl alcohol (31.0 mL, 299 mmol) were added to toluene (46 mL) and stirred. Concentrated sulfuric acid (1 mol%) was then slowly added to this solution. A Dean-Stark trap was used and the mixture was allowed to stir at reflux for 2 hours. The solvent was then removed in *vacuo*, and the crude product was used without further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.26-7.33 (m, 10H), 5.14 (s, 4H), 3.53 (q, *J* = 7.3 Hz, 1H), 1.57 (d, *J* = 7.3 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 169.8, 135.4, 128.6, 128.5, 128.4, 128.3, 128.1, 67.1, 46.2, 13.6; IR (neat) cm^{-1} 2033, 2944, 1728, 1498, 1454, 1381, 1324, 1210, 1147, 1076, 1027, 956, 904, 733, 694; Exact mass calcd for $C_{18}H_{18}O_4Na$ [M+Na]$^+$: 321.1103. Found: 321.1127.

[90] Liang, Y.-F.; Jiao, N. *Angew. Chem. Int. Ed.* **2014**, *53*, 548.
[91] Wolfe, S.; Ro, S.; Kim, C.-K.; Shi, Z. *Can. J. Chem.* **2001**, *79*, 1238.



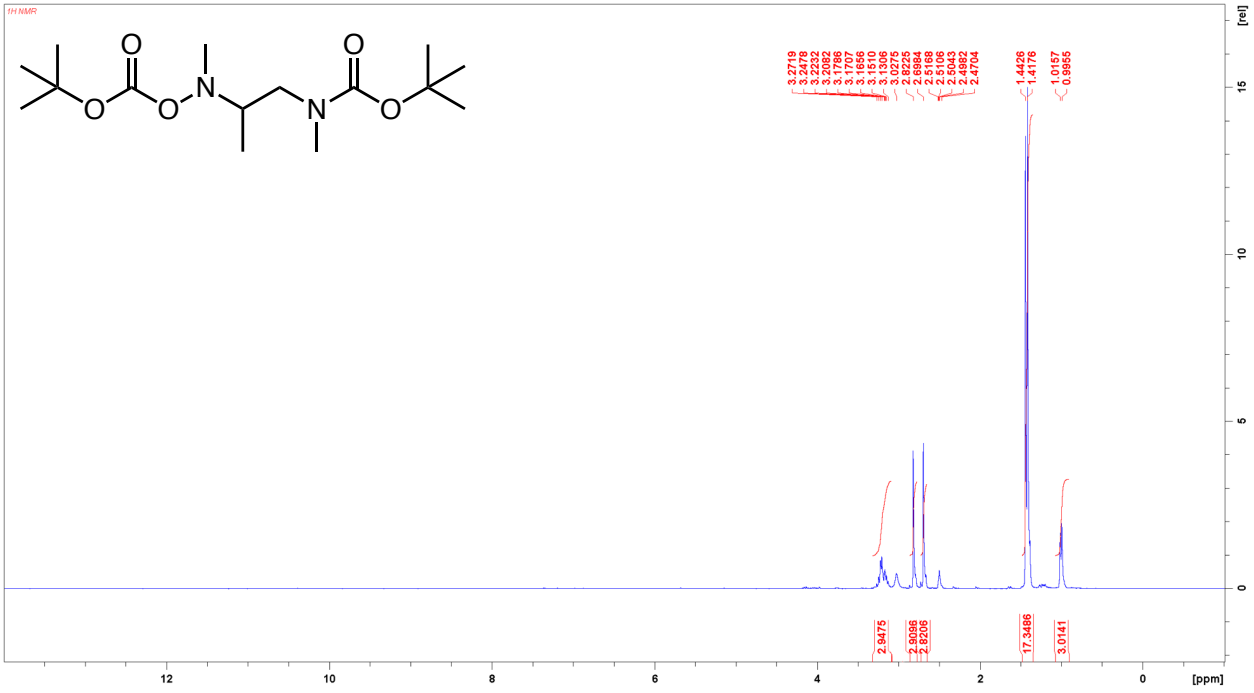
Dibenzyl 2-hydroxy-2-methylmalonate (prepared according to the literature procedure):[90] Dibenzyl 2-methylmalonate (5.40 g, 18.1 mmol), triethylphosphite (6.20 mL, 36.2 mmol), and catalytic cesium carbonate (1.18 g, 3.62 mmol) were added to DMSO (72.0 mL). The reaction flask was then put under vacuum, re-filled with oxygen (3x), and stirred overnight at room temperature. The solution was then diluted with ethyl acetate, and then washed with brine, and extracted with ethylacetate (3x). The combined organic layers were then washed with Na_2SO_4 and then the solvent was removed in *vacuo*. The crude oil was then purified by column chromatography (1:20 ethyl acetate: hexane, Rf = 0.15) to produce 3.33 g (58 %) of the desired product. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.24-7.34 (m, 10H), 5.17 (s, 4H), 3.79 (s, 1H), 1.67 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 170.6, 134.8, 129.1, 128.6, 128.5, 128.4, 128.3, 128.1, 76.3, 68.0, 21.6; IR (neat) cm^{-1} 2482, 3034, 1732, 1498, 1455, 1379, 1266, 1217, 1154, 1105, 964, 905; Exact mass calcd for $C_{18}H_{18}O_5Na$ $[M+Na]^+$: 337.1052. Found: 337.1075.

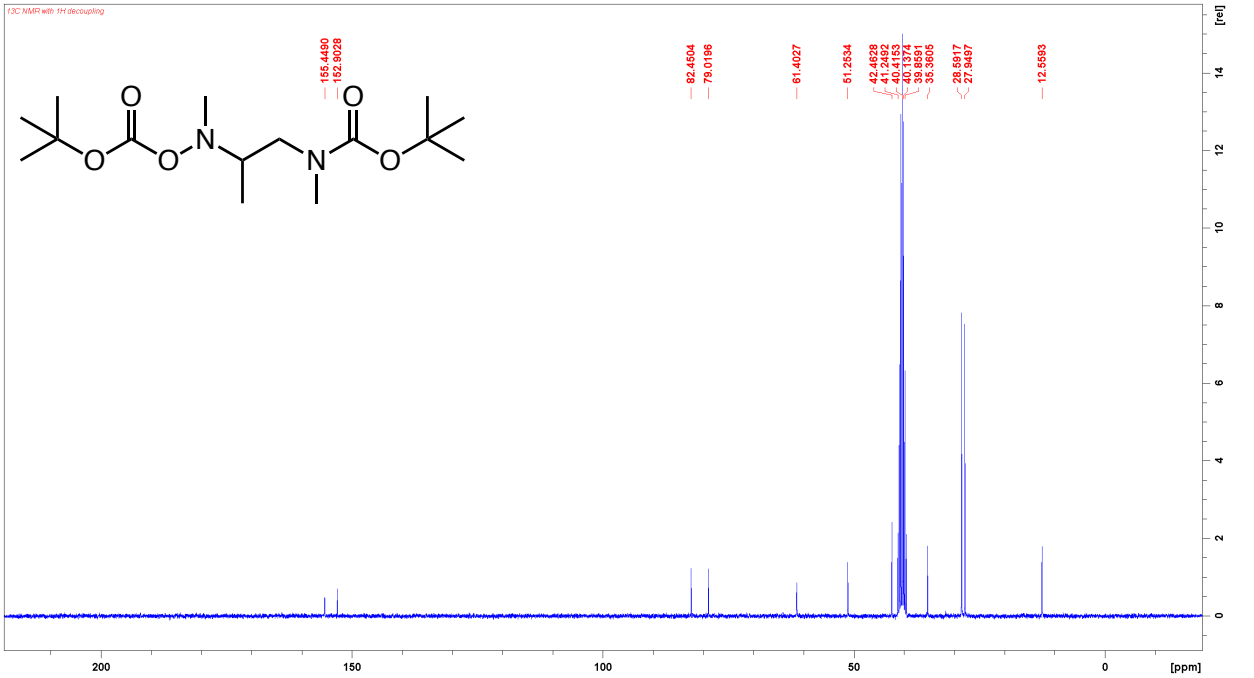
Benzyl 4-methyl-5-oxooxazolidine-4-carboxylate di-benzyl 2-amino-2-methylmalonate

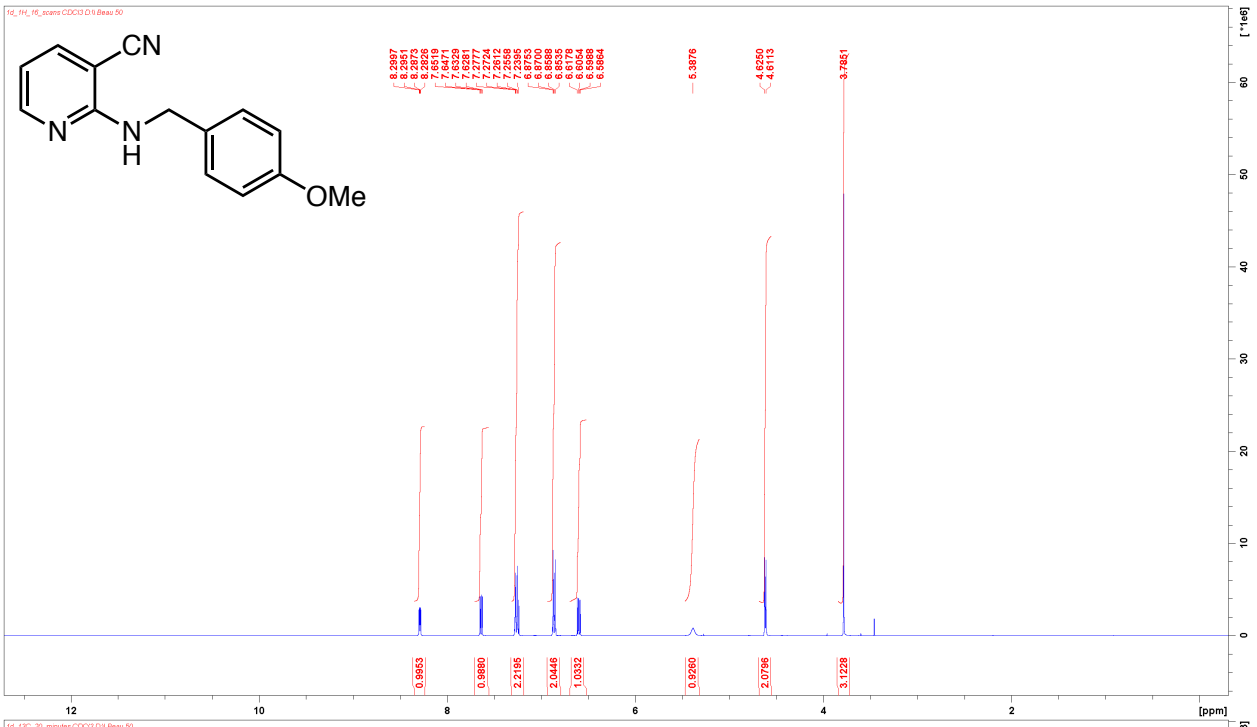


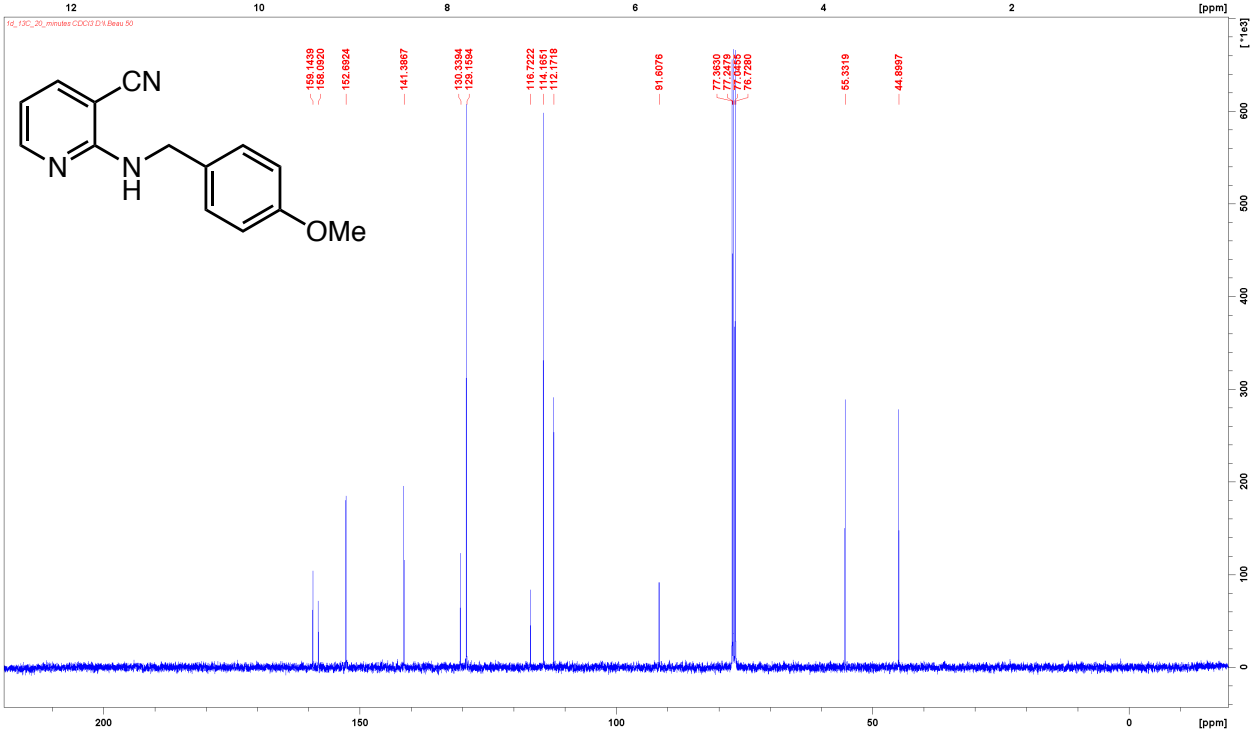
(0.125 g, 0.400 mmol) was added to a flame dried flask charged with a stir bar, followed by the addition of dry toluene (5.00 mL), diphenyl phosphate (0.100 g, 0.400 mmol), and formaldehyde (0.0298 mL, 0.400 mmol). The reaction mixture was stirred for 24 hours at 80 °C and the solvent was removed in *vacuo*. Attempts of purifying this crude solid with 1: 20 MeOH: CH_2Cl_2, or with 1: 20 EtOAc: Hexane all failed, however a crude ^{1}H NMR was obtained. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.25-7.37 (m, 5H), 5.05 (d, *J* = 12.0 Hz, 1H), 5.00 (s, 1H), 4.74 (d, *J* = 12.0 Hz, 1H), 4.30 (s, 1H), 1.75 (s, 3H).

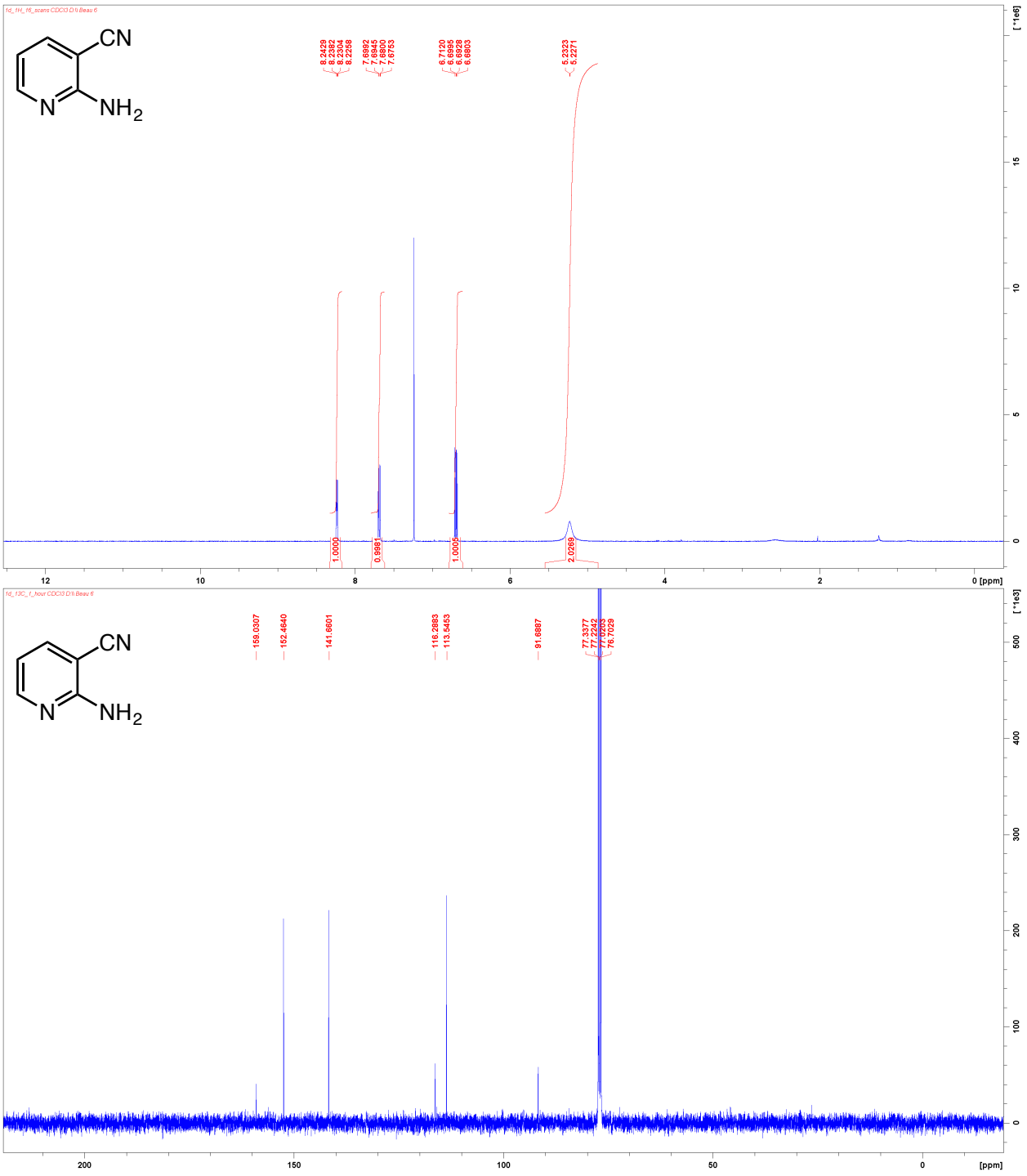
Spectra

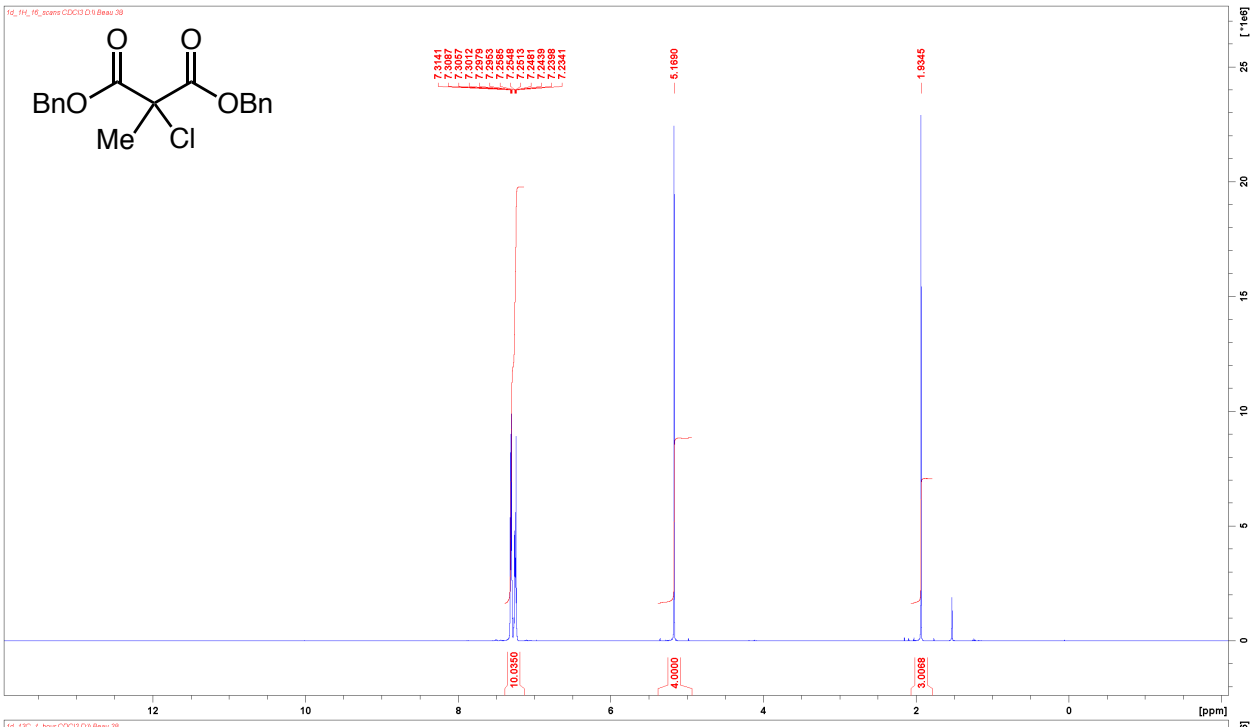

1H NMR
3.2719
3.2478
3.2232
3.2002
3.1786
3.1707
3.1656
3.1510
3.1396
3.1275
2.8225
2.6984
2.5168
2.5106
2.5043
2.4982
2.4704
1.4426
1.4176
1.0157
0.9955
2.9475
2.8086
2.7856
17.3486
3.0141
[rel]
[ppm]

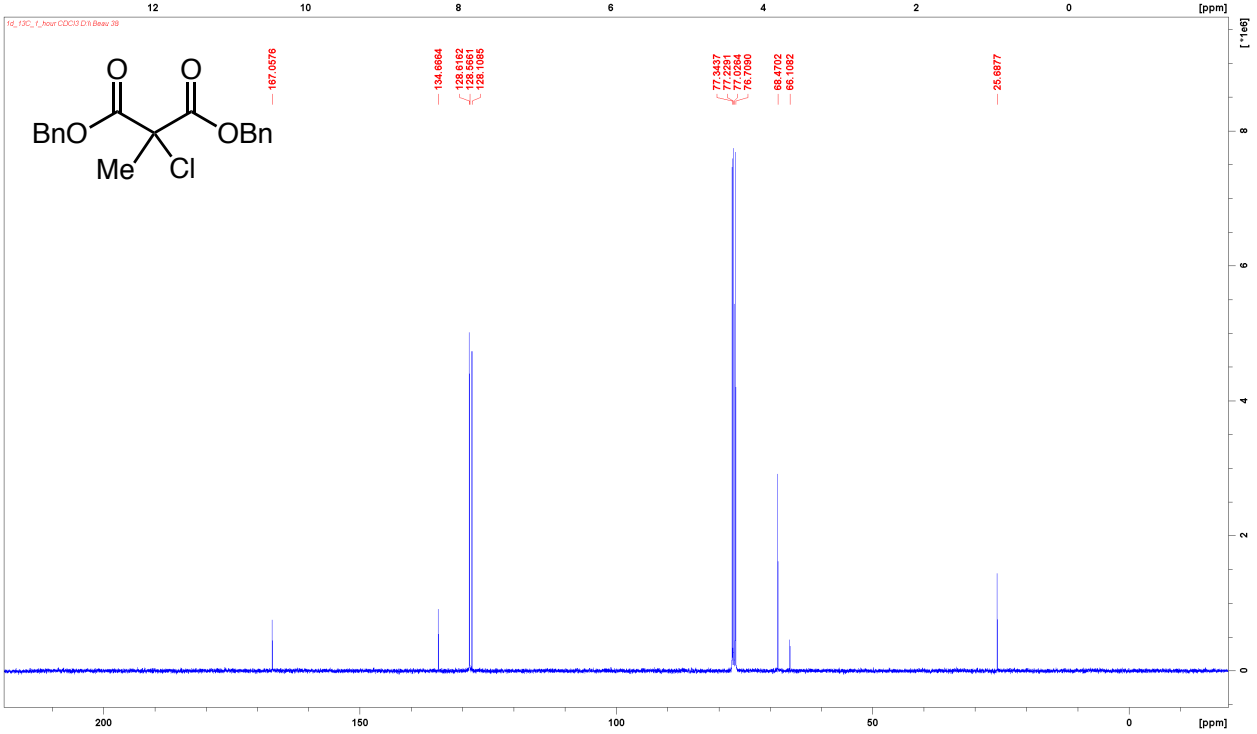

13C NMR with 1H decoupling
155.4490
152.9028
82.4504
79.0198
61.4027
51.2534
42.4628
41.2482
40.4153
40.1374
39.8591
35.3605
28.5917
27.9497
12.5593
[rel]
[ppm]

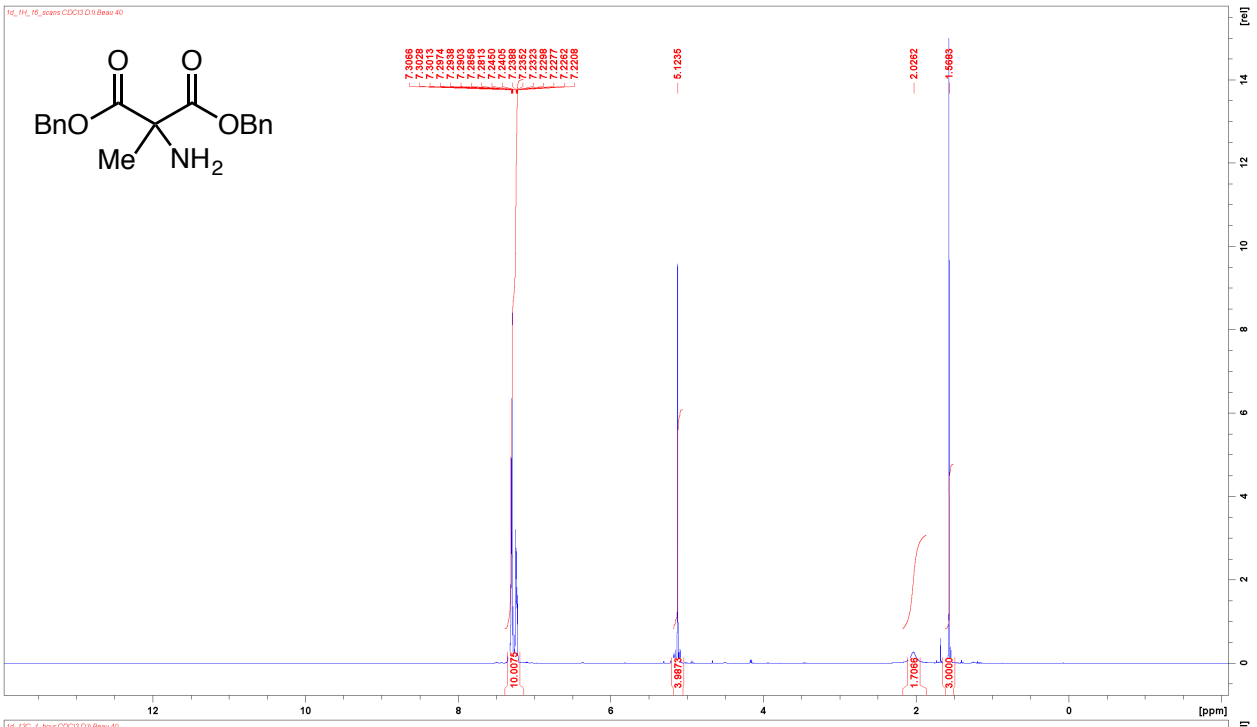

CN
N
N
H
OMe
8.2907
8.2851
8.2873
8.2826
8.2516
7.6471
7.6329
7.6281
7.2777
7.2724
7.2612
7.2558
7.2505
6.8753
6.8700
6.8588
6.8535
6.6178
6.6054
6.5998
6.5884
5.3876
4.6250
4.6113
3.7851
0.9953
0.9880
2.2195
2.0446
1.0332
0.9260
2.0796
3.1228
12
10
8
6
4
2
[ppm]
[*1e5]

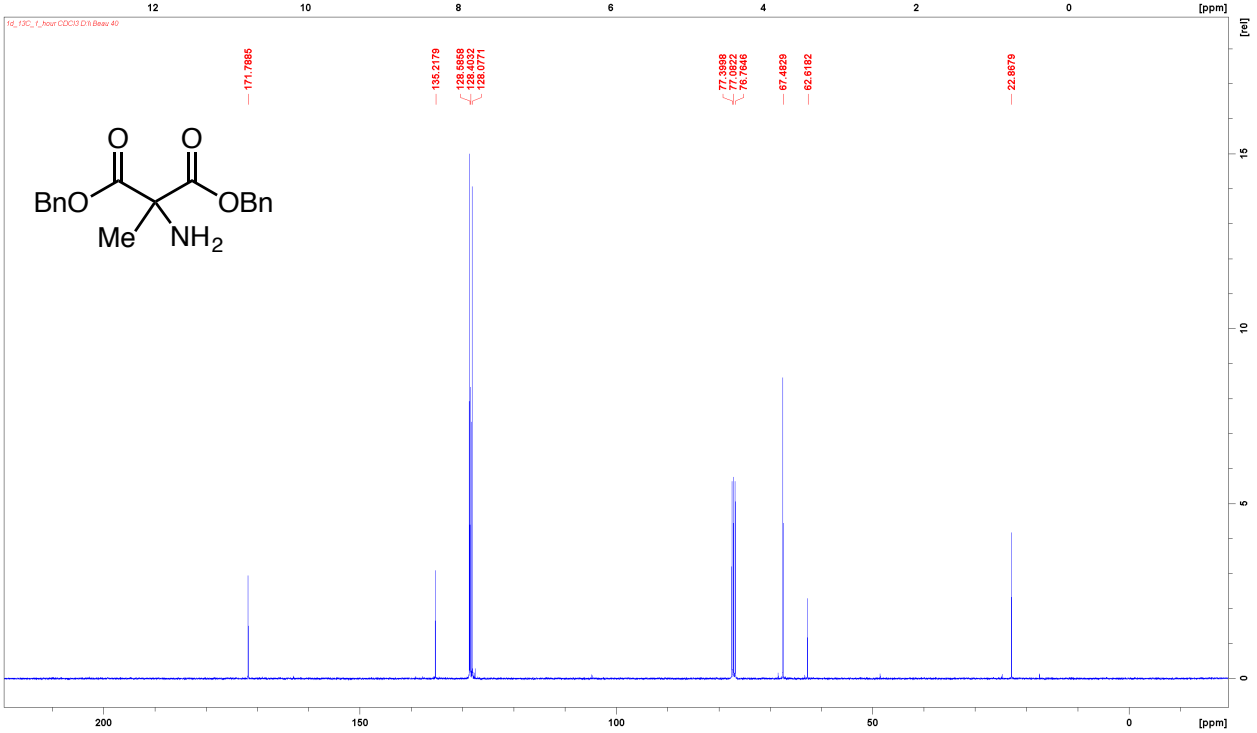

CN
N
N
H
OMe
159.1439
158.0920
152.6924
141.3867
130.3384
128.1584
116.7222
114.1651
112.1718
91.6076
77.3830
77.2419
77.0435
76.7280
55.3319
44.8997
200
150
100
50
0
[ppm]
[*1e3]

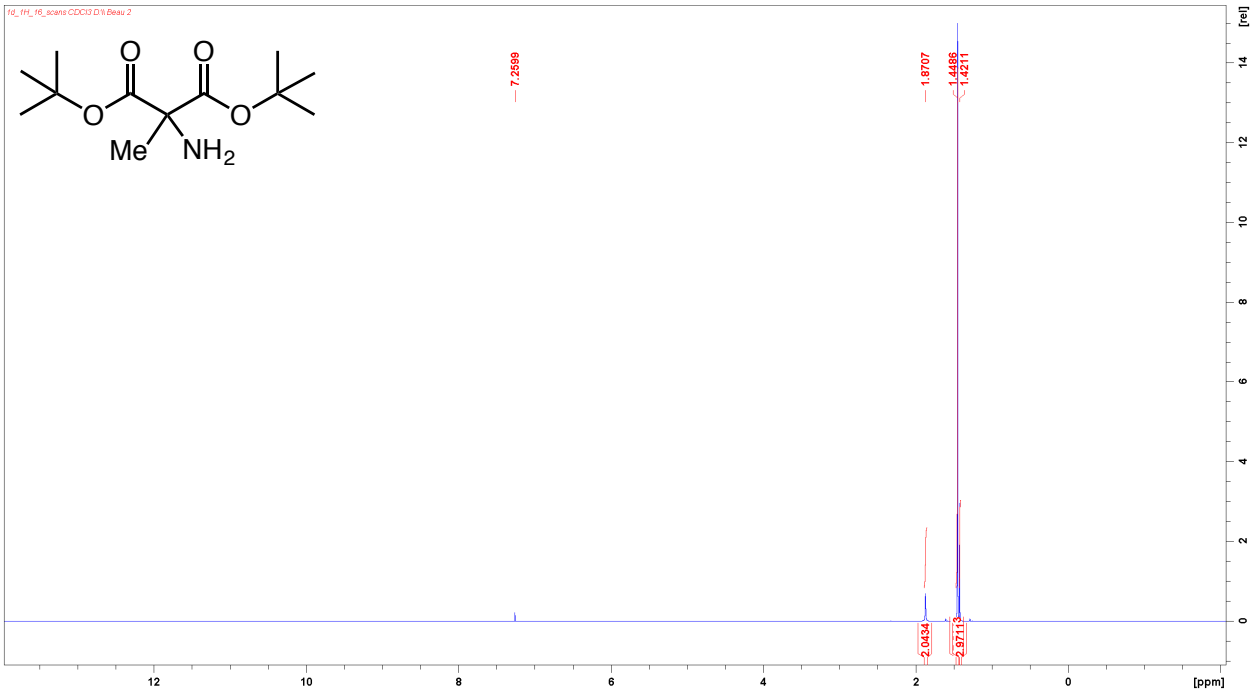

CN
NH2
8.2429
8.2362
8.2304
8.2268
7.6992
7.6845
7.6800
7.6753
6.7120
6.6985
6.6903
6.6863
5.2323
5.2271
1.0000
0.9981
1.0005
2.0269
12
10
8
6
4
2
0 [ppm]
[*1e5]
159.0307
152.4640
141.6601
116.2883
113.5453
91.6887
77.5377
77.3212
77.0203
76.7029
200
150
100
50
0
[ppm]
[*1e3]

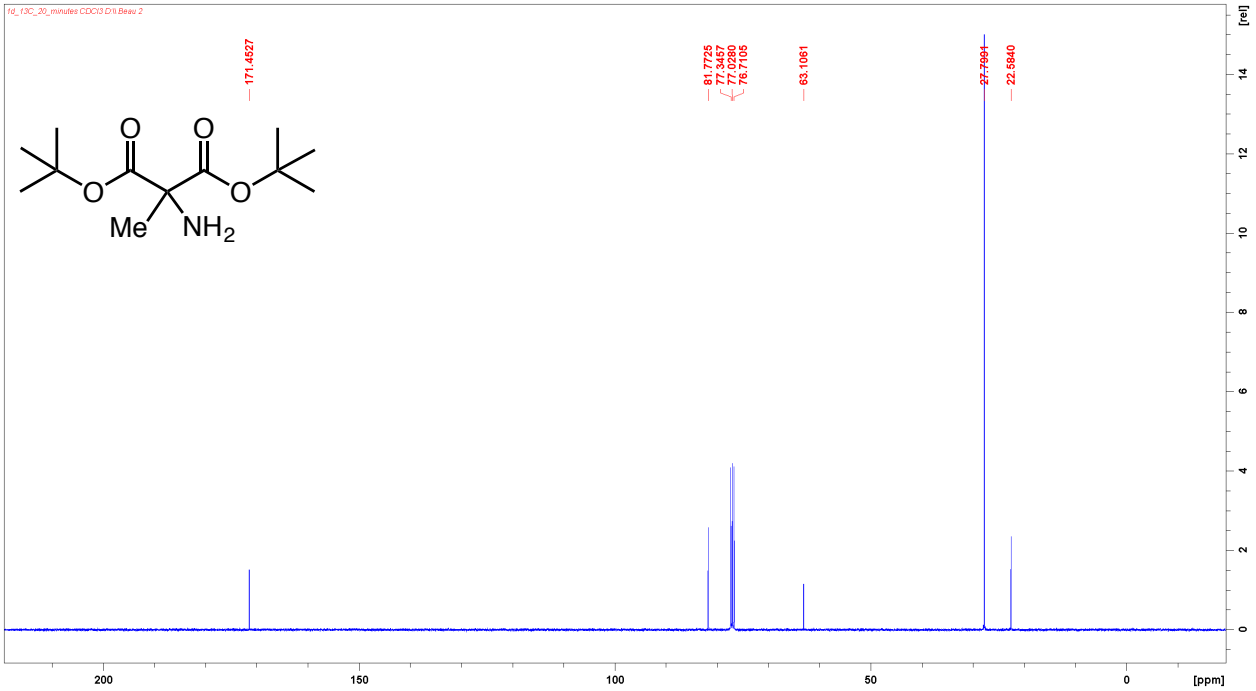

BnO
OBn
Me
Cl
7.3141
7.3057
7.3012
7.2879
7.2653
7.2585
7.2548
7.2513
7.2491
7.2439
7.2388
7.2341
5.1690
1.9345
10.0350
4.0000
3.0068
[ppm]

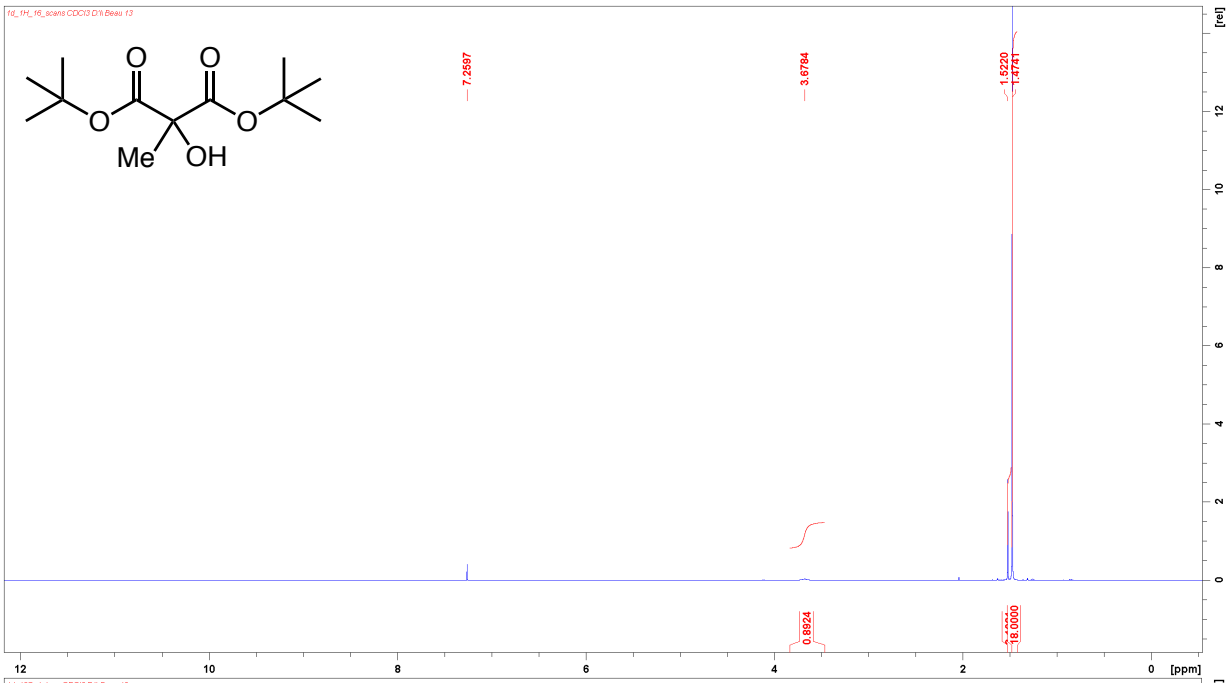

BnO
OBn
Me
Cl
167.0576
134.6684
128.6162
128.5661
128.1085
77.3437
77.2431
77.0264
76.7090
68.4702
66.1082
25.6877
[ppm]

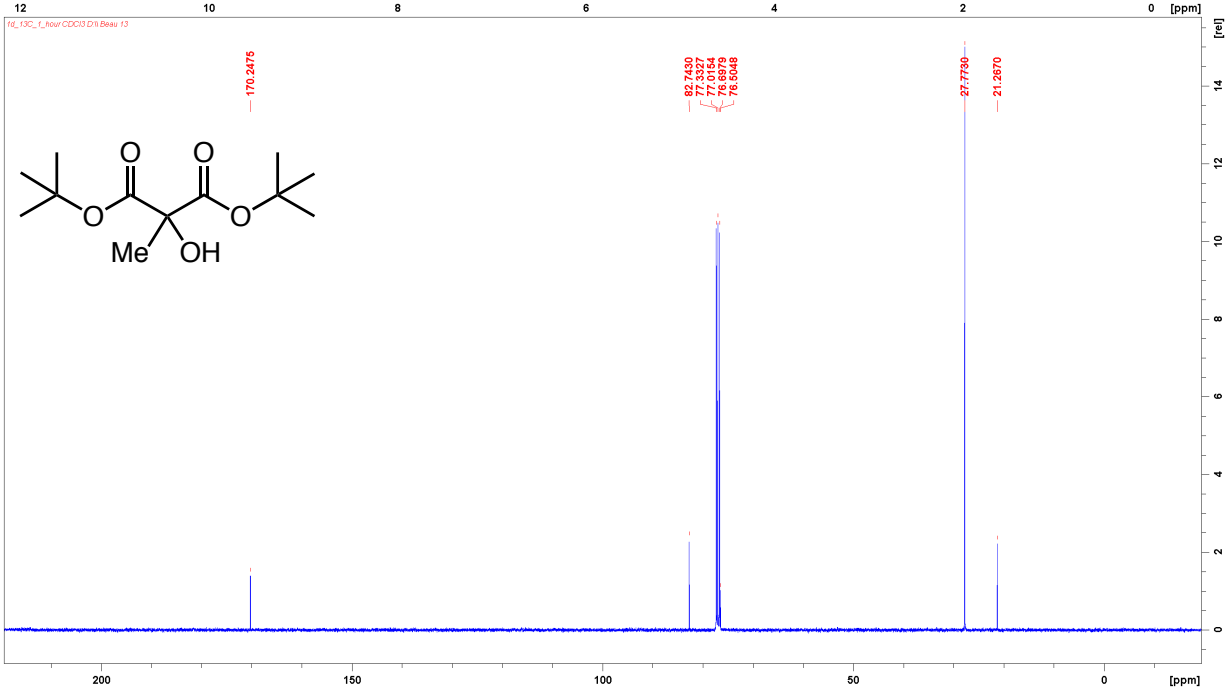

BnO
OBn
Me
NH2
7.3066
7.3029
7.3013
7.2974
7.2938
7.2900
7.2858
7.2813
7.2460
7.2405
7.2388
7.2352
7.2313
7.2289
7.2277
7.2262
7.2208
5.1235
2.0262
1.5683
10.0075
3.9872
1.7069
3.0000
[rel]
[ppm]

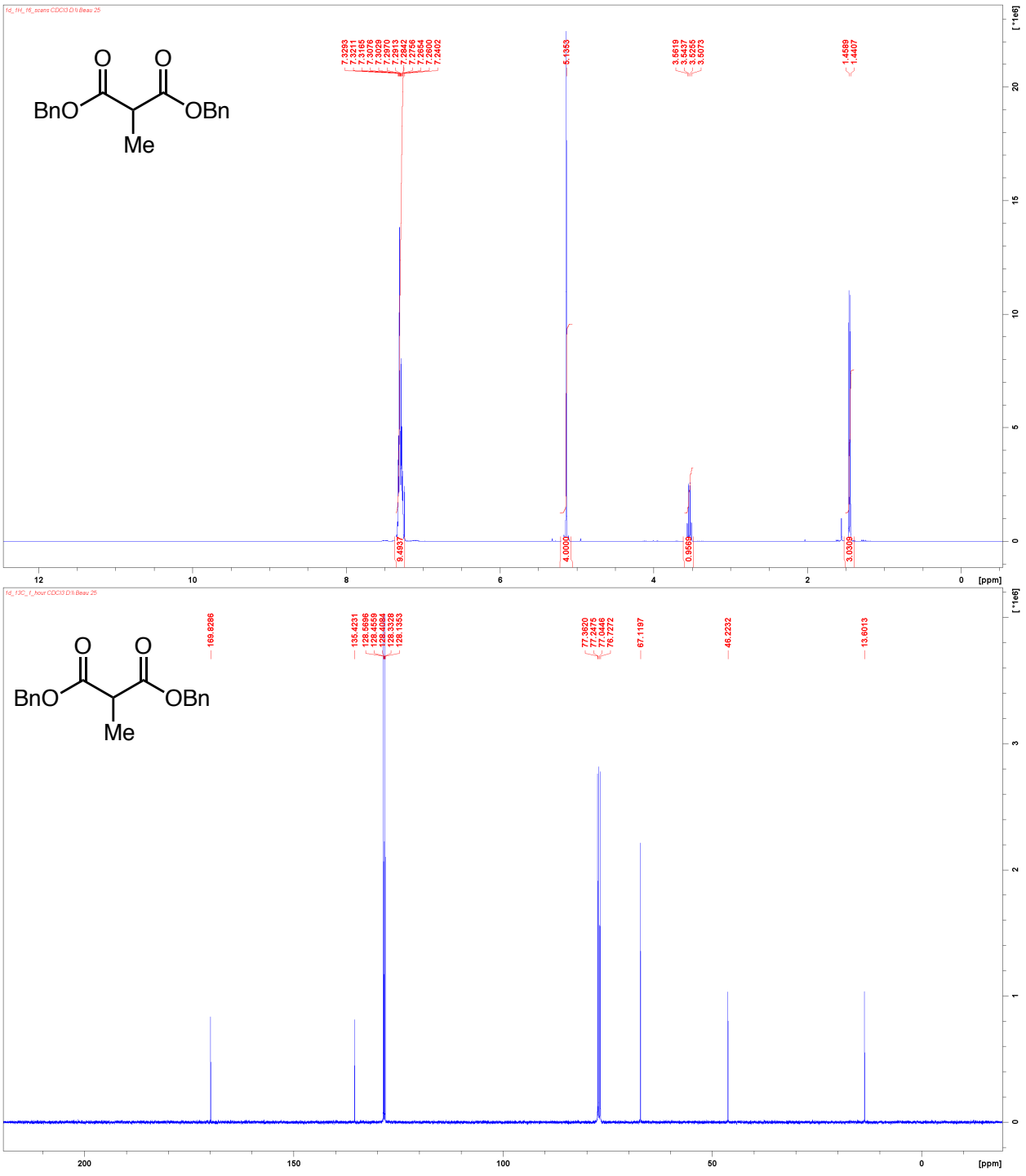

BnO
OBn
Me
NH2
171.7885
135.2179
128.5858
128.4032
128.0771
77.3998
77.0822
76.7646
67.4829
62.6182
22.8679
[rel]
[ppm]

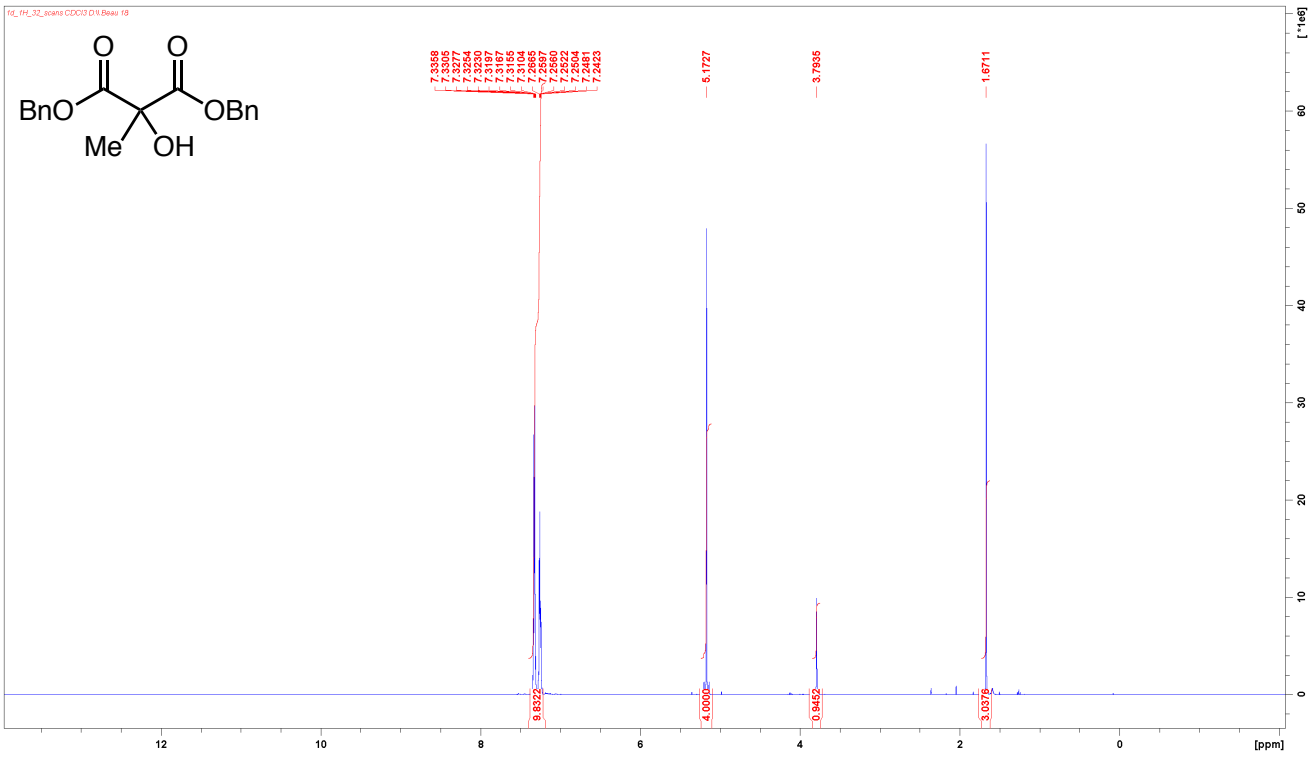

1d_1H_16_scans CDCl3 D:\\Beau 2
O
O
O
O
Me
NH2
7.2599
1.8707
1.4486
1.4211
2.0434
2.9711
[rel]
[ppm]

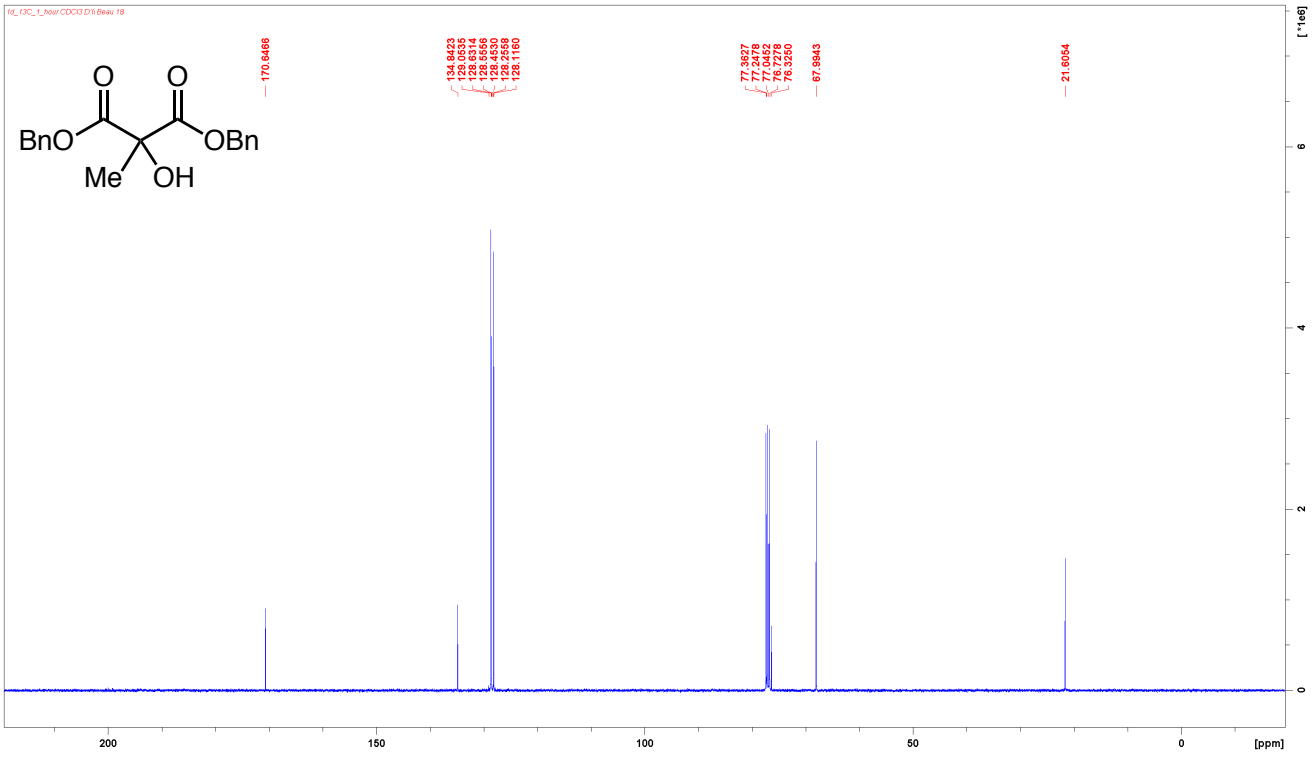

1d_13C_20_minutes CDCl3 D:\\Beau 2
O
O
O
O
Me
NH2
171.4527
81.7725
77.3457
77.0280
76.7105
63.1061
27.7991
22.5840
[rel]
[ppm]


7.2597
3.6784
1.5220
1.4741
0.8924
18.0000
[rel]
[ppm]
Me
OH


170.2475
82.7460
77.3327
77.0154
76.6979
76.6048
27.7730
21.2670
[rel]
[ppm]
Me
OH


BnO
OBn
Me
7.3093
7.3231
7.3165
7.3076
7.3029
7.2970
7.2913
7.2842
7.2795
7.2654
7.2600
7.2402
5.1353
3.5616
3.5437
3.5255
3.5073
1.4589
1.4407
9.4937
4.0000
0.9569
3.0309
[ppm]
[*1e6]
169.8286
135.4231
128.5696
128.4559
128.4084
128.3328
128.1353
77.3620
77.2405
77.0446
76.7272
67.1197
46.2232
13.6013


BnO
OBn
Me
OH
7.3358
7.3305
7.3277
7.3254
7.3230
7.3197
7.3167
7.3155
7.3104
7.2965
7.2597
7.2560
7.2522
7.2504
7.2481
7.2423
5.1727
3.7935
1.6711
9.8322
4.0000
0.9452
3.0375
12
10
8
6
4
2
0
[ppm]


BnO
OBn
Me
OH
170.6466
134.8423
129.0555
128.6314
128.5556
128.4530
128.2583
128.1160
77.3827
77.1738
77.0452
76.7278
76.3250
67.9943
21.6054
200
150
100
50
0
[ppm]

Side Products