Synthesis of Novel Isoprenoid Diphosphate Analogs as Chemical Tools to Investigate Protein Geranylgeranylation

Kayla Jo Temple

Purdue University

Follow this and additional works at: http://docs.lib.purdue.edu/open_access_dissertations

Part of the Biochemistry Commons, and the Organic Chemistry Commons

Recommended Citation


This document has been made available through Purdue e-Pubs, a service of the Purdue University Libraries. Please contact epubs@purdue.edu for additional information.
This is to certify that the thesis/dissertation prepared

By Kayla Jo Temple

Entitled
Synthesis of Novel Isoprenoid Diphosphate Analogs as Chemical Tools to Investigate Protein Geranylgeranylation

For the degree of Doctor of Philosophy

Is approved by the final examining committee:

Richard Gibbs

Jean-Christophe Rochet

Laurie Parker

Mahdi Abu-Omar

To the best of my knowledge and as understood by the student in the Research Integrity and Copyright Disclaimer (Graduate School Form 20), this thesis/dissertation adheres to the provisions of Purdue University’s “Policy on Integrity in Research” and the use of copyrighted material.

Approved by Major Professor(s): Richard Gibbs

Approved by: Richard Borch 08/20/2013

Head of the Graduate Program Date
SYNTHESIS OF NOVEL ISOPRENOID DIPHOSPHATE ANALOGS AS CHEMICAL TOOLS TO INVESTIGATE PROTEIN GERANYLGERANYLATION

A Dissertation
Submitted to the Faculty
of
Purdue University
by
Kayla Jo Temple

In Partial Fulfillment of the
Requirements for the Degree
of
Doctor of Philosophy

December 2013
Purdue University
West Lafayette, Indiana
For my family, especially my mother.

My struggles over the past six years have been nothing when compared to yours.

Your courage, grace, and strength have truly been inspiring.
I would like to express my deepest appreciation and gratitude to my Ph.D. thesis advisor Dr. Richard A. Gibbs. His patience and mentorship provided the foundation on which my abilities as a scientist and chemist could develop and grow. I am also thankful to my committee members, Dr. Jean-Christophe Rochet, Dr. Laurie Parker, and Dr. Madhi Abu-Omar for taking the time to offer guidance in my development throughout graduate school. Our collaboration with the Fierke laboratory at the University of Michigan, especially the work of Elia Wright, was instrumental in providing the biological data for this project. I thank you for all of your diligent work on such a short time line. I would also like to thank the Department of Medicinal Chemistry and Molecular Pharmacology for providing with such a wonderful opportunity.

I am grateful to all of my labmates in the Gibbs lab (both past and present) who created a wonderful working environment. I would like to thank those who have gone before me for sharing their knowledge and offering not only their support but their friendship as well: Dr. Amanda Krzysiak, Dr. Andrew Placzek, Dr. Michelle Mynderse, Dr. Animesh Aditya, Dr. Joel Bergman, Dr. Jiao Song, Dr. Jaimeen Majmudar, and Dr. Liza Shrestha. I would also like to thank those who will come after me: Feifei Zhao, Danelle Rolle, and Qianwei Knauf. It was a pleasure getting to know and work with all of you.

I am grateful for everyone in my family, especially my parents, for their love and support during my time in Graduate School. I would not be the person I am today nor would I have been able to accomplish so much without them. I love you all.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>List of Tables</th>
<th>vi</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Figures</td>
<td>vii</td>
</tr>
<tr>
<td>List of Schemes</td>
<td>xv</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xvi</td>
</tr>
<tr>
<td>Abstract</td>
<td>xviii</td>
</tr>
<tr>
<td>Chapter 1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Posttranslational Modifications</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Protein Prenylation</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Ras Family of Proteins</td>
<td>4</td>
</tr>
<tr>
<td>1.4 Targeting Protein Prenylation &amp; Alternative Prenylation in Cancer</td>
<td>6</td>
</tr>
<tr>
<td>1.5 The CaaX Prenyltransferase Enzymes</td>
<td>7</td>
</tr>
<tr>
<td>1.5.1 Mechanism of Catalysis and Kinetics of CaaX Prenyltransferases</td>
<td>9</td>
</tr>
<tr>
<td>1.5.2 Comparing the Isoprenoid Binding Pockets of FTase &amp; GGTase-I</td>
<td>14</td>
</tr>
<tr>
<td>1.6 GGPP Analogs as Chemical Tools</td>
<td>16</td>
</tr>
<tr>
<td>1.7 <em>In vitro</em> Fluorescence Screening Assay for FTase &amp; GGTase-I Activity</td>
<td>18</td>
</tr>
<tr>
<td>1.8 Protein Prenylation &amp; Other Diseases</td>
<td>20</td>
</tr>
<tr>
<td>1.9 Significance of Work</td>
<td>20</td>
</tr>
<tr>
<td>Chapter 2. Synthesis &amp; Biochemical Evaluation of Aryl-Modified Geranylgeranyl Pyrophosphate Analogs</td>
<td>22</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>22</td>
</tr>
<tr>
<td>2.2 Synthesis of Aryl-Modified Geranylgeranyl Pyrophosphate Analogs</td>
<td>28</td>
</tr>
<tr>
<td>2.3 Biochemical Evaluation of Aryl-Modified Geranylgeranyl Pyrophosphate Analogs</td>
<td>32</td>
</tr>
<tr>
<td>2.4 Conclusions</td>
<td>36</td>
</tr>
<tr>
<td>2.5 Experimental Procedures Utilized for the Synthesis &amp; Biochemical Evaluation of Aryl-Modified Geranylgeranyl Pyrophosphate Analogs</td>
<td>38</td>
</tr>
</tbody>
</table>
CHAPTER 3. SYNTHESIS & BIOCHEMICAL EVALUATION OF SATURATED GERANYLGERANYL PYROPHOSPHATE ANALOGS ........................................................... 54

3.1 Introduction ....................................................................................................................... 54
3.2 Synthesis of Saturated Geranylgeranyl Pyrophosphate Analogs ...................................... 58
3.3 Biochemical Evaluation of Saturated Geranylgeranyl Pyrophosphate Analogs ............... 63
3.4 Conclusions ....................................................................................................................... 68
3.5 Experimental Procedures Utilized for the Synthesis & Biochemical Evaluation of Saturated Geranylgeranyl Pyrophosphate Analogs ........................................................... 71

CHAPTER 4. SYNTHESIS & BIOCHEMICAL EVALUATION OF FRAME-SHIFTED GERANYLGERANYL PYROPHOSPHATE ANALOGS ........................................................... 95

4.1 Introduction ....................................................................................................................... 95
4.2 Synthesis of Frame-Shifted Geranylgeranyl Pyrophosphate Analogs ............................ 100
4.3 Biochemical Evaluation of Frame-Modified Geranylgeranyl Pyrophosphate Analogs .. 106
4.4 Conclusions ..................................................................................................................... 111
4.5 Experimental Procedures Utilized for the Synthesis & Biochemical Evaluation of Frame-Shifted Geranylgeranyl Pyrophosphate Analogs ...................................................... 114

CHAPTER 5. SYNTHESIS & BIOCHEMICAL EVALUATION OF ALKYNYL-TAGGED GERANYLGERANYL PYROPHOSPHATE ANALOGS ......................................................... 142

5.1 Introduction ..................................................................................................................... 142
5.2 Synthesis of Alkynyl-Tagged Geranylgeranyl Pyrophosphate Analogs ........................... 147
5.3 Biochemical Evaluation of Alkynyl-Tagged Geranylgeranyl Pyrophosphate Analogs .. 153
5.4 Conclusions ..................................................................................................................... 157
5.5 Experimental Procedures Utilized for the Synthesis & Biochemical Evaluation of Alkynyl-Tagged Geranylgeranyl Pyrophosphate Analogs ...................................................... 159

CHAPTER 6. CONCLUSIONS & FUTURE DIRECTIONS ...................................................... 175

6.1 Aryl-Modified GGPP Analogs ...................................................................................... 175
6.2 Saturated GGPP Analogs .......................................................................................... 177
6.3 Frame-Shifted GGPP Analogs .................................................................................... 179
6.4 Alkynyl-GGPP Analogs ............................................................................................. 181

LIST OF REFERENCES ............................................................................................................. 188

APPENDIX: NMR SPECTRA OF FINAL ALCOHOLS & PYROPHOSPHATES .................. 201

VITA ............................................................................................................................................ 325
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1. Summary of FTase &amp; GGTase-I requirements.</td>
<td>12</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Figure 1.1.</strong> Post-translational modifications of CaaX or CXC-containing peptides by prenyltransferase enzymes.</td>
<td>3</td>
</tr>
<tr>
<td><strong>Figure 1.2.</strong> Post translational modifications of Ras proteins by FTase &amp; GGTase-I and a simplified version of the Ras signaling pathway.</td>
<td>4</td>
</tr>
<tr>
<td><strong>Figure 1.3.</strong> Structural representation of FTase and GGTase-I</td>
<td>9</td>
</tr>
<tr>
<td><strong>Figure 1.4.</strong> Transition state model of protein prenylation</td>
<td>11</td>
</tr>
<tr>
<td><strong>Figure 1.5.</strong> Reaction pathway of farnesylation</td>
<td>13</td>
</tr>
<tr>
<td><strong>Figure 1.6.</strong> Reaction pathway of geranylgeranylation</td>
<td>13</td>
</tr>
<tr>
<td><strong>Figure 1.7.</strong> Comparison of FTase &amp; GGTase-I β-subunits within the binding pocket</td>
<td>15</td>
</tr>
<tr>
<td><strong>Figure 1.8.</strong> Examples of farnesylated pyrophosphates containing affinity tags or fluorophores utilized in the past</td>
<td>17</td>
</tr>
<tr>
<td><strong>Figure 1.9.</strong> Modifications of the farnesyl scaffold explored by our laboratory</td>
<td>18</td>
</tr>
<tr>
<td><strong>Figure 1.10.</strong> Single cuvette fluorometric assay</td>
<td>19</td>
</tr>
<tr>
<td><strong>Figure 2.1.</strong> Aryl-modified FPP analogs previously evaluated</td>
<td>25</td>
</tr>
<tr>
<td><strong>Figure 2.2.</strong> Aromatic rich GGTase-I binding site</td>
<td>25</td>
</tr>
<tr>
<td><strong>Figure 2.3.</strong> Aromatic groups as isoprenoid mimics</td>
<td>26</td>
</tr>
<tr>
<td><strong>Figure 2.4.</strong> Comparing protein prenylation with our laboratory’s nanomolar Icmt inhibitor</td>
<td>26</td>
</tr>
<tr>
<td><strong>Figure 2.5.</strong> Overlay of aryl-modified GGPP analogs and GGPP in the GGTase-I binding pocket</td>
<td>27</td>
</tr>
<tr>
<td><strong>Figure 2.6.</strong> Bar graphs of substrate activity represented in RFI of aryl-modified GGPP analogs 2.6a-d versus GGPP</td>
<td>34</td>
</tr>
<tr>
<td><strong>Figure 2.7.</strong> Bar graphs of substrate activity represented in RFI of aryl-modified GGPP analogs 2.6e-f and 2.15 versus GGPP</td>
<td>34</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Figure 2.8.</strong></td>
<td>Monitoring continuous changes in fluorescence of aryl-modified</td>
</tr>
<tr>
<td><strong>Figure 3.1.</strong></td>
<td>Inhibitors of GGTase-I that contain saturated hydrocarbon moieties.</td>
</tr>
<tr>
<td><strong>Figure 3.2.</strong></td>
<td>Conventional method of isoprene unit labeling</td>
</tr>
<tr>
<td><strong>Figure 3.3.</strong></td>
<td>Saturated GGPP analogs synthesized</td>
</tr>
<tr>
<td><strong>Figure 3.4.</strong></td>
<td>Bar graphs of substrate activity represented in RFI of saturated GGPP analogs versus GGPP</td>
</tr>
<tr>
<td><strong>Figure 3.5.</strong></td>
<td>Bar graphs of substrate activity represented in RFI of saturated GGPP analogs versus GGPP</td>
</tr>
<tr>
<td><strong>Figure 3.6.</strong></td>
<td>Bar graphs of substrate activity represented in RFI of saturated GGPP analogs versus GGPP</td>
</tr>
<tr>
<td><strong>Figure 3.7.</strong></td>
<td>Bar graphs of substrate activity represented in RFI of saturated GGPP analogs versus GGPP</td>
</tr>
<tr>
<td><strong>Figure 3.8.</strong></td>
<td>Monitoring continuous changes in fluorescence of saturated GGPP analogs versus GGPP</td>
</tr>
<tr>
<td><strong>Figure 4.1.</strong></td>
<td>Frame-shifted analog numbering scheme &amp; previously synthesized FPP analogs....</td>
</tr>
<tr>
<td><strong>Figure 4.2.</strong></td>
<td>Newly synthesized frame-shifted analogs.</td>
</tr>
<tr>
<td><strong>Figure 4.3.</strong></td>
<td>Bar graphs of substrate activity represented in RFI of 4.11, 4.17, 4.21, 4.32, &amp; 1,2,2,1-OPP versus GGPP</td>
</tr>
<tr>
<td><strong>Figure 4.4.</strong></td>
<td>Bar graphs of substrate activity represented in RFI of 4.28 &amp; 4.40a-c versus GGPP.</td>
</tr>
<tr>
<td><strong>Figure 4.5.</strong></td>
<td>Monitoring continuous changes in fluorescence of frame-shifted GGPP analogs versus GGPP. Endpoint assays of 4.11 &amp; 4.17 are also included.</td>
</tr>
<tr>
<td><strong>Figure 4.6.</strong></td>
<td>Preliminary inhibitor assay results for 4.32</td>
</tr>
<tr>
<td><strong>Figure 5.1.</strong></td>
<td>Potential alkynyl pyrophosphate cellular probes for proteomic studies</td>
</tr>
<tr>
<td><strong>Figure 5.2.</strong></td>
<td>Structural overlay of alkynyl-GGPP analogs with GGPP</td>
</tr>
<tr>
<td><strong>Figure 5.3.</strong></td>
<td>Bar graphs of substrate activity represented in RFI of alkynyl-GGPP analogs versus GGPP.</td>
</tr>
<tr>
<td><strong>Figure 5.4.</strong></td>
<td>Monitoring continuous changes in fluorescence of alkynyl-GGPP .................</td>
</tr>
<tr>
<td><strong>Figure 5.5.</strong></td>
<td>Endpoint assay results for slow alkynyl-GGPP analogs with</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Figure 6.1.</strong> Summary of project findings and proposed future analogs for synthesis..................</td>
<td>183</td>
</tr>
<tr>
<td><strong>Figure 6.2.</strong> Structure of GGPP and CVLL or CVIL bound in the β-subunit of GGTase-I ............</td>
<td>184</td>
</tr>
<tr>
<td><strong>Figure 6.3.</strong> Aryl-modified analogs bound in the exit grove of GGTase-I. .............................</td>
<td>184</td>
</tr>
<tr>
<td><strong>Figure 6.4.</strong> Analyzing the distances between the Zn(^{2+}) ion or aromatic residues in the binding pocket of GGTase-I with isoprenoid chains.. ..........................................................</td>
<td>185</td>
</tr>
<tr>
<td><strong>Figure 6.5.</strong> Analyzing the affects of increasing the number of carbons between the α and β- isoprene units to three carbons.................................................................</td>
<td>186</td>
</tr>
<tr>
<td><strong>Figure 6.6.</strong> Triazole-containing alkynyl-GGPP analogs bound in GGTase-I. .........................</td>
<td>187</td>
</tr>
</tbody>
</table>

**Appendix Figure**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Figure A. 1.</strong> (^1)H NMR of 2.5a.................................................................</td>
<td>202</td>
</tr>
<tr>
<td><strong>Figure A. 2.</strong> (^{13})C NMR of 2.5a...............................................................</td>
<td>203</td>
</tr>
<tr>
<td><strong>Figure A. 3.</strong> (^1)H NMR of 2.6a.................................................................</td>
<td>204</td>
</tr>
<tr>
<td><strong>Figure A. 4.</strong> (^{31})P NMR of 2.6a.........................................................</td>
<td>205</td>
</tr>
<tr>
<td><strong>Figure A. 5.</strong> (^1)H NMR of 2.5b.................................................................</td>
<td>206</td>
</tr>
<tr>
<td><strong>Figure A. 6.</strong> (^{13})C NMR of 2.5b...............................................................</td>
<td>207</td>
</tr>
<tr>
<td><strong>Figure A. 7.</strong> (^1)H NMR of 2.6b.................................................................</td>
<td>208</td>
</tr>
<tr>
<td><strong>Figure A. 8.</strong> (^{31})P NMR of 2.6b...............................................................</td>
<td>209</td>
</tr>
<tr>
<td><strong>Figure A. 9.</strong> (^1)H NMR of 2.5c.................................................................</td>
<td>210</td>
</tr>
<tr>
<td><strong>Figure A. 10.</strong> (^{13})C NMR of 2.5c...............................................................</td>
<td>211</td>
</tr>
<tr>
<td><strong>Figure A. 11.</strong> (^1)H NMR of 2.6c.................................................................</td>
<td>212</td>
</tr>
<tr>
<td><strong>Figure A. 12.</strong> (^{31})P NMR of 2.6c...............................................................</td>
<td>213</td>
</tr>
<tr>
<td><strong>Figure A. 13.</strong> (^1)H NMR of 2.5d.................................................................</td>
<td>214</td>
</tr>
<tr>
<td><strong>Figure A. 14.</strong> (^{13})C NMR of 2.5d...............................................................</td>
<td>215</td>
</tr>
<tr>
<td><strong>Figure A. 15.</strong> (^1)H NMR of 2.6d.................................................................</td>
<td>216</td>
</tr>
<tr>
<td><strong>Figure A. 16.</strong> (^{31})P NMR of 2.6d...............................................................</td>
<td>217</td>
</tr>
<tr>
<td>Appendix Figure</td>
<td>Page</td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td>Figure A. 17.  $^1$H NMR of 2.5e.</td>
<td>218</td>
</tr>
<tr>
<td>Figure A. 18.  $^{13}$C NMR of 2.5e.</td>
<td>219</td>
</tr>
<tr>
<td>Figure A. 19.  $^1$H NMR of 2.6e.</td>
<td>220</td>
</tr>
<tr>
<td>Figure A. 20.  $^{31}$P NMR of 2.6e.</td>
<td>221</td>
</tr>
<tr>
<td>Figure A. 21.  $^1$H NMR of 2.5f.</td>
<td>222</td>
</tr>
<tr>
<td>Figure A. 22.  $^{13}$C NMR of 2.5f.</td>
<td>223</td>
</tr>
<tr>
<td>Figure A. 23.  $^{19}$F NMR of 2.5f.</td>
<td>224</td>
</tr>
<tr>
<td>Figure A. 24.  $^1$H NMR of 2.6f.</td>
<td>225</td>
</tr>
<tr>
<td>Figure A. 25.  $^{31}$P NMR of 2.6f.</td>
<td>226</td>
</tr>
<tr>
<td>Figure A. 26.  $^1$H NMR of 2.14.</td>
<td>227</td>
</tr>
<tr>
<td>Figure A. 27.  $^{13}$C NMR of 2.14.</td>
<td>228</td>
</tr>
<tr>
<td>Figure A. 28.  $^1$H NMR of 2.15.</td>
<td>229</td>
</tr>
<tr>
<td>Figure A. 29.  $^{31}$P NMR of 2.15.</td>
<td>230</td>
</tr>
<tr>
<td>Figure A. 30.  $^1$H NMR of 3.2a.</td>
<td>231</td>
</tr>
<tr>
<td>Figure A. 31.  $^{13}$C NMR of 3.2a.</td>
<td>232</td>
</tr>
<tr>
<td>Figure A. 32.  $^1$H NMR of 3.3a.</td>
<td>233</td>
</tr>
<tr>
<td>Figure A. 33.  $^{31}$P NMR of 3.3a.</td>
<td>234</td>
</tr>
<tr>
<td>Figure A. 34.  $^1$H NMR of 3.2b.</td>
<td>235</td>
</tr>
<tr>
<td>Figure A. 35.  $^{13}$C NMR of 3.2b.</td>
<td>236</td>
</tr>
<tr>
<td>Figure A. 36.  $^1$H NMR of 3.3b.</td>
<td>237</td>
</tr>
<tr>
<td>Figure A. 37.  $^{31}$P NMR of 3.3b.</td>
<td>238</td>
</tr>
<tr>
<td>Figure A. 38.  $^1$H NMR of 3.2c.</td>
<td>239</td>
</tr>
<tr>
<td>Figure A. 39.  $^{13}$C NMR of 3.2c.</td>
<td>240</td>
</tr>
<tr>
<td>Figure A. 40.  $^1$H NMR of 3.3c.</td>
<td>241</td>
</tr>
<tr>
<td>Figure A. 41.  $^{31}$P NMR of 3.3c.</td>
<td>242</td>
</tr>
<tr>
<td>Appendix Figure</td>
<td>Page</td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td>Figure A. 42</td>
<td>(^1)H NMR of 3.6a</td>
</tr>
<tr>
<td>Figure A. 43</td>
<td>(^13)C NMR of 3.6a</td>
</tr>
<tr>
<td>Figure A. 44</td>
<td>(^1)H NMR of 3.7a</td>
</tr>
<tr>
<td>Figure A. 45</td>
<td>(^31)P NMR of 3.7a</td>
</tr>
<tr>
<td>Figure A. 46</td>
<td>(^1)H NMR of 3.6b</td>
</tr>
<tr>
<td>Figure A. 47</td>
<td>(^13)C NMR of 3.6b</td>
</tr>
<tr>
<td>Figure A. 48</td>
<td>(^1)H NMR of 3.7b</td>
</tr>
<tr>
<td>Figure A. 49</td>
<td>(^31)P NMR of 3.7b</td>
</tr>
<tr>
<td>Figure A. 50</td>
<td>(^1)H NMR of 3.6c</td>
</tr>
<tr>
<td>Figure A. 51</td>
<td>(^13)C NMR of 3.6c</td>
</tr>
<tr>
<td>Figure A. 52</td>
<td>(^1)H NMR of 3.7c</td>
</tr>
<tr>
<td>Figure A. 53</td>
<td>(^31)P NMR of 3.7c</td>
</tr>
<tr>
<td>Figure A. 54</td>
<td>(^1)H NMR of 3.6d</td>
</tr>
<tr>
<td>Figure A. 55</td>
<td>(^13)C NMR of 3.6d</td>
</tr>
<tr>
<td>Figure A. 56</td>
<td>(^1)H NMR of 3.7d</td>
</tr>
<tr>
<td>Figure A. 57</td>
<td>(^31)P NMR of 3.7d</td>
</tr>
<tr>
<td>Figure A. 58</td>
<td>(^1)H NMR of 3.15a</td>
</tr>
<tr>
<td>Figure A. 59</td>
<td>(^13)C NMR of 3.15a</td>
</tr>
<tr>
<td>Figure A. 60</td>
<td>(^1)H NMR of 3.16a</td>
</tr>
<tr>
<td>Figure A. 61</td>
<td>(^31)P NMR of 3.16a</td>
</tr>
<tr>
<td>Figure A. 62</td>
<td>(^1)H NMR of 3.15b</td>
</tr>
<tr>
<td>Figure A. 63</td>
<td>(^13)C NMR of 3.15b</td>
</tr>
<tr>
<td>Figure A. 64</td>
<td>(^1)H NMR of 3.16b</td>
</tr>
<tr>
<td>Figure A. 65</td>
<td>(^31)P NMR of 3.16b</td>
</tr>
<tr>
<td>Figure A. 66</td>
<td>(^1)H NMR of 3.20</td>
</tr>
<tr>
<td>Figure A. 67</td>
<td>$^{13}$C NMR of 3.20</td>
</tr>
<tr>
<td>Figure A. 68</td>
<td>$^1$H NMR of 3.21</td>
</tr>
<tr>
<td>Figure A. 69</td>
<td>$^{31}$P NMR of 3.21</td>
</tr>
<tr>
<td>Figure A. 70</td>
<td>$^1$H NMR of 3.25</td>
</tr>
<tr>
<td>Figure A. 71</td>
<td>$^{13}$C NMR of 3.25</td>
</tr>
<tr>
<td>Figure A. 72</td>
<td>$^1$H NMR of 3.26</td>
</tr>
<tr>
<td>Figure A. 73</td>
<td>$^{31}$P NMR of 3.26</td>
</tr>
<tr>
<td>Figure A. 74</td>
<td>$^1$H NMR of 4.10</td>
</tr>
<tr>
<td>Figure A. 75</td>
<td>$^{13}$C NMR of 4.10</td>
</tr>
<tr>
<td>Figure A. 76</td>
<td>$^1$H NMR of 4.11</td>
</tr>
<tr>
<td>Figure A. 77</td>
<td>$^{31}$P NMR of 4.11</td>
</tr>
<tr>
<td>Figure A. 78</td>
<td>$^1$H NMR of 4.16</td>
</tr>
<tr>
<td>Figure A. 79</td>
<td>$^{13}$C NMR of 4.16</td>
</tr>
<tr>
<td>Figure A. 80</td>
<td>$^1$H NMR of 4.17</td>
</tr>
<tr>
<td>Figure A. 81</td>
<td>$^{31}$P NMR of 4.17</td>
</tr>
<tr>
<td>Figure A. 82</td>
<td>$^1$H NMR of 4.20</td>
</tr>
<tr>
<td>Figure A. 83</td>
<td>$^{13}$C NMR of 4.20</td>
</tr>
<tr>
<td>Figure A. 84</td>
<td>$^1$H NMR of 4.21</td>
</tr>
<tr>
<td>Figure A. 85</td>
<td>$^{31}$P NMR of 4.21</td>
</tr>
<tr>
<td>Figure A. 86</td>
<td>$^1$H NMR of 4.27</td>
</tr>
<tr>
<td>Figure A. 87</td>
<td>$^{13}$C NMR of 4.27</td>
</tr>
<tr>
<td>Figure A. 88</td>
<td>$^1$H NMR of 4.28</td>
</tr>
<tr>
<td>Figure A. 89</td>
<td>$^{31}$P NMR of 4.28</td>
</tr>
<tr>
<td>Figure A. 90</td>
<td>$^1$H NMR of 4.31</td>
</tr>
<tr>
<td>Figure A. 91</td>
<td>$^{13}$C NMR of 4.31</td>
</tr>
<tr>
<td>Appendix Figure</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
</tr>
<tr>
<td>Figure A. 92.</td>
<td>1H NMR of 4.32.</td>
</tr>
<tr>
<td>Figure A. 93.</td>
<td>31P NMR of 4.32.</td>
</tr>
<tr>
<td>Figure A. 94.</td>
<td>1H NMR of 4.39a.</td>
</tr>
<tr>
<td>Figure A. 95.</td>
<td>13C NMR of 4.39a.</td>
</tr>
<tr>
<td>Figure A. 96.</td>
<td>1H NMR of 4.40a.</td>
</tr>
<tr>
<td>Figure A. 97.</td>
<td>31P NMR of 4.40a.</td>
</tr>
<tr>
<td>Figure A. 98.</td>
<td>1H NMR of 4.39b.</td>
</tr>
<tr>
<td>Figure A. 99.</td>
<td>13C NMR of 4.39b.</td>
</tr>
<tr>
<td>Figure A. 100.</td>
<td>1H NMR of 4.40b.</td>
</tr>
<tr>
<td>Figure A. 101.</td>
<td>31P NMR of 4.40b.</td>
</tr>
<tr>
<td>Figure A. 102.</td>
<td>1H NMR of 4.39c.</td>
</tr>
<tr>
<td>Figure A. 103.</td>
<td>13C NMR of 4.39c.</td>
</tr>
<tr>
<td>Figure A. 104.</td>
<td>1H NMR of 4.40c.</td>
</tr>
<tr>
<td>Figure A. 105.</td>
<td>31P NMR of 4.40c.</td>
</tr>
<tr>
<td>Figure A. 106.</td>
<td>1H NMR of 5.3.</td>
</tr>
<tr>
<td>Figure A. 107.</td>
<td>1H NMR of 5.5.</td>
</tr>
<tr>
<td>Figure A. 108.</td>
<td>13C NMR of 5.5.</td>
</tr>
<tr>
<td>Figure A. 109.</td>
<td>31P NMR of 5.6.</td>
</tr>
<tr>
<td>Figure A. 110.</td>
<td>1H NMR of 5.6.</td>
</tr>
<tr>
<td>Figure A. 111.</td>
<td>1H NMR of 5.7.</td>
</tr>
<tr>
<td>Figure A. 112.</td>
<td>31P NMR of 5.7.</td>
</tr>
<tr>
<td>Figure A. 113.</td>
<td>1H NMR of 5.10.</td>
</tr>
<tr>
<td>Figure A. 114.</td>
<td>13C NMR of 5.10.</td>
</tr>
<tr>
<td>Figure A. 115.</td>
<td>1H NMR of 5.11.</td>
</tr>
<tr>
<td>Figure A. 116.</td>
<td>31P NMR of 5.11.</td>
</tr>
<tr>
<td>Appendix Figure</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
</tr>
<tr>
<td>Figure A. 117.</td>
<td>318</td>
</tr>
<tr>
<td>H NMR of 5.13.</td>
<td></td>
</tr>
<tr>
<td>Figure A. 118.</td>
<td>319</td>
</tr>
<tr>
<td>C NMR of 5.13.</td>
<td></td>
</tr>
<tr>
<td>Figure A. 119.</td>
<td>320</td>
</tr>
<tr>
<td>H NMR of 5.14.</td>
<td></td>
</tr>
<tr>
<td>Figure A. 120.</td>
<td>321</td>
</tr>
<tr>
<td>P NMR of 5.14.</td>
<td></td>
</tr>
<tr>
<td>Figure A. 121.</td>
<td>322</td>
</tr>
<tr>
<td>H NMR of 5.24.</td>
<td></td>
</tr>
<tr>
<td>Figure A. 122.</td>
<td>323</td>
</tr>
<tr>
<td>C NMR of 5.24.</td>
<td></td>
</tr>
<tr>
<td>Figure A. 123.</td>
<td>324</td>
</tr>
<tr>
<td>P NMR of 5.25.</td>
<td></td>
</tr>
</tbody>
</table>
**LIST OF SCHEMES**

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 2.1</td>
<td>Synthesis of Aryl-Modified GGPP analogs.</td>
<td>30</td>
</tr>
<tr>
<td>Scheme 2.2</td>
<td>Synthesis of “TAB” pyrophosphate.</td>
<td>31</td>
</tr>
<tr>
<td>Scheme 3.1</td>
<td>Synthesis of α-containing pyrophosphates.</td>
<td>60</td>
</tr>
<tr>
<td>Scheme 3.2</td>
<td>Synthesis of α &amp; β-containing pyrophosphate.</td>
<td>60</td>
</tr>
<tr>
<td>Scheme 3.3</td>
<td>Synthesis of α &amp; ω-containing pyrophosphates.</td>
<td>61</td>
</tr>
<tr>
<td>Scheme 3.4</td>
<td>Synthesis of tetrahydro-GGPP.</td>
<td>62</td>
</tr>
<tr>
<td>Scheme 3.5</td>
<td>Synthesis of dihydro-GGPP.</td>
<td>62</td>
</tr>
<tr>
<td>Scheme 4.1</td>
<td>Synthesis of 3,3,1-OPP.</td>
<td>103</td>
</tr>
<tr>
<td>Scheme 4.2</td>
<td>Synthesis of 2,3,1-OPP.</td>
<td>103</td>
</tr>
<tr>
<td>Scheme 4.3</td>
<td>Synthesis of 1,2,2,1-OPP</td>
<td>104</td>
</tr>
<tr>
<td>Scheme 4.4</td>
<td>Synthesis of 3,2,1-OPP.</td>
<td>104</td>
</tr>
<tr>
<td>Scheme 4.5</td>
<td>Synthesis of 2,2,2,2-OPP</td>
<td>105</td>
</tr>
<tr>
<td>Scheme 4.6</td>
<td>Synthesis of 4,2,1-OPP. 5,2,1-OPP, &amp; 6,2,1-OPP.</td>
<td>105</td>
</tr>
<tr>
<td>Scheme 5.1</td>
<td>Synthesis of terminal alkynyl-GGPP analogs.</td>
<td>150</td>
</tr>
<tr>
<td>Scheme 5.2</td>
<td>Synthesis of triazole-containing alkynyl-GGPP analogs.</td>
<td>151</td>
</tr>
<tr>
<td>Scheme 5.3</td>
<td>Synthesis of 7-propargyl GGPP.</td>
<td>152</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CENP</td>
<td>Centromere protein</td>
</tr>
<tr>
<td>FPP</td>
<td>Farnesyl pyrophosphate</td>
</tr>
<tr>
<td>FTase</td>
<td>Farnesyl transferase</td>
</tr>
<tr>
<td>FTI</td>
<td>Farnesyl transferase inhibitor</td>
</tr>
<tr>
<td>GDP</td>
<td>Guanosine diphosphate</td>
</tr>
<tr>
<td>GEF</td>
<td>Guanine nucleotide exchange factors</td>
</tr>
<tr>
<td>GGPP</td>
<td>Geranylgeranyl pyrophosphate</td>
</tr>
<tr>
<td>GGTase-I</td>
<td>Geranylgeranyl transferase-I</td>
</tr>
<tr>
<td>GGTI</td>
<td>Geranylgeranyl transferase inhibitor</td>
</tr>
<tr>
<td>GPP</td>
<td>Geranyl pyrophosphate</td>
</tr>
<tr>
<td>GRB</td>
<td>Growth factor receptor-bound protein</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>HEPPSO</td>
<td>N-2-hydroxyethylpiperazine-N’-2-hydroxypropanesulfonic acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-Performance Liquid Chromatography</td>
</tr>
<tr>
<td>Icmt</td>
<td>Isocysteine carboxymethyl transferase</td>
</tr>
<tr>
<td>LMNA</td>
<td>Lamin A</td>
</tr>
<tr>
<td>MEK/MAPK</td>
<td>Mitogen-activated protein kinase pathway</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein data bank</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PTase</td>
<td>Prenyl transferases</td>
</tr>
<tr>
<td>PTIs</td>
<td>Prenyl transferase inhibitors</td>
</tr>
<tr>
<td>RCE-1</td>
<td>Ras converting enzyme-1</td>
</tr>
<tr>
<td>RFI</td>
<td>Relative fluorescence increase</td>
</tr>
<tr>
<td>RTK</td>
<td>Receptor tyrosine kinase</td>
</tr>
<tr>
<td>SH2</td>
<td>Src-homogy 2</td>
</tr>
<tr>
<td>SOS</td>
<td>Son of Sevenless</td>
</tr>
<tr>
<td>TAB</td>
<td>Triazole and biphenyl containing Icmt Analog</td>
</tr>
<tr>
<td>ZACA</td>
<td>Zirconium-catalyzed asymmetric carbo-alumination</td>
</tr>
</tbody>
</table>
Many proteins require prenylation in order to be biologically functional. Some such proteins include the small Ras and Rho GTPase superfamilies, nuclear lamins A and B, and the kinesin motor proteins CENP-E and F. Prenyltransferase (PTase) inhibition is currently being explored as a possible treatment not only for cancer but for a wide variety of other diseases.

Clinical studies revealed that the effectiveness of farnesyltransferase inhibitors (FTIs) to treat Ras-dependent tumors is determined by which isoform of Ras is overactive. Unfortunately the majority of Ras-dependent tumors have a mutation in either the N- or K-Ras isoforms; both of these isoforms can be alternatively prenylated by GGTase-I and, therefore, do not respond to FTI treatment. This sparked our interest in developing GGTase-I inhibitors and exploring requirements needed for alternative prenylation by GGTase-I.

Clinical studies also brought about the discovery that FTIs were effective toward some Ras-independent tumors (e.g. breast cancer, chronic & acute myeloid leukemia, multiple myeloma, and advanced myelodyplastic syndrome). Presumably, these results are due to the prenylation of one or more essential proteins required for tumorigenesis. The identity of the protein(s) responsible for the observed antitumor affect in Ras-independent tumors remains elusive. Identifying tumors reliant on proteins that are solely prenylated by one prenyltransferase could open up new avenues for therapeutic intervention by FTIs or GGTIs. Thus, identifying...
prenylated proteins and the prenyltransferase(s) required for this modification is of great interest and importance.

Chemical tools capable of modulating prenylation of specific proteins would allow researchers to more precisely investigate proteins’ individual roles in the cell as well as the function of their lipid moieties. To this end we use a combinatorial approach in which we screen isoprenoid pyrophosphate analogs against a synthetic Dansyl-GCaaX peptide library (the minimal recognition sequence of PTases; Dansyl-G = Dansyl-glycine, C = Cys, a = aliphatic amino acid, X = a small subset of amino acids, which in general designates which PTase modifies the CaaX sequence). This approach revealed that for each pyrophosphate analog, both FTase and GGTase-I exhibit unique patterns of reactivity among various CaaX sequences. Our laboratory has also developed a tagging-via-substrate proteomic method to identify farnesylated proteins within cells. The aim of this research focuses not only on extending our current techniques into the realm of geranylgeranylation in order to study the enzymatic requirements of GGTase-I, but also on developing cellular probes that would allow for the identification of geranylgeranylated proteins.

The initial goal of this project was to advance our knowledge of GGTase-I substrate specificity in terms of both prenyl and protein substrates and to investigate GGTase-I versus FTase substrate specificity. The overall goal of this project was the development of biologically useful chemical tools that could in the future be developed into proteomic probes for GGTase-I in order to identify and characterize geranylgeranylated proteins.
CHAPTER 1. INTRODUCTION

1.1 Posttranslational Modifications

Posttranslational modifications are chemical modifications that proteins undergo following their biosynthesis resulting in enhanced protein biodiversity. The human genome predicts 30,000 genes; however, due to posttranslational modifications, the human proteome is hypothesized to contain 300,000 – 3,000,000 different forms of proteins.\(^1\) The dynamicity of proteins and their ability to change in the presence of various cellular stimuli via posttranslational modifications are what dictate various cellular functions and activities.

Posttranslational modifications can be classified into two general groups: covalent modifications and covalent cleavage. The majority of covalent modifications (e.g. acetylation, glycosylation, methylation, lipidation, phosphorylation, and ubiquitination) can be categorized in accordance with the type of amino acid side chain modified, the extent of reversibility of the modification, and classification of the modifying enzyme.\(^1\) Covalent cleavage or hydrolytic cleavage of peptide backbones in proteins is catalyzed by proteases or, less commonly, by autocatalytic cleavage.\(^1\)

Protein lipidation is a posttranslational modification that aids in membrane targeting by covalently attaching at least one lipid anchor to a protein. Many proteins involved in human diseases are modified by the covalent linkage of fatty acids or isoprenoid groups. The attachment of hydrophobic groups to proteins aids in regulating protein structure and function.
Major types of protein lipidation include: N-myristoylation, S-acetylation, S-prenylation, palmitylation, and the attachment of glycosyl phosphotidylinositol anchors.¹

### 1.2 Protein Prenylation

After protein prenylation’s first appearance in the late 1970’s in fungal peptide pheromones, it was realized in the late 1980’s that the Ras superfamily of proteins underwent protein prenylation, a type of posttranslational modification, which localized the proteins to the plasma membrane.²⁻³ Many proteins require prenylation in order to be biologically functional; these proteins include critical proteins such as the small Ras and Rho GTPase superfamilies, nuclear lamins A and B, and the kinesin motor proteins CENP-E and F.⁴

Prenylation, a type of lipidation, occurs on a cysteine four residues from the C-terminus. Prenylated proteins contain a C-terminal “CaaX box” sequences, where ‘C’ denotes cysteine, ‘a’ is typically an aliphatic amino acid, and ‘X’ represents a small subset of amino acid residues.⁵ Proteins containing a CaaX box are recognized by prenyl transferases located in the cytosol. The prenyl transferase enzymes catalyze the formation of a thioether bond between the cysteine residue of the CaaX box and isoprenyl lipids.⁶ There are three categories of prenyltransferases in mammalian cells: farnesyl transferase (FTase), geranylgeranyl transferase-I (GGTase-I), and geranylgeranyl transferase-II (GGTase-II aka RabGGTase). FTase and GGTase-I are CaaX prenyltransferases. FTase catalyzes the covalent attachment of a 15-carbon farnesyl isoprenoid (farnesyl pyrophosphate, FPP)⁷ while GGTase-I catalyzes the attachment of a 20-carbon geranylgeranyl isoprenoid (geranylgeranyl pyrophosphate, GGPP) to cysteine.⁸ Both isoprenoid chains are derived from the mevalonate pathway. In comparison, GGTase-II catalyzes the attachment of two 20-carbon geranylgeranyl isoprenoids to a C-terminal CXC or CC motif.⁹

The three types of protein prenylation modifications are shown in Figure 1.1. After covalent
attachment of the isoprenoid(s), the protein then relocates to the endoplasmic reticulum where it undergoes proteolytic cleavage of the “-aaX” residues by the endoprotease Ras-converting enzyme-1 (Rce-1). To eliminate the charge of the free carboxylate, the C-terminal cysteine is methyl-esterified by isoprenylcysteine carboxyl methyl transferase (Icmt), utilizing S-adenosyl methionine as the methyl donor. Upon completion of these modifications, the newly isoprenylated protein can be anchored in the membrane and regulate various cellular functions (Figure 1.2) such as cell signaling (Ras & others), cell division (CENP-E & CENP-F), and organelle structure (lamins).

Figure 1.1. Post-translational modifications of CaaX or CXC-containing peptides by prenyltransferase enzymes: A) FTase; B) GGTase-I; C) RabGGTase.
1.3 Ras Family of Proteins

It has been estimated that approximately 0.5-2% of all mammalian proteins are prenylated, but roughly only 60 proteins have been identified thus far. Of the known prenylated proteins, many exhibit a plethora of cellular functions including cell signaling, cell mobility, cell division, organelle structure, and vascularization. Due to its diverse functionalities, it is not surprising that protein prenylation is currently being explored as possible treatments not only for cancer but for a wide variety of other diseases such as neurodegradation, Progeria, arteriosclerosis, as well as parasitic and viral infections.
The RAS superfamily of GTPases is a well-studied class consisting of over one hundred small monomeric G proteins that act as molecular switches. Members of this superfamily include the Ras family of proteins: H-Ras, N-Ras, & K-Ras (K-Ras4a & K-Ras4b). In addition to prenylation, all three isoforms of Ras require additional modifications for proper membrane localization. H-Ras and N-Ras both require palmitoylation for membrane localization while K-Ras does not need to be alternatively lipidated. Instead, K-Ras has a polylysine sequence located near the prenylated cysteine residue that offers additional membrane affinity for localization to the plasma membrane.

Ras acts as a molecular switch and cycles between an active-GTP bound and an inactive-GDP bound state that is controlled predominately by guanine nucleotide exchange factors (GEFS). In its active-GTP bound state Ras localizes to the inner leaflet of the plasma membrane where it can interact with other cofactors and initiate a signaling cascade. At the plasma membrane, growth factors bind to membrane-bound receptor tyrosine kinases (RTK) which results in RTK phosphorylation. This allows for docking proteins, such as growth factor receptor-bound protein 2 (GRB2), to bind to the phosphorylated RTK via Src-homology 2 (SH2) domains. Next, via an SH3 domain, GRB2 can associate with Son of Sevenless (SOS), a GEF, thus activating SOS. Activated SOS converts the membrane-anchored, inactive-GDP bound Ras to the active-GTP bound Ras. Activated Ras can then associate with, and in turn activate, Raf-1 which leads to the upregulation of cell growth and proliferation via the MAPK pathway (Figure 1.2).

Oncogenic Ras is stabilized in an active GTP-bound state that promotes cellular signaling and, consequently, tumorigenesis. The constitutive activation of these proteins was linked to a single point mutation in the Ras gene and implicates the Ras proteins in a number of cancers. Approximately 30% of all tumors have an activating mutation in one of the RAS genes with high incidence rates in pancreatic (~90%), colon (~50%), lung (~40%) and acute myeloid leukemia.
cancers (~20%).

The constitutive activation of Ras contributes to deregulation of tumor-cell growth, programmed cell death, invasiveness, and angiogenesis. Moreover, Ras farnesylation is required for proper cell signaling and was implicated as the cause of oncogenic transformations in cells.

1.4 Targeting Protein Prenylation & Alternative Prenylation in Cancer

When it was discovered that the Ras family of proteins is farnesylated and their function depends on their association with the inner face of the plasma membrane, FTase as a drug target along with its biochemical mechanisms became of great interest among the scientific community. Over the past two decades, several FTase inhibitors (FTIs) have been developed and evaluated as potential cancer therapeutics in an effort to treat Ras-dependent tumors. These FTIs include: 1) FPP analogs (non-peptidomimetic) that compete with the isoprenoid substrates, 2) peptidomimetic inhibitors that mimic the CaaX sequence of target proteins such as Ras, and 3) bisubstrate analogs which mimic both the isoprenoid and the CaaX sequence.

Several FTIs showed promising results, both in vitro and in vivo, in preclinical experiments with low nanomolar IC$_{50}$ values; however, clinical trials brought about more questions than answers. These studies revealed that the effectiveness of FTIs to treat Ras-dependent tumors is reliant upon which isoform of Ras is overactive. FTIs are general effective toward H-Ras diseases. This is a consequence of the fact that H-Ras can only be prenylated by FTase. Unfortunately, the majority of Ras-dependent tumors have a mutation in either the N- or K-Ras isoforms; in particularly, K-Ras4B is the main oncogenic form of Ras. It was discovered that both of these isoforms of Ras can be alternatively prenylated by GGTase-I. Due to alternative prenylation, N- and K-Ras tumors do not respond to FTI treatment.
Moreover, preclinical studies brought about the revelation that although FTIs are not effective for treating the Ras-dependent tumors they were originally envisioned for (due to alternative prenylation), FTIs were effective toward some Ras-independent tumors (e.g. breast cancer, chronic & acute myeloid leukemia, multiple myeloma, and advanced myelodyplastic syndrome). Presumably, these results are due to the prenylation of one or more essential proteins required for tumorigenesis. This finding sparked the quest to identify “Protein X,” the true target(s) responsible for the observed antitumor effects in Ras-independent tumors. The identity of the proteins(s) responsible for the observed antitumor affect in Ras-independent tumors remains elusive.

1.5 The CaaX Prenyltransferase Enzymes

In the early 1990’s, two enzymes were identified that catalyze the CaaX protein prenylation reactions: farnesyltransferase and geranylgeranyltransferase-I. As previously mentioned, the primary functions of these cytosolic enzymes are to covalently attach isoprenoid chains to the sulfur of a C-terminal cysteine via a thioether linkage. This posttranslational modification serves as a method to localize and anchor proteins to cellular membranes, or other cellular locations, to ensure proper cellular functioning.

FTase catalyzes the transfer of a 15-carbon isoprenoid onto a cysteine residue. Mammalian FTase is a heterodimeric protein consisting of a 46 kD α-subunit and a 48 kD β-subunit that, as revealed by X-ray crystal structure, are predominantly alpha-helical. The active site of this enzyme is located in a groove found between the α- and β-subunits; thus, both subunits are essential for substrate binding and catalysis. For protein recognition, FTase uses a C-terminal “CaaX” motif. Generally, the “X” residues control enzyme-substrate recognition between FTase and GGTase-I. The “X” residues that designate farnesylation are serine, methionine, or
glutamine. On the other hand, GGTase-I recognizes protein substrates where the “X” residues are typically leucine, phenylalanine, and occasionally methionine. A list of some known farnesylated and geranylgeranylated proteins can be found in Table 1.1.

Mammalian GGTase-I is also a heterodimeric zinc metalloenzyme consisting of a 46 kD α-subunit and a 43 kD β-subunit that are predominantly alpha-helical. In fact, FTase and GGTase-I have the same α-subunit but differ in their β-subunits. The extensive interface at the α- and β-subunits essentially buries ~20% of the accessible surface area of each subunit. Unlike most subunit interfaces, the α/β-interface of both FTase and GGTase-I exhibits greater hydrophilic character resulting in nearly double the number of hydrogen bonds.

Although the β-subunits share only ~25% sequence homology, they have very similar structures consisting of 14 α-helices in FTase and 13 α-helices in GGTase-I (Figure 1.3). The α-α barrel shown in Figure 1.3C is made up of twelve of these α-helices. The core of this barrel consists of six parallel helices while the other six helices are parallel with each another but antiparallel in regard to the core helices and form the outside of the barrel. In both enzymes, one end of the α-α barrel is open to solvent while the opposing end is blocked by a loop formed from the C-terminal residues of the β-subunit. This conformation creates the active site which is a deep, funnel-shaped cavity in the center of the barrel. The active site cavity has a depth of 14Å and a diameter of 15Å.
1.5.1 Mechanism of Catalysis and Kinetics of CaaX Prenyltransferases

Both FTase and GGTase-I have been identified in a number of various species including mammals, protists, plants, and fungi. The CaaX prenyltransferases are essential for the function of these organisms and elimination of these enzymes have severe detrimental effects and in some instances, lethality.\textsuperscript{50, 52-54} As mentioned previous, both FTase and GGTase-I are cytosolic heterodimeric zinc metalloenzymes with unique, yet similar, kinetics of binding and catalytic mechanisms. In fact, all three prenyltransferases have a conserved prenyl binding site and have an ordered binding mechanisms.\textsuperscript{6, 8, 55} The catalytic cycle begins with the binding of the pyrophosphate substrate (FPP or GGPP depending on the enzyme) to the active site located at the interface of the α- and β-subunits. Following FPP/GGPP binding, the CaaX protein or peptide binds to the active site to form a ternary complex.\textsuperscript{8, 56, 57} It is believed that the CaaX protein binds to the active site as the thiol but is rapidly deprotonated and binds tightly to the zinc atom as the thiolate.\textsuperscript{58-60}

In the case of FTase, the zinc atom is embedded within the active site in the β-subunit and coordinates to three important amino acids in the β-subunit (D297 β, C299 β, and H362 β) and is located 2.5 Å from the cysteine sulfur of the CaaX box.\textsuperscript{6, 8} The Zn\textsuperscript{2+} atom is present in

---

**Figure 1.3.** Structural representation of FTase (A) and GGTase-I (B) with the α-subunit shown in red. (C) Overlay of the β-subunits of FTase (blue) and GGTase-I (yellow).\textsuperscript{6}
stoichiometric amounts and has been shown to be crucial for enzyme activity.\textsuperscript{58, 61} In addition to Zn\textsuperscript{2+}, Mg\textsuperscript{2+} is also present in the mechanism of action. Although it is not essential for enzyme catalysis, Mg\textsuperscript{2+} has been shown to enhance the rate of FTase activity by several hundred fold.\textsuperscript{62} It is believed that the catalytic role of Zn\textsuperscript{2+} in farnesylation is to generate and coordinate to the cysteine thiolate, while the Mg\textsuperscript{2+} plays the important role of positioning the FPP substrate prior to catalysis.\textsuperscript{56, 63, 64} There are also implications that Mg\textsuperscript{2+} aids in the stabilization of the diphosphate leaving group that results from the chemical lipidation with farnesyl pyrophosphate.\textsuperscript{62} Taken together, Zn\textsuperscript{2+} and Mg\textsuperscript{2+} ensure the efficient function of FTase by properly orienting FPP and forming/activating the cysteine thiolates (\textbf{Figure 1.4}).

Analogous to FTase, the Zn\textsuperscript{2+} atom in GGTase-I also coordinates to three strictly conserved residues (D269β, C271β, and H321β), as well as to the thiolate group of the cysteine residue of the CaaX box.\textsuperscript{8} Kinetic studies revealed that although FTase requires millimolar amounts of Mg\textsuperscript{2+} for full catalytic efficiently, GGTase-I is Mg\textsuperscript{2+} independent.\textsuperscript{65} It has been hypothesized that FTase requires Mg\textsuperscript{2+} in order to stabilize the negative charge on the phosphate group that develops as the bond between the α-phosphate and the C\textsubscript{1} atom of the farnesyl group breaks.\textsuperscript{62} In fact, structural studies show that the Mg\textsuperscript{2+} coordinates to residue D352β of FTase and the pyrophosphate moiety of FPP.\textsuperscript{6} Sequence alignment of FTase and GGTase-I revealed that the D352β residue of FTase corresponded to a lysine residue in GGTase-I (K311β).\textsuperscript{8} FTase and GGTase-I superimposition revealed that the lysine residue of GGTase-I adopts a conformation that positions the positively charged side chain amine (N\textsubscript{ε}) at the site of the Mg\textsuperscript{2+} in FTase.\textsuperscript{6} Mutagenesis studies confirm these theories.\textsuperscript{63, 66} Mutating the D352β residue of FTase to a lysine abolished Mg\textsuperscript{2+} dependence while mutating the K311β of GGTase-I to either alanine or aspartate introduced Mg\textsuperscript{2+} dependence (\textbf{Figure 1.4}).\textsuperscript{66}

After the binding of the pyrophosphate and CaaX protein to the enzyme, the following chemical step proceeds quickly via a mechanism that is still highly controversial. This chemical
The rate limiting step of protein prenylation is the product release. To induce product release, a second molecule of the isoprenoid pyrophosphate must bind to the active site. This causes the newly added prenyl motif of the CaaX protein to move into the “exit groove” located in the β-subunit where it is then transferred to the endoplasmic reticulum for further modifications by RCE-1 and ICMT (Figures 1.5 & 1.6).

**Figure 1.4.** Transition state model of protein prenylation. Hydrogen bonds are shown as dashed red lines. Red amino acid residues correspond to GGTase-I & blue amino acid residues correspond to FTase. (Figure adapted from Lane & Beece).
Table 1.1. Summary of FTase & GGTase-I requirements.\textsuperscript{6}

<table>
<thead>
<tr>
<th></th>
<th>Farnesytransferase (FTase)</th>
<th>Geranylgeranyltransferase-I (GGTase-I)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid Donor Substrate</strong></td>
<td>Farnesyl diphosphate (FPP) - 15 Carbon</td>
<td>Geranylgeranyl diphosphate (GGPP) - 20 Carbons</td>
</tr>
<tr>
<td><strong>Protein Recognition Motif</strong></td>
<td>Caax</td>
<td>Caax</td>
</tr>
<tr>
<td></td>
<td>X = Ala, Gln, Ser, Met, Phe</td>
<td>X = Leu, Phe, sometimes Met</td>
</tr>
<tr>
<td><strong>Select Protein Substrates</strong></td>
<td>Ras, nuclear lamins, Transducin y subunit, Rhodopsin kinase, centromeric proteins</td>
<td>Rho, Rac, Rap, heterotrimeric G protein y subunits</td>
</tr>
<tr>
<td><strong>Subunit Composition (mammalian)</strong></td>
<td>48 kDa (α)</td>
<td>identical</td>
</tr>
<tr>
<td></td>
<td>46 kDa (β)</td>
<td>25% identical</td>
</tr>
<tr>
<td></td>
<td>43 kDa (β)</td>
<td></td>
</tr>
<tr>
<td><strong>Metal requirements</strong></td>
<td>( \text{Zn}^{2+}, \text{Mg}^{2+} )</td>
<td>( \text{Zn}^{2+} )</td>
</tr>
<tr>
<td></td>
<td>( \text{Zn}^{2+} ) required for catalysis and peptide binding</td>
<td></td>
</tr>
<tr>
<td><strong>Reaction</strong></td>
<td>![Reaction Diagram]</td>
<td>![Reaction Diagram]</td>
</tr>
</tbody>
</table>
Figure 1.5. Reaction pathway of farnesylation (PDB: 1FT1, 1FT2, 1K2P, 1K2O).\textsuperscript{6}

Figure 1.6. Reaction pathway of geranylgeranylation (PDB: 1N4P, 1N4Q, 1N4S)
1.5.2 Comparing the Isoprenoid Binding Pockets of FTase & GGTase-I

Interestingly, FTase is capable of binding to geranyl pyrophosphate (GPP, 10 carbon isoprenoid chain) and GGPP. In fact, FTase can catalyze reactions with either GPP or FPP, although GPP is a much poorer substrate than FPP. On the other hand, it has been shown that GGPP is a competitive inhibitor of FTase. When bound to FTase, GGPP hinders proper CaaX binding/alignment in such a manner that catalysis cannot occur. Conversely, FPP can act as a substrate for GGTase-I, albeit with 300-fold less affinity than GGPP. These findings can be partially explained by taking a closer look at the enzymes’ active sites.

The major portion of the enzyme that interacts with the isoprene diphosphate is the β-subunit. As mentioned previously, the β-subunits of FTase and GGTase-I are only ~25% homologous; however, the portion of the β-subunits that interact with the isoprene are strikingly similar (Figure 1.7A). Comparing the binding pockets of FTase and GGTase-I shows that FPP binds to FTase in an extended conformation, but GGPP binds in such a way that the last isoprene unit of GGPP is nearly perpendicular with respect to the rest of the molecule within the active site (Figure 1.7B). This can be attributed to the fact that W102β and W106β in FTase-I correspond to T49β and F53β, respectively, in GGTase-I. The differences in the amino acids within the active site allow for a larger binding pocket in GGTase-I. Thus, this could explain why GGPP acts as a competitive inhibitor of FTase. The isoprenoid chain of GGPP can be recognized by and bind into the FTase binding pocket; however, due to the longer chain of GGPP, the diphosphate head group cannot orientate properly to coordinate with the zinc ion (Figures 1.7C & D). Therefore, the geranylgeranyl isoprenoid chain cannot be transferred to the Cys residue of a protein to form the thioether linkage via FTase.
Figure 1.7. Comparison of FTase & GGTase-I β-subunits within the binding pocket. Amino acid residues that interact with the isoprene unit are shown as sticks; FPP (red) and GGPP (pink). (A) Overlay of FTase (blue) and GGTase-I (yellow) reveals several identical amino acids (green). (B) Unique amino acids of the β-subunits within the binding pocket are shown. (C) Surface view of FTase. A much smaller binding pocket is available due to W102β and W106β. GGPP does not fit properly into the cavity. (D) Surface view of GGTase-I. The W102β and W106β of FTase-I correspond to T49β and F53β in GGTase-I allowing for a larger isoprenoid pocket. (PDB: 1N4P (FTase) and 1K2O (GGTase)).
While the reaction mechanisms of the prenyltransferases have been vastly studied and are well understood, there are many unanswered questions pertaining to their \textit{in vivo} function(s). Bioinformatic analysis predicts that there are hundreds of prenylated proteins, but only a small percentage of proteins have been experimentally confirmed. Thus, the true number of prenylated proteins, referred to as the prenylome, remains unknown. Moreover, FTase and GGTase-I have been shown to display overlapping substrate specificities with a subset of substrates; however, the \textit{in vivo} extent and the physiological significance of this overlap have been studied on a very limited number of substrates.

The identification of the elusive “Protein-X targets” can be accomplished via a variety of different techniques. One such method is the use of prenyltransferase inhibitors (PTIs) to evaluate the role of individual proteins; however, this approach can be inconvenient due to the non-specific nature of PTIs. It is presumed that FTIs and GGTIs (GGTase-I inhibitors) affect farnesylation and geranylgeranylation, respectively, on a global level. Hence, upon inhibitor administration, monitoring the cellular effect(s) of a single protein would be an extremely difficult and daunting task when taken into consideration that it is predicted ~2% of all mammalian proteins are prenylated (i.e. 6,000-60,000 proteins).

Fortunately, there are other more attractive approaches that our laboratory has taken advantage of in the past. One such approach is the use of unnatural pyrophosphate analogs. These unnatural analogs can be very beneficial when they behave as either selective substrates or selective inhibitors of specific CaaX proteins. For example, if we could design and synthesize a GGPP analog that was a selective inhibitor for K-Ras, we could evaluate the role of geranylgeranyl-KRas in cells without the interference of other geranylgeranylated proteins.
Another approach utilized for elucidating the biological effects of one prenylated protein from another is with the use of farnesylated pyrophosphate analogs containing either a fluorophore and/or an affinity tag (Figure 1.8). Such analogs provide researchers with a means to purify farnesylated proteins from cell lysates and/or a method to visualize farnesylated proteins in vivo. While PTIs are a great tool to study global prenylation, selective FPP/GGPP analogs and affinity-labeled FPP/GPP analogs are far superior chemical tools to aid in the elucidation of the prenylome and “protein-X”. In the past, these approaches have been largely focused on farnesylation; hence, unique GGPP analogs are still largely unexplored.

Figure 1.8. Examples of farnesylated pyrophosphates containing affinity tags or fluorophores utilized in the past.

Many of the modifications made to the FPP scaffold that our laboratory has investigated are shown in Figure 1.9. These analogs have been extensively screened with various libraries of dansylated-CaaX containing peptides and many show activity in our in vitro fluorescence assays as either substrates or inhibitors. Only three of the eight modifications shown have been extended to develop analog libraries of GGPP. With the exception of the 3-substituted analogs, the modified GGPP analogs show similar activity/selectivity trends in reference to their FPP counterparts. Thus, the remaining five modifications shown in Figure 1.9 (blue) provide an
opportunity to develop novel GGPP analogs that could provide insight into protein geranylgeranylation. By developing selective GGPP analogs in combination with an affinity tag and/or fluorophore for purification and/or visualization would provide investigators with novel chemical tools for monitoring protein geranylgeranylation in vivo.

**Figure 1.9.** Modifications of the farnesyl scaffold explored by our laboratory. Green: These modifications have been evaluated on the geranylgeranyl scaffold. Blue: These modifications have yet to be investigated utilizing a geranylgeranyl scaffold allowing for new opportunity.

1.7 *In vitro* Fluorescence Screening Assay for FTase & GGTase-I Activity

In order to determine the activity of FTase and GGTase-I, a fluorescence-based approach was originally developed by Pompliano and coworkers and later modified by Poulter and colleagues. This approach takes advantage of two facts: 1) the minimal recognition sequence of FTase and GGTase-I is the tetrapeptide CaaX sequence, and 2) the unique properties of the
dansyl fluorophore. In a simple assay buffer solution, the dansyl group emits at a wavelength of 550 nm with a 340 nm excitation; however, upon lipidation of a fluorescently labeled peptide, the fluorophore undergoes a blue shift to an emission of 505 nm and also leads to a large increase in fluorescence (Figure 1.10). Although it is not quite understood how the increase in hydrophobic environment elicits such a response, it has been proven that these assays are robust and reproducible. Due to the added hydrophilic character, prenylation can be confirmed by HPLC. With our laboratories optimized conditions, the unprenylated proteins have a retention time of ~1-2 minutes whereas the prenylated product has a retention time of ~23 minutes. Therefore, by utilizing pentapetides of the form dansyl-GCaaX, we can observe the extent of protein prenylation by monitoring the change in fluorescence of the dansyl group that results from lipidation.

**Figure 1.10.** Single cuvette fluorometric assay.
1.8 Protein Prenylation & Other Diseases

Due to the vastness of protein prenylation, it is not surprising that recent studies have brought to light prenylation as a key player in several diseases. While protein prenylation is most well-known and investigated for its pivotal role in cancer, it has also been implicated or could be a potential target in a variety of other diseases such as neurodegeneration (e.g. Alzheimer’s),
atherosclerosis/restenosis,
angio genesis,
retinal degradation,
prenature aging (e.g. Hutchinson-Gilford progeria syndrome), osteoporosis,
parasitic disease (e.g. malaria),
Hepatitis δ virus, Costello Syndrome, renal disease, and asthma. It is important to remember that protein prenylation is present in all types of cells and a key regulator of a variety of cellular functions. Thus, identifying prenylated proteins and understanding their individual cellular roles will aid researchers to determine the best approach to a given disease and perhaps even uncover novel therapeutic targets. The first step is designing selective substrates or inhibitors of this cellular pathway to be used as chemical tools to probe the prenylome.

1.9 Significance of Work

The prenylome is believed to consist of 60-600 thousand proteins; however, only a small percentage of these proteins have been confirmed. Moreover, there is a grey area to protein prenylation in which a subset of known proteins can be prenylated by either FTase or GGTase-I. This can make therapeutic intervention difficult for diseases reliant on prenylation. For example, FTIs were originally designed for Ras driven tumors; however, K-Ras, the major form of oncogenic Ras, can be geranylgeranylated by GGTase-I, rendering FTIs ineffective. On the other hand, Hutchinson-Gilford progeria syndrome and Costello syndrome are genetic disorders caused by mutations of the LMNA (lamin A) and H-RAS genes, respectively. Both lamin A and H-Ras are farnesylated proteins; thus, FTIs are being explored as treatment options for these disorders.
and preliminary findings are promising. While FTIs and GGTIs have the potential to be highly effective therapeutic treatments, researchers still lack the ability to determine which diseases FTIs and GGTIs would be effective against. Hence, possessing tools that can identify prenylated proteins, unravel their biological significance, and/or determine which prenyltransferase is responsible for said prenylation would be of great significance.

Much work has been focused on the elucidation of farnesylated proteins. The use of unnatural FPP analogs as selective substrates or inhibitors of FTase has been employed in an effort to reveal each farnesylated proteins’ biological impact. The development of selective geranylgeranylationing substrates or inhibitors would allow researchers to evaluate one or a few proteins at a time. Thus, these analogs should greatly increase the amount of information obtained from a single biological assay about a given proteins’ physiological significance.

The overall goal of this research is to identify geranylgeranylated proteins and to evaluate the enzymatic requirements of FTase and GGTase-I utilizing unnatural GGPP analogs. Based on the crystallographic analysis of FTase and GGTase-I by the Beese laboratory, as well as our laboratory’s success with the development of unnatural FPP analogs, we have synthesized a small library of unnatural GGPP analogs with frame-modifications. This library can be classified into four subgroups: saturated, frame-shifted, alkynyl-tagged, and ω-modified GGPP analogs.
CHAPTER 2. SYNTHESIS & BIOCHEMICAL EVALUATION OF ARYL-MODIFIED GERANYLGERANYL PYROPHOSPHATE ANALOGS

2.1 Introduction

In the past, structural studies of FTase by the Beese group have unveiled a hydrophobic binding pocket rich with aromatic amino acid residues such as Tyr, Trp, and Phe. Such findings sparked many researchers, our group included, to explore the possibility of pi-pi stacking interactions between these aromatic amino acids and FPP analogs containing aromatic motifs. Many of the previously synthesized aromatic FPP analogs have aryl-modifications at the terminal isoprene and have displayed some interesting biochemical results (Figure 2.1). While these aryl-modifications have been greatly explored as FTase substrates and inhibitors, little remains known of these modifications in GGTase-I binding ability.

Previously, our laboratory has concentrated on generating GGPP analogs containing substitutions either at the 3 position, the 7 position, or both. Although some of these analogs have been shown to act as efficient substrates of GGTase-I, others have high nanomolar IC\text{50} values. The synthesis of aryl-modified analogs would allow us to investigate greater structural diversity in GGPP analogs. Although some of the aromatic residues (W102, Y361) in FTase correspond to non-aromatic residues in GGTase-I (T49, F53, respectively) in order to allow for a more spacious binding pocket to accommodate the longer isoprene chain of GGPP, structural studies of GGTase-I have revealed that it too has a hydrophobic binding pocket abundant with aromatic residues (Figure 2.2). The potential of aryl-containing GGPP analogs to participate in
pi-pi stacking interactions with the aromatic amino acid residues of the GGTase-I binding pocket prompted us to synthesize and evaluate a small library of aryl-modified GGPP analogs.

When considering which analogs to evaluate, our goal was to select analogs that best mimicked the terminal isoprene unit (Figure 2.3). In order to do this, we overlaid several potential compounds with GGPP in the GGTase-I binding pocket using PyMol (Figure 2.5). The two analogs that best simulate the isoprene unit were 2.6d and 2.6e, which both contain methyl-substituted benzene rings. The addition of a methyl substituent on the aromatic ring allows the molecule to mimic both terminal CH$_3$ groups of the isoprene unit. Analogs 2.6a and 2.6b also aligned well with GGPP. While they lack the extra CH$_3$ of 2.6d-e, both of these analogs provide the double bond of the terminal isoprene unit. In comparison, 2.6a-b may provide great insight into the need or lack thereof the methyl substitution of the aromatic ring. Analog 2.6e was included to determine if hydrophobic bulk would be sufficient to bind to GGTase-I or, as we hypothesize, aromaticity would be more beneficial.

Due to its unique characteristics such as small size and high electronegativity, fluorine has been used to alter physical properties and binding interactions.\textsuperscript{104} In general, addition of fluorine or fluorinated groups results in an increase in lipophilicity of organic molecules, especially aromatic compounds.\textsuperscript{105} Fluorine also has three sets of lone-pair elections that it can share with electron-deficient atoms such as acidic hydrogens bound to heteroatoms. More recently, polar C-F bond-protein interactions have been shown to be crucial in stabilizing fluorine-containing compounds and their protein targets. These types of interactions have been found between C-F bonds and polar functional groups such as carbonyls (C-F···C=O) and guanidinium ion moieties (C-F···C(NH$_2$)(=NH)) of amino acid side chains.\textsuperscript{105} Thus, it is not surprising that fluorine substituents have been known to enhance binding interactions; therefore, compound 2.6f was chosen to evaluate the effects of electronics on the aromatic ring.
Recently, our lab has synthesized a potent hIcmt inhibitor designated “TAB” (Figure 2.4). Unfortunately, there is no crystal structure of Icmt; however, it is known that both farnesylated and geranylgeranylated proteins bind to hIcmt and are methylated after they are proteolytically cleaved by Rec-1. Thus, it stands to reason that Icmt and the prenyltransferases have similar prenyl-binding pockets. Therefore, we wished to evaluate 2.15 as a potential inhibitor of FTase and GGTase-I.
Figure 2.1. Aryl-modified FPP analogs previously evaluated.\textsuperscript{12, 82}

Figure 2.2. Aromatic rich GGTase-I binding site. Amino acids of the β-subunit that interact with GGPP (cyan) are shown in yellow. (A) Interacting amino acids are shown as sticks; (B) Surface view of binding pocket.
Figure 2.3. Aromatic groups as isoprenoid mimics.

Figure 2.4. Comparing protein prenylation with our laboratory’s nanomolar Icmt inhibitor. Blue box represents isoprenoid mimic and purple circle corresponds to the protein.
Figure 2.5. Overlay of aryl-modified GGPP analogs and GGPP (green) in the GGTase-I binding pocket.
2.2 Synthesis of Aryl-Modified Geranylgeranyl Pyrophosphate Analogs

The synthesis of the aryl-modified GGPP analogs was designed in such a way that all compounds could be generated from a common intermediate, 2.3. Additionally, the availability of a wide variety of commercially available Grignard reagents and benzylic/phenyllic halides in addition to the ease of introduction of the aryl-motifs motivated us to explore this synthetic route. To begin the synthesis, THP-protected farnesol (2.1) underwent oxidation in the presence of SeO\textsubscript{2} followed by a NaBH\textsubscript{4} reduction to generate alcohol 2.2\textsuperscript{107,109}. Next, diethyl chlorophosphate is subjected to a displacement reaction in the presence of 2.2 and DIEA to generate diethyl phosphate 2.3 in 74\% yield. There were a few advantages of choosing this type of intermediate. One advantage to using diethyl phosphate 2.3 is that it can be stored for longer periods of time than the corresponding allylic halides which are unstable and easily degrade. More so, the corresponding allylic halides generally undergo Grignard displacement reactions to give a mixture of S\textsubscript{N}2 and S\textsubscript{N}2’ products usually in fairly equal quantities and isolations of one isomer are not facile.\textsuperscript{110} Thus, with common intermediate 2.3 in hand, a similar method as Snyder & Treitler was employed and a variety of Grignard reagents could be utilized in an S\textsubscript{N}2 displacement reaction to generate the aryl-modified GGPP analogs 2.4 a-f.\textsuperscript{111} These analogs were first deprotected using PPTS in EtOH to generate alcohols 2.5 a-f and then converted into the corresponding pyrophosphates (2.6 a-f) utilizing the method of Davisson \textit{et al.}\textsuperscript{112,113}

The synthesis of the “TAB-pyrophosphate” 2.15 was accomplished according to the procedure of Bergman \textit{et al.}\textsuperscript{106} It began with the conversion of 4-bromobut-1-yne (2.7) to alcohol 2.8 using Negishi’s zirconium-catalyzed asymmetric carbo-alumination (ZACA) reaction.\textsuperscript{114}
Next, alcohol 2.8 was THP-protected using a standard procedure to generate compound 2.9. The second half of the molecule was generated by subjecting biphenyl-iodide 2.10 to the 1-(trimethylsilyl)-1-propyne (2.11) anion followed by TMS deprotection with TBAF to afford alkyne 2.12. Alcohol 2.9 was then converted into the corresponding azide in situ by displacement of the primary bromide with sodium azide. Utilizing standard Cu(I) mediated conditions, biphenyl alkyne (2.12) was then “clicked” with the freshly generated azide resulting in a 1,4-disubstituted 1,2,3-triazole (2.13). These analogs were first deprotected using PPTS in EtOH to generate alcohol 2.14. Halogenations of triazole-containing compounds via standard Corey-Kim conditions using NCS has been revealed to be problematic in the past. Thus, alcohol 2.14 was first converted in to the mesylate and then converted into the corresponding pyrophosphate (2.15) utilizing the method of Davisson et al. \(^{112,113}\)
Scheme 2.1. Synthesis of Aryl-Modified GGPP analogs. (a) i. DHP, PPTS, DCM; ii. SeO$_2$, t-BuOOH, salicylic acid, DCM; ii. NaBH$_4$, EtOH (37% - 3 steps); (b) DIEA, (EtO)$_2$POCl, Et$_2$O (74%); (c) R-MgX, THF, o/n ; (d) PPTS, EtOH, 70°C; (e) NCS, DMS, DCM, 2.5 hr.; (f) (NBu$_4$)$_3$HP$_2$O$_7$, ACN, 3 hr.
Scheme 2.2. Synthesis of “TAB” pyrophosphate. (a) Me₃Al, Cp₂ZrCl₂, DCM, 0°C, 18 hr then (CH₂O)n, 3hr (83%); (b) PPTS, DHP, DCM (79%); (c) i. TMS-propyne, n-BuLi, THF, -78°C; ii. K₂CO₃, MeOH, 12 hr (36% - 2 Steps); (d) NaN₃, CuSO₄·5H₂O, Sodium ascorbate, DMF, 55°C (20%); (e) PPTS, EtOH, 70°C (85%); (f) MsCl, DMAP, DCM, 2.5 hrs; (g) (NBu₃)₂HP₂O₇, ACN, 3 hr (89%).
2.3 Biochemical Evaluation of Aryl-Modified Geranylgeranyl Pyrophosphate Analogs

Previously, our laboratory and others have shown that aryl-modified FPP analogs can behave as substrates or inhibitors of FTase with various CaaX-peptides. Thus, we aimed to explore these modifications when applied to GGTase-I. The aryl-modified GGPP analogs synthesized (2.6a-f & 2.15) were evaluated for their biochemical activity in an in vitro continuous spectrofluorometric assay with GGTase and the co-substrate CaaX-peptide dansyl-GCVLL (the CaaX sequence of cdc42). All biochemical assays were performed in our collaborator Dr. Carol Fierke’s laboratory at the University of Michigan by Elia Wright.

Among the aryl-modified compounds tested, six of the seven compounds synthesized displayed substrate activity. It was not surprising that 2.15 was not a substrate as it was based off of an Icmt inhibitor recently synthesized in our laboratory. As mentioned previously, due to the fact that both farnesyl and geranylgeranyl proteins are further processed by Icmt, we hypothesized that Icmt and the prenyltransferases have similar prenyl-binding pockets (Figure 2.4). Therefore, our original motive for synthesizing this compound was to determine if this isoprene-mimic could also inhibit GGTase-I. Further studies are currently underway to test the ability of 2.15 to act as an inhibitor of protein prenylation.

The remaining six compounds (2.6e-f) all displayed varying degrees of substrate activity. From these results (Figures 2.6 & 2.7) it is evident that chain length plays a role in substrate ability. For example, the homobenzyl analog 2.6a is the same overall length as GGPP and displays substrate activity comparable to GGPP. On the other hand, removing one methylene unit to afford the benzyl analog 2.6b greatly diminished the substrate ability. In the case of both 2.6a and 2.6b, it appears that our original hypothesis proved true. By adding a methyl substituent on the benzene ring to mimic the terminal isoprene, substrate activity increased (2.6d and 2.6e); however, it is important to note that the shorter carbon chain analog (2.6e) still displayed
significantly less substrate ability than analogs that have the same overall carbon chain length as GGPP (e.g. 2.6d).

The results of the remaining two analogs (2.6c and 2.6f) were interesting. We had hypothesized that our aromatic compounds would have the added benefit of being able to participate in additional favorable interactions with the binding site (such as pi-pi stacking). Thus, we believed the aromatic compounds would display a greater degree of activity than a non-aromatic counterpart due to the large number of aromatic residues in the binding pocket of GGTase-I. This did not prove to be true in the case of analog 2.6c. By comparing analogs 2.6c and 2.6b, the only difference is the lack of aromaticity in 2.6c; however, 2.6c displays slightly greater substrate activity than its aromatic counterpart 2.6b. Thus, hydrophobicity may play a greater role than aromaticity; however, further studies are needed before a definite conclusion can be reached.

Due to its small size, high electronegativity, and unique chemical reactivity, fluorine is becoming more and more common place in medicinal chemistry and drug discovery. In fact, fluorine’s unique nature has been linked to enhancing binding interactions, changing physical properties (lipophilicity/solubility), metabolic stability, and selective reactivities. Additionally, the incorporation of fluorine into our laboratory’s “TAB” compound resulted in a more potent Icmt inhibitor. Assuming the Icmt and GGTase-I binding pockets are similar, we hypothesized that a fluorinated aryl-GGPP compound could be beneficial to enzyme activity. Thus, the last analog, 2.6f, was a difluoro compound whose substituent potions were selected based on previous data of a “TAB” derivative. The in vitro analysis revealed that 2.6f displayed substrate activity comparable to GGPP (Figure 2.6) and may even be more efficient as a substrate than GGPP (Figure 2.7).
Figure 2.6. Bar graphs of substrate activity represented in RFI of aryl-modified GGPP analogs 2.6a-d versus GGPP (+ control) with 5 μM dansyl-GCVLL and 50 nM GGTase-I. Error bars represent mean ± SD (n = 3). *Values given are for 10 μM analog at 2 hours.

Figure 2.7. Bar graphs of substrate activity represented in RFI of aryl-modified GGPP analogs 2.6e-f and 2.15 versus GGPP (+ control) with 5 μM dansyl-GCVLL and 50 nM GGTase-I. Error bars represent mean ± SD (n = 3). *Values given are for 10 μM analog at 2 hours.
Figure 2.8. Monitoring continuous changes in fluorescence of aryl-modified GGPP analogs versus GGPP (+ control) at various concentrations (1 µM, 5 µM, & 10 µM) with 5 µM dansyl-GCVLL and 50 nM GGtase-I. Experiments were performed in triplicate and data points represent the mean.
2.4 Conclusions

The goal of this aim was to focus on the synthesis of a small library of aryl-modified GGPP analogs. While many aromatic derivatives of FPP have been synthesized and investigated in the past, these modifications had yet to be extended into the realm of GGTase-I. Additionally, the majority of the FPP analogs previously explored contained either an amine or ether linkage between the isoprene chain and the aromatic moiety.

This aim focused on designing a robust synthetic route which would allow us to quickly and efficiently produce a small library of aryl-modified analogs (2.6a-f). Moreover, this route allowed us to produce compounds that contain an all carbon backbone which more effectively mimics the natural isoprenoid chain of GGPP. The synthesis was dependent on the allylic diethyl phosphate 2.3 which proved to be more stable than the corresponding allylic halide and could be stored over time. This allowed us to produce larger quantities of the common intermediate 2.3 which could rapidly be converted into several aryl-modified GGPP analogs.

Upon in vitro biochemical evaluation of these analogs, it was discovered that all of these analogs displayed substrate activity; however, the length of the carbon chain plays an important role. Having two methylene units between the aromatic ring and the terminal double bond (homobenzyl moiety) provides compounds that have substrate ability comparable to GGPP. By decreasing the methylene unit to one (benzyl moiety) the substrate activity was greatly reduced. Another important factor that increased substrate activity was the introduction of fluorine substituents on the homobenzyl ring (2.6f). This resulted in an analog that appears to be comparable to GGPP. Unfortunately, the analogs were tested at only three concentrations (1, 5, & 10 µM) and many of the reactions did not reach completion at 5 or 10 µM of analog (Figure 2.8). In order to determine accurate $k_{cat}$ and $K_m$ values, these analogs are currently being retested at more concentrations.
Moreover, we also synthesized an aromatic analog (2.15) that was based on a nanomolar inhibitor of Icmt previously developed in our laboratory. If the Icmt and GGTase-I isoprenoid binding pockets are similar, then 2.15 has the potential of being an inhibitor of GGTase-I. As expected, the in vitro substrate assay revealed that 2.15 did not display substrate activity; further testing is currently underway to determine whether or not 2.15 is an inhibitor of GGTase-I.

We have successfully synthesized a library of aryl-modified GGPP compounds in which the ω-isoprene units has been replaced by an aromatic group. The preliminary biochemical evaluation of these compounds revealed several intriguing results. At a later date, these compounds will be screened with an expansive library of dansyl-GCaaX peptides to determine if these analogs globally or selectively geranylgeranylate proteins.
2.5 Experimental Procedures Utilized for the Synthesis & Biochemical Evaluation of Aryl-Modified Geranylgeranyl Pyrophosphate Analogs

**General Experimental Procedures:** All reactions were performed with oven-dried or flame-dried glassware and under dry argon gas. All commercial reagents and solvents were used directly without subsequent purification. For the organometallic coupling reactions, anhydrous THF was freshly distilled from sodium and benzophenone. All other anhydrous solvents were purchased from Acros Organics as extra dry solvents and were bottled over molecular sieves. Reactions were monitored by thin layer chromatography and visualized with one or more of the following: UV light, iodine, vanillin solution, potassium permanganate solution, dinitrophenylhydrazine solution, and/or phosphomolybdic acid solution. All products were purified using flash chromatography silica gel 60 M purchased from Macherey-Nagel. All reactions involving either triphenyl phosphine or triphenyl phosphine oxide were first dry-loaded with sodium sulfate before column purification. All NMR spectra were taken either on a 300 MHz Bruker ARX300 or a 500 MHz Bruker DRX500 spectrometer. Low-resolution MS (EI/CI) were recorded with a Hewlett Packard Engine and low-resolution MS (ESI) were taken on a Thermoquest LCQ. All high-resolution mass spectra were recorded on a FinniganMAT XL95.

(2E,6E,10E)-2,6,10-trimethyl-12-((tetrahydro-2H-pyran-2-yl)oxy)dodeca-2,6,10-trien-1-ol

(2.2a):

To an Erlenmeyer flask charged with \( t \)-BuOOH (2.0 eq, 40 mmol, 70% in water) and 60 mL of CH\(_2\)Cl\(_2\) is added MgSO\(_4\). The solution was then filtered into a round bottom flask equipped with a magnetic stir bar. Next, SeO\(_2\) (0.12 eq, 2.4 mmol) and salicylic acid (0.5 eq, 10 mmol) were added to the reaction mixture. While the solution stirred, THP-protected farnesol, 2.1 (1.0 eq, 20 mmol) was added dropwise to the reaction vessel. The reaction mixture was
allowed to stir for 12 hours. The CH\textsubscript{2}Cl\textsubscript{2} was removed and the resulting residue is resuspended in Et\textsubscript{2}O. Next, 10\% NaOH was added to solution, the organic layer was removed, and the aqueous layer was extracted 3 × Et\textsubscript{2}O (40 mL). The organic layers are combined, washed with brine, dried with MgSO\textsubscript{4}, filtered, and concentrated. In an oven-dried round bottom flask, the crude reaction product was diluted with 80 mL of ethanol and NaBH\textsubscript{4} (1.0 eq, 20 mmol) was added in several portions to the reaction vessel. The reaction was allowed to stir for 1 hour and then diluted with 200 mL of water. The aqueous layer was extracted 3 × Et\textsubscript{2}O (40 mL) and the organic layers were combined, washed 2 × H\textsubscript{2}O, washed with brine, dried with MgSO\textsubscript{4}, filtered, and concentrated. The crude reaction product was purified by column chromatography using 15\% Ethyl Acetate/Hexanes as the mobile phase to afford 2.2 in 37\% yield. \textsuperscript{1}H NMR (300 MHz, Chloroform-d) δ 5.41 – 5.25 (m, 2H), 5.08 (t, J = 6.3 Hz, 1H), 4.59 (t, J = 3.4 Hz, 1H), 4.19 (dd, J = 11.9, 6.4 Hz, 1H), 4.06 – 3.91 (m, 3H), 3.85 (ddd, J = 11.1, 7.4, 3.3 Hz, 1H), 3.47 (dt, J = 10.4, 4.7 Hz, 1H), 2.24 – 1.90 (m, 7H), 1.81 – 1.24 (m, 17H). \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ 140.07 (s), 134.77 (s), 134.68 (s), 125.62 (s), 124.07 (s), 120.53 (s), 97.54 (s), 68.67 (s), 63.50 (s), 62.11 (s), 39.48 (s), 39.18 (s), 30.55 (s), 26.09 (s), 26.02 (s), 25.39 (s), 19.44 (s), 16.31 (s), 15.89 (s), 13.59 (s).

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\textsuperscript{1}H NMR (300 MHz, Chloroform-d) δ 5.41 – 5.25 (m, 2H), 5.08 (t, J = 6.3 Hz, 1H), 4.59 (t, J = 3.4 Hz, 1H), 4.19 (dd, J = 11.9, 6.4 Hz, 1H), 4.06 – 3.91 (m, 3H), 3.85 (ddd, J = 11.1, 7.4, 3.3 Hz, 1H), 3.47 (dt, J = 10.4, 4.7 Hz, 1H), 2.24 – 1.90 (m, 7H), 1.81 – 1.24 (m, 17H). \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ 140.07 (s), 134.77 (s), 134.68 (s), 125.62 (s), 124.07 (s), 120.53 (s), 97.54 (s), 68.67 (s), 63.50 (s), 62.11 (s), 39.48 (s), 39.18 (s), 30.55 (s), 26.09 (s), 26.02 (s), 25.39 (s), 19.44 (s), 16.31 (s), 15.89 (s), 13.59 (s).}
\end{tikzpicture}
\end{center}

Diethyl ((2E,6E,10E) - 2,6,10 - trimethyl -12-(tetrahydro-2H-pyran-3-y1)oxy) dodeca-2,6,10-trien-1-yl) phosphate (2.3a):

To a round bottom flask equipped with a magnetic stir bar was added alcohol 2.2 (1.0 eq, 8.1 mmol) in 10 mL of CH\textsubscript{2}Cl\textsubscript{2}. Next, Et\textsubscript{3}N (7 eq, 56.7 mmol) was added to the reaction vessel and the mixture was cooled to 0°C, where diethyl chlorophosphate (5.5 eq, 44.8 mmol) was added dropwise to the reaction. The reaction was allowed to warm to room temperature and stirred for 12 hours. Next, 10\% NH\textsubscript{4}Cl\textsubscript{(aq)} was added to solution, the organic layer was removed,
and the aqueous layer was extracted $3 \times \text{Et}_2\text{O}$ (20 mL). The organic layers are combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was purified by column chromatography using 50% Ethyl Acetate/Hexanes as the mobile phase to afford diethyl phosphate 2.3 in 74% yield. NMR: $^1\text{H}$ NMR (300 MHz, Chloroform-d) $\delta$ 5.46 (t, $J = 6.4$ Hz, 1H), 5.33 (t, $J = 5.6$ Hz, 1H), 5.09 (t, $J = 6.1$ Hz, 1H), 4.67 – 4.54 (m, 1H), 4.36 (d, $J = 6.9$ Hz, 2H), 4.21 (dd, $J = 11.9$, 6.4 Hz, 1H), 4.16 – 3.93 (m, 5H), 3.87 (ddd, $J = 11.0$, 6.9, 3.7 Hz, 1H), 3.49 (ddd, $J = 11.3$, 6.3, 3.5 Hz, 1H), 2.27 – 1.89 (m, 9H), 1.69 – 1.62 (m, 6H), 1.63 – 1.41 (m, 8H), 1.31 (td, $J = 7.1$, 1.0 Hz, 6H). $^{13}\text{C}$ NMR (75 MHz, Chloroform-d) $\delta$ 140.28, 134.79, 130.54 (d, $J = 6.9$ Hz), 129.94, 124.47, 120.76, 97.94, 73.42 (d, $J = 5.8$ Hz), 63.81, 63.77, 63.73, 62.44, 39.72, 39.12, 30.87, 26.52, 26.43, 25.65, 19.79, 16.58, 16.35, 16.26, 16.14, 13.74. $^{31}\text{P}$ NMR (122 MHz, Chloroform-d) $\delta$ -0.25.

**Representative procedure for the synthesis of 2.5a-c utilizing commercially available Grignard reagents:**

![Diagram](https://via.placeholder.com/150)

2-(((2E,6E,10E)-3,7,11-trimethyl-13-phenyltrideca-2,6,10-trien-1-yl)oxy)tetrahydro-2H-pyran (2.4a):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added diethyl phosphate 2.3 (1.0 eq, 0.5 mmol) dissolved in 2 mL of THF and cooled to 0°C. Next, benzylmagnesium bromide (2 M in THF, 5 eq, 2.5 mmol) was added dropwise to the reaction mixture and the reaction was allowed to stir for 12 hours. The reaction was quenched with 10% NH$_4$Cl, the organic layer was removed, and the aqueous layer was extracted $3 \times \text{Et}_2\text{O}$ (5 mL). The organic layers are combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was purified by column chromatography using 5%
Ethyl Acetate/Hexanes as the mobile phase to afford 2.4a in 76% yield. NMR: $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 7.33 – 7.23 (m, 2H), 7.24 – 7.14 (m, 3H), 5.38 (t, $J = 6.9$ Hz, 1H), 5.13 (q, $J = 6.6$ Hz, 2H), 4.64 (t, 1H), 4.26 (dd, $J = 11.9$, 6.4 Hz, 1H), 4.04 (dd, $J = 11.9$, 7.4 Hz, 1H), 3.91 (ddd, $J = 11.1$, 7.3, 3.3 Hz, 1H), 3.52 (dt, $J = 10.5$, 4.8 Hz, 1H), 2.81 – 2.65 (m, 2H), 2.33 – 2.22 (m, 2H), 2.19 – 2.02 (m, 6H), 2.02 – 1.92 (m, 2H), 1.92 – 1.79 (m, 1H), 1.70 (s, 3H), 1.67 (s, 3H), 1.63 (s, 1H), 1.61 (s, 3H), 1.59 – 1.47 (m, 4H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 142.66, 140.45, 135.36, 134.61, 128.55, 128.38, 125.81, 124.90, 124.12, 120.75, 97.96, 63.83, 62.46, 41.84, 39.84, 39.80, 34.99, 30.91, 26.80, 26.49, 25.71, 19.83, 16.64, 16.35, 16.22.

2-(((2E,6E,10E)-3,7,11-trimethyl-12-phenyldodeca-2,6,10-trien-1-yl)oxy)tetrahydro-2H-pyran (2.4b):

Yield: 46%. NMR: $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 7.34 – 7.24 (m, 2H), 7.23 – 7.13 (m, 3H), 5.38 (t, $J = 7.3$ Hz, 1H), 5.24 (t, $J = 6.3$ Hz, 1H), 5.13 (t, $J = 6.4$ Hz, 1H), 4.71 – 4.57 (m, 1H), 4.25 (dd, $J = 11.9$, 6.4 Hz, 1H), 4.04 (dd, $J = 11.9$, 7.4 Hz, 1H), 3.90 (ddd, $J = 11.2$, 7.1, 3.8 Hz, 1H), 3.58 – 3.45 (m, 1H), 3.28 (s, 2H), 2.08 (tdd, $J = 16.4$, 8.7, 5.2 Hz, 8H), 1.69 (s, 3H), 1.67 – 1.44 (m, 10H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 140.46, 135.25, 134.46, 129.00, 128.34, 126.57, 126.05, 124.29, 120.76, 97.97, 63.84, 62.48, 46.44, 39.83, 30.92, 26.82, 26.49, 25.71, 19.83, 16.64, 16.19, 15.99.

2-(((2E,6E,10E)-12-cyclohexyl-3,7,11-trimethylldodeca-2,6,10-trien-1-yl)oxy)tetrahydro-2H-pyran (2.4c):

Yield: 54%. NMR: $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.38 – 5.30 (m, 1H), 5.15 – 4.91 (m, 2H), 4.64 – 4.55 (m, 1H), 4.21 (dd, 1H), 4.00 (dd, $J = 11.9$, 7.4 Hz, 1H), 3.87 (ddd, $J = 11.2$, 7.1, 3.8 Hz, 1H), 3.60 – 3.36 (m, 1H), 2.19 – 1.90 (m, 7H), 1.88 – 1.77 (m, 3H), 1.73 – 1.34
Representative procedure for the synthesis of 2.4d-f utilizing freshly synthesized Grignard reagents:

![diagram](image)

2-(((2E,6E,10E)-3,7,11-trimethyl-13-(o-tolyl)trideca-2,6,10-trien-1-yl)oxy)tetrahydro-2H-pyran (2.4d):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added magnesium powder (40 eq, 20 mmol) and 6 mL of anhydrous Et₂O and an iodide chip was added to the round bottom and the mixture was stirred for 5 minutes. Next, 2-methylbenzyl chloride (10 eq, 5 mmol) in 2 mL of Et₂O was added dropwise to the reaction vessel over a 10 minute period and the reaction mixture was stirred for 4 hours. After the allotted time, the reaction mixture was cooled to 0°C where diethyl phosphate 2.3 (1.0 eq, 0.5 mmol) dissolved in 2 mL of THF was added dropwise to the mixture and the reaction was allowed to stir for 12 hours. The reaction was quenched with 10% NH₄Cl, the organic layer was removed, and the aqueous layer was extracted 3 × Et₂O (5 mL). The organic layers are combined, washed with brine, dried with MgSO₄, filtered, and concentrated. The crude reaction product was purified by column chromatography using 5% Ethyl Acetate/Hexanes as the mobile phase to afford 2.4d in 61% yield. NMR: ¹H NMR (300 MHz, Chloroform-d) δ 7.20 – 6.99 (m, 4H), 5.44 – 5.32 (m, 1H), 5.21 – 5.04 (m, 2H), 4.62 (dd, ̂J = 4.3, 2.7 Hz, 1H), 4.24 (dd, ̂J = 11.8, 6.4 Hz, 1H), 4.03 (dd, ̂J = 11.9, 7.4 Hz, 1H), 3.89 (ddd, ̂J = 11.1, 7.6, 3.3 Hz, 1H), 3.62 – 3.46 (m, 1H), 2.73 – 2.58 (m, 2H), 2.31 (s, 3H), 2.25 – 2.17 (m, 2H), 2.16 – 1.93 (m, 8H), 1.91 – 1.78 (m, 1H), 1.68 (s, 6H), 1.60 (s,
3H), 1.59 – 1.47 (m, 5H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 140.89, 140.50, 136.00, 135.40, 134.96, 130.27, 128.96, 126.08, 126.01, 124.77, 124.17, 120.75, 97.99, 63.86, 62.50, 40.59, 39.87, 39.83, 32.58, 30.93, 26.86, 26.52, 25.71, 19.84, 19.47, 16.67, 16.41, 16.25.

2-(((2E,6E,10E)-3,7,11-trimethyl-12-(m-tolyl)dodeca-2,6,10-trien-1-yloxy)tetrahydro-2H-pyran (2.4e):

Yield (45%). NMR: $^1$H NMR (300 MHz, Chloroform-d) δ 7.20 – 7.11 (m, 1H), 6.99 (t, $J$ = 9.2 Hz, 3H), 5.37 (t, $J$ = 6.3 Hz, 1H), 5.23 (t, $J$ = 6.3 Hz, 1H), 5.12 (t, $J$ = 6.2 Hz, 1H), 4.62 (dd, $J$ = 4.2, 2.6 Hz, 1H), 4.24 (dd, $J$ = 11.8, 6.4 Hz, 1H), 4.03 (dd, $J$ = 11.9, 7.4 Hz, 1H), 3.89 (ddd, $J$ = 11.2, 7.3, 3.7 Hz, 1H), 3.51 (ddd, 1H), 3.23 (s, 2H), 2.32 (s, 3H), 2.20 – 1.98 (m, 7H), 1.85 (ddd, $J$ = 17.9, 8.1, 5.4 Hz, 2H), 1.68 (s, 3H), 1.63 – 1.46 (m, 11H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 140.56, 140.42, 137.80, 135.26, 134.52, 129.78, 128.20, 126.78, 126.41, 126.00, 124.23, 120.75, 97.92, 63.80, 62.44, 46.36, 39.81, 30.89, 26.84, 26.47, 25.69, 21.60, 19.80, 16.62, 16.19, 15.97.

2-(((2E,6E,10E)-13-(3,4-difluorophenyl)-3,7,11-trimethyltrideca-2,6,10-trien-1-yloxy)tetrahydro-2H-pyran (2.4f):

Yield (65%). NMR: $^1$H NMR (300 MHz, Chloroform-d) δ 7.08 – 6.95 (m, 2H), 6.89 – 6.78 (m, 1H), 5.34 (tq, $J$ = 7.6, 6.0, 1.4 Hz, 1H), 5.14 – 4.99 (m, 1H), 4.60 (dd, $J$ = 4.5, 2.6 Hz, 1H), 4.22 (dd, $J$ = 11.9, 6.4 Hz, 1H), 4.00 (dd, $J$ = 11.9, 7.4 Hz, 1H), 3.87 (ddd, $J$ = 11.1, 7.3, 3.5 Hz, 1H), 3.49 (ddd, $J$ = 10.6, 5.5, 3.6 Hz, 1H), 2.62 (dd, $J$ = 9.3, 6.6 Hz, 2H), 2.29 – 1.72 (m, 11H), 1.66 (s, 3H), 1.63 – 1.42 (m, 11H). $^{19}$F NMR (282 MHz, Chloroform-d) δ -112.23 (ddd, $J$ = 20.7, 11.5, 8.2 Hz), -115.88 – -116.29 (m).
Representative procedure for the THP-deprotection of 2.5a-f:

(2E,6E,10E)-3,7,11-trimethyl-13-phenyltrideca-2,6,10-trien-1-ol (2.5a):

In a scintillation vial equipped with a magnetic stir bar, 2.4a (1.0 eq, 0.38 mmol) was dissolved in 5.0 mL of absolute EtOH and PPTS (0.1 eq, 0.038 mmol) was added to the vial. The reaction mixture was heated to 75°C and stirred for 12 hours. Next, the reaction was cooled to room temperature where it was poured into a separator funnel containing water and Et₂O and the aqueous layer was extracted 3 × 5 mL Et₂O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using 15% Ethyl Acetate/Hexanes as the mobile phase to afford 2.5 a in 65% yield. NMR: ^1^H NMR (300 MHz, Chloroform-d) δ 7.33 – 7.24 (m, 2H), 7.24 – 7.15 (m, 3H), 5.43 (tq, J = 7.0, 5.4, 2.7, 1.3 Hz, 1H), 5.13 (tq, J = 8.1, 6.8, 5.6, 1.4 Hz, 2H), 4.16 (d, 2H), 2.76 – 2.66 (m, 2H), 2.34 – 2.21 (m, 2H), 2.21 – 1.92 (m, 8H), 1.70 (s, 3H), 1.67 (s, 3H), 1.61 (s, 3H). ^1^C NMR (75 MHz, Chloroform-d) δ 142.59, 139.89, 135.42, 128.48, 128.32, 125.75, 124.80, 123.93, 123.43, 59.51, 41.77, 39.71, 39.67, 34.91, 26.72, 26.43, 16.43, 16.29, 16.14. MS (EI) m/z [M⁺+H]; MS (CI) m/z [M⁺+H].

(2E,6E,10E)-3,7,11-trimethyl-12-phenyldodeca-2,6,10-trien-1-ol (2.5b):

Yield (53%). NMR: ^1^H NMR (300 MHz, Chloroform-d) δ 7.28 (ddd, J = 7.7, 6.3, 1.6 Hz, 2H), 7.24 – 7.13 (m, 3H), 5.47 – 5.36 (m, 1H), 5.29 – 5.21 (m, 2H), 5.17 – 5.09 (m, 2H), 4.16 (d, J = 6.9 Hz, 2H), 2.27 – 1.86 (m, 9H), 1.69 (s, 7H), 1.62 (s, 3H), 1.54 (s, 3H). ^1^C NMR (75 MHz, Chloroform-d) δ 140.65, 139.98, 135.38, 134.50, 129.01, 128.35, 126.54, 126.06, 124.16, 123.51, 59.59, 46.43, 39.79, 39.74, 26.81, 26.50, 16.51, 16.19, 15.99. MS (EI) m/z 298 [M⁺]; 280 [M⁺-H₂O]; MS (CI) m/z 281 [(M⁺+H)-H₂O].
(2E,6E,10E)-12-cyclohexyl-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (2.5c):

Yield (59%). NMR: $^1$H NMR (300 MHz, Chloroform-$d$) δ 5.41 (td, $J = 6.6, 6.2, 1.7$ Hz, 1H), 5.10 (t, 1H), 5.03 (t, 1H), 4.15 (d, $J = 6.8$ Hz, 2H), 2.05 (tt, $J = 15.2, 5.8$ Hz, 7H), 1.82 (d, $J = 7.1$ Hz, 2H), 1.75 – 1.48 (m, 15H), 1.34 – 1.02 (m, 5H), 0.91 – 0.59 (m, 2H). $^{13}$C NMR (75 MHz, Chloroform-$d$) δ 139.95, 135.46, 133.69, 125.52, 123.90, 123.41, 59.52, 48.17, 39.88, 39.68, 37.88, 35.59, 33.40, 26.84, 26.76, 26.67, 26.56, 26.43, 16.42, 16.11. MS (EI) m/z 304 [M$^+$]; 286 [M$^+$-H$_2$O]; MS (Cl) m/z 309 [(M$^+$+H)-H$_2$O].

(2E,6E,10E)-3,7,11-trimethyl-13-(o-tolyl)trideca-2,6,10-trien-1-ol (2.5d):

Yield (61%). NMR: $^1$H NMR (300 MHz, Chloroform-$d$) δ 7.19 – 7.04 (m, 4H), 5.41 (tq, $J = 7.0, 5.5, 2.6, 1.3$ Hz, 1H), 5.20 – 5.03 (m, 2H), 4.14 (d, $J = 6.9$ Hz, 2H), 2.74 – 2.60 (m, 2H), 2.31 (s, 3H), 2.26 – 2.15 (m, 2H), 2.15 – 1.89 (m, 8H), 1.67 (s, 6H), 1.60 (s, 3H). $^{13}$C NMR (75 MHz, Chloroform-$d$) δ 140.85, 139.98, 135.97, 135.50, 134.98, 130.25, 128.94, 126.07, 126.00, 124.72, 124.03, 123.48, 59.58, 40.57, 39.80, 39.76, 32.55, 26.82, 26.51, 19.46, 16.50, 16.39, 16.22. MS (EI) m/z 308 [M$^+$-H$_2$O]; MS (Cl) m/z 309 [(M$^+$+H)-H$_2$O].

(2E,6E,10E)-3,7,11-trimethyl-12-(m-tolyl)dodeca-2,6,10-trien-1-ol (2.5e):

Yield (71%). NMR: $^1$H NMR (300 MHz, Chloroform-$d$) δ 7.22 – 7.09 (m, 1H), 6.98 (t, $J = 8.8$ Hz, 3H), 5.47 – 5.38 (m, 1H), 5.29 – 5.20 (m, 1H), 5.18 – 5.08 (m, 1H), 4.14 (d, $J = 7.0$ Hz, 2H), 3.25 (d, $J = 10.4$ Hz, 2H), 2.32 (s, 3H), 2.19 – 1.91 (m, 8H), 1.68 (d, $J = 1.3$ Hz, 3H), 1.61 (d, $J = 1.4$ Hz, 3H), 1.53 (d, $J = 1.3$ Hz, 3H). $^{13}$C NMR (75 MHz, Chloroform-$d$) δ 140.54,
Representative procedure for the synthesis of pyrophosphates 2.6a-f.

To an oven-dried round bottom flask equipped with a magnetic stir bar was added NCS (2.5 eq, 0.39 mmol) in 0.8 mL of CH$_2$Cl$_2$ and cooled to -30°C where dimethyl sulfide (2.5 eq, 0.39 mmol) was added dropwise to the reaction. Following the addition, the mixture is then placed in a 0°C ice bath and stirred for 5 minutes before being recooled back to -30°C. Next, alcohol 2.5a (1 eq, 0.16 mmol) is dissolved in 0.3 mL of CH$_2$Cl$_2$ and added dropwise to the
reaction mixture. The mixture is then placed in a 0°C ice bath and stirred for 2.5 hours coming to room temperature. After the allotted time, brine is added to the reaction mixture and the organic layer was extracted. The aqueous layer was further extracted 3 x 5 mL CH₂Cl₂ and the organic layers were combined, dried with magnesium sulfate, and concentrated. The crude reaction product was used immediately in the following step.

To another oven-dried round bottom flask equipped with a magnetic stir bar was added tris (tetrabutylammonium) hydrogen pyrophosphate (3.0eq, 0.47 mmol) dissolved in 2.0 mL of acetonitrile. Next, a solution of crude allylic chloride dissolve in 1.4 mL acetonitrile was added dropwise to the reaction vessel. The reaction was stirred for 3 hours at room temperature and then the solvent was removed by rotary evaporation at 34°C. The residue was dissolve in a minimal amount of ion exchange NH₃HCO₃ buffer (700mg NH₃HCO₃, 1 L of deionized H₂O, 20 mL of isopropanol) and the resulting solution was passed through a Dowex AG 50 x 8 ion exchange column (2 x 8 cm) using the NH₃HCO₃ buffer as an eluent and 25 mL was collected in a flask. The resulting solution was lyophilized for 3-5 hours. The resulting residue was then redissolved in deionized watered and purified by cellulose flash column chromatography (3 x 15 cm) using isopropanol:deionized H₂O:acetonitrile: NH₃HCO₃ buffer (500 mL : 250 mL : 250 mL : 4 g) as the eluent. In a beaker was collected 40 mL of eluent, then twenty-four 2.5 mL fractions were collected. Typically, fractions 12-18 were collected and the organic solvents were removed by rotary evaporation at 34°C. The resulting solution was then lyophilized to afford pyrophosphate (2.6a) as a white fluffy solid in 58% yield. 

^1H NMR (500 MHz, Deuterium Oxide) δ 7.02 – 6.91 (m, 3H), 6.92 – 6.81 (m, 2H), 5.35 (s, 1H), 5.11 (s, 2H), 4.40 (s, 2H), 2.42 (s, 2H), 1.95 (d, J = 45.4 Hz, 10H), 1.80 (s, 3H), 1.62 (s, 6H). ^31P NMR (202 MHz, Deuterium Oxide) δ -10.77 (d, J = 19.3 Hz), -13.91 (d, J = 17.9 Hz). HRMS 471.1706 [M+2H]⁺, calculated 471.1702 (C₂₂H₃₅O₇P₂).
(2E,6E,10E)-3,7,11-trimethyl-12-phenyldodeca-2,6,10-trien-1-yl diphosphate (2.6b):

Yield (51%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 6.99 (s, 2H), 6.92 (s, 3H), 5.51 – 5.01 (m, 3H), 4.44 (s, 2H), 3.19 (s, 2H), 2.03 (d, $J = 41.5$ Hz, 8H), 1.68 (s, 3H), 1.58 (s, 3H), 1.55 (s, 3H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -10.06, -13.94. HRMS 457.1554 [M+2H]$^+$, calculated 547.1545 (C$_{21}$H$_{31}$O$_7$P$_2$).

(2E,6E,10E)-12-cyclohexyl-3,7,11-trimethylldodeca-2,6,10-trien-1-yl diphosphate (2.6c):

Yield (60%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 5.41 (s, 1H), 5.14 (s, 1H), 5.08 (s, 1H), 4.44 (s, 2H), 2.03 (d, $J = 53.2$ Hz, 8H), 1.83 (d, $J = 6.9$ Hz, 2H), 1.69 (s, 3H), 1.69 – 1.59 (m, 6H), 1.60 (s, 3H), 1.55 (s, 3H), 1.29 – 1.04 (m, 5H), 0.81 (d, $J = 11.5$ Hz, 2H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -12.36, -14.33. HRMS 463.2022 [M+2H]$^+$, calculated 463.2015 (C$_{21}$H$_{37}$O$_7$P$_2$).

(2E,6E,10E)-3,7,11-trimethyl-13-(o-tolyl)trideca-2,6,10-trien-1-yl diphosphate (2.6d):

Yield (75%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 6.80 (s, 4H), 5.36 (s, 1H), 5.01 (s, 2H), 4.42 (s, 2H), 2.39 (s, 2H), 2.24 – 1.79 (m, 13H), 1.64 (s, 3H), 1.47 (s, 3H), 1.43 (s, 3H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -11.24 (d, $J = 19.1$ Hz), -13.98 (d, $J = 17.6$ Hz). HRMS 485.1860 [M+2H]$^+$, calculated 485.1858 (C$_{23}$H$_{35}$O$_7$P$_2$).
(2E,6E,10E)-3,7,11-trimethyl-12-(m-tolyl)dodeca-2,6,10-trien-1-yl diphosphate (2.6e):

Yield (87%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 6.81 (s, 3H), 6.70 (s, 2H), 5.39 (s, 1H), 5.09 (d, $J = 26.3$ Hz, 2H), 4.43 (s, 2H), 2.98 (s, 2H), 2.09 (s, 3H), 2.00 (s, 8H), 1.65 (s, 3H), 1.50 (s, 3H), 1.36 (s, 3H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -10.17 (d, $J = 16.2$ Hz), -13.92 (d, $J = 16.2$ Hz). HRMS 471.1704 [M+2H]$,^2$ calculated 471.1702 (C$_{22}$H$_{33}$O$_7$P$_2$).

(2E,6E,10E)-13-(3,4-difluorophenyl)-3,7,11-trimethyltrideca-2,6,10-trien-1-yl diphosphate (2.6f):

Yield (94%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 6.82 (s, 1H), 6.69 (d, $J = 26.5$ Hz, 2H), 5.36 (s, 1H), 4.99 (s, 1H), 4.94 (s, 1H), 4.41 (s, 2H), 2.44 (s, 2H), 2.00 (s, 2H), 1.91 (s, 6H), 1.78 (s, 2H), 1.63 (s, 3H), 1.47 (s, 6H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -9.98 (d, $J = 13.0$ Hz), -13.75 (d, $J = 10.6$ Hz). HRMS 507.1518 [M+2H]$^2$, calculated 507.1513 (C$_{22}$H$_{31}$F$_2$O$_7$P$_2$).

(E)-5-bromo-3-methylpent-2-en-1-ol (2.8):

Cp$_2$ZrCl$_2$ (0.25 eq, 3.75mmol) was dissolved in 15 mL dichloromethane and the solution was cooled to 0°C, where a Me$_3$Al solution (2.0 M in heptanes, 2.5eq, 37.5mmol) was added dropwise. After stirring the reaction mixture at 0°C for 30 minutes, 4-bromo-1-butyne (1.0 eq, 15 mmol) was diluted in 7.5 mL of dichloromethane and added to the reaction mixture dropwise. The reaction mixture was allowed to slowly warm to room temperature and stirred an additional 12 hours. Next, the reaction mixture was cooled to 0°C, where (CH$_2$O)$_n$ (5 eq, 75 mmol) was added in several portions. The reaction continued to stir for 3 hours after which it was slowly poured into an ice-cold 10% HCl$_{(aq)}$ solution. The solution was then filtered over a pad of Celite
extracted with $3 \times 50$ mL dichloromethane, washed with brine, dried with magnesium sulfated, filtered, and concentrated. Column chromatography (30% Ethyl acetate in Hexanes) afforded 2.8 in 85% yield.

(E)-2-((5-bromo-3-methylpent-2-en-1-yl)oxy)tetrahydro-2H-pyran (2.9): (E)-5-bromo-3-methylpent-2-en-1-ol (1 eq, 31.32 mmol) was dissolved in 100 mL of dichloromethane where PPTS (0.1 eq, 3.13 mmol) and 2,3-dihydropyran (3.0 eq, 94 mmol) were added to the reaction vessel. The reaction was allowed to stir for 1 hour at room temperature and was then concentrated down and loaded directly onto a silica flash column (30% Ethyl acetate in Hexanes) to afford 2.9 in 79% yield. $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.46 – 5.38 (m, 1H), 4.60 (t, $J = 3.5$ Hz, 1H), 4.21 (dd, $J = 12.2$, 6.3 Hz, 1H), 4.02 (dd, $J = 12.2$, 7.3 Hz, 1H), 3.91 – 3.79 (m, 1H), 3.43 (t, $J = 7.5$ Hz, 2H), 2.56 (t, $J = 7.4$ Hz, 2H), 1.89 – 1.71 (m, 1H), 1.67 (s, 3H), 1.64 (d, $J = 1.0$ Hz, 1H), 1.61 – 1.43 (m, 5H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 136.82 (s), 124.04 (s), 98.09 (s), 77.65 (s), 77.23 (s), 76.80 (s), 63.56 (s), 62.58 (s), 42.83 (s), 30.99 (d, $J = 19.6$ Hz), 25.66 (s), 19.81 (s), 16.23 (s).

4-(but-3-yn-1-yl)-1,1'-biphenyl (2.12):

An oven-dried round bottom flask equipped with a magnetic stir bar was charged with 1-(trimethylsilyl)propyne (2.5 eq, 7.5 mmol) dissolved in 9.5 mL of anhydrous THF and cooled to -78°C. Next, n-BuLi (2.5 eq, 7.5 mmol) was slowly added to the mixture and the reaction was allowed to stir 1.5 hours. After the allotted time, 2.10a (1.0 eq, 3 mmol) in 15 mL of THF was added dropwise to the reaction vessel at -78°C. The reaction was allowed to stir for an additional 12 hours after which time the reaction was quenched by the addition of 10% NH$_4$Cl. The organic layer was removed, and the aqueous layer was extracted $3 \times$ Et$_2$O (5 mL). The organic layers
were combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was then dissolved in a saturated K$_2$CO$_3$/MeOH solution and stirred for an additional 4 hours. Next, the reaction was poured into a separator funnel containing water and Et$_2$O and the aqueous layer was extracted 3 × 15 mL Et$_2$O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using 2.5% Ethyl Acetate/Hexanes as the mobile phase to afford 2.12 in 36% yield. NMR: $^1$H NMR (300 MHz, Chloroform-d) δ 7.62 – 7.49 (m, 4H), 7.42 (t, $J = 7.8$ Hz, 2H), 7.37 – 7.26 (m, 3H), 2.88 (t, $J = 7.5$ Hz, 2H), 2.52 (td, $J = 7.5$, 2.6 Hz, 2H), 1.99 (t, $J = 2.6$ Hz, 1H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 139.70, 139.53, 129.04, 128.93, 127.35, 127.22, 83.99, 69.22, 34.67, 20.75.

(E)-4-(2-([1,1'-biphenyl]-4-yl)ethyl)-1-(3-methyl-5-((tetrahydro-2H-pyran-2-yl)oxy)pent-3-en-1-yl)-1H-1,2,3-triazole (2.13):

To a scintillation vial equipped with a magnetic stir bar was added bromide 2.9 (1.0 eq, 0.85 mmol) and 4.5 mL of DMF. Next, NaN$_3$ (3 eq, 2.55 mmol), sodium ascorbate (0.5 eq, 0.43 mmol), and CuSO$_4$·H$_2$O (0.25 eq, 0.21 mmol) are added sequentially to the vial. Alkyne 2.12 (1.2 eq, 1.02 mmol) was then dissolved in 2.3 mL of DMF and added to the vial. The reaction was heated to 70°C and allowed to stir for 36 hours. The reaction was then quenched with 10% NH$_4$Cl and the aqueous layer was extracted 3 × EtOAC (10 mL). The organic layers were combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was purified by column chromatography using 60% Ethyl Acetate/Hexanes as the mobile phase to afford 2.13 in 20% yield. $^1$H NMR (300 MHz, Chloroform-d) δ 7.60 – 7.20 (m, 9H), 7.11 (s, 1H), 5.33 (s, 1H), 4.53 (t, $J = 3.6$ Hz, 1H), 4.39 (t, $J = 7.4$ Hz, 2H), 4.23 – 3.91 (m, 2H), 3.83 (s, 1H), 3.50 (s, 1), 3.03 (q, $J = 3.3$ Hz, 4H), 2.55 (t, $J = 7.4$ Hz, 2H), 1.68 (s, 5H), 1.51 (tq, $J
$= 7.3, 4.3, 2.9$ Hz, $4H$). $^{13}C$ NMR (75 MHz, Chloroform-$d$) $\delta$ 147.27, 141.04, 140.46, 139.12, 135.13, 129.02, 128.86, 127.21, 127.08, 124.46, 120.95, 98.10, 63.42, 62.50, 48.63, 40.26, 35.35, 30.80, 27.58, 25.56, 19.72, 16.41.

(E)-5-(4-(2-((1,1'-biphenyl)-4-yl)ethyl)-1H-1,2,3-triazol-1-yl)-3-methylpent-2-en-1-ol (2.14):

In a scintillation vial equipped with a magnetic stir bar, 2.13 ($1.0$ eq, $0.17$ mmol) was dissolved in $5.0$ mL of absolute EtOH and PPTS ($0.1$ eq, $0.02$ mmol) was added to the vial. The reaction mixture was heated to $75^\circ$C and stirred for 12 hours. Next, the reaction was cooled to room temperature where it was poured into a separator funnel containing water and EtOAc and the aqueous layer was extracted $3 \times 5$ mL EtOAc. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using $5\%$ MeOH/CH$_2$Cl$_2$ as the mobile phase to afford 2.14 in $85\%$ yield. $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.65 – 6.97 (m, 9H), 5.46 – 5.21 (m, 1H), 4.38 (t, $J = 7.2$ Hz, 2H), 4.07 (d, $J = 6.7$ Hz, 2H), 3.03 (h, $J = 1.9$ Hz, 4H), 2.53 (t, $J = 7.2$ Hz, 2H), 1.67 (s, 3H). $^{13}C$ NMR (75 MHz, Chloroform-$d$) $\delta$ 147.41, 147.38, 141.09, 140.50, 140.47, 139.19, 134.52, 129.13, 128.96, 127.32, 127.27, 127.14, 127.09, 121.08, 59.21, 48.66, 40.31, 35.35, 27.57, 16.33. MS (ESI) $m/z$ 348 [M$^+$+H].

(E)-5-(4-(2-((1,1'-biphenyl)-4-yl)ethyl)-1H-1,2,3-triazol-1-yl)-3-methylpent-2-en-1-yl diphosphate (2.15):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added methanesulfonyl chloride ($1.3$ eq, $0.09$ mmol), DMAP ($1.5$ eq, $0.10$ mmol) and $0.4$ mL of dichloromethane. The mixture was cooled to $0^\circ$C and a solution of alcohol 2.14 in $0.2$ mL of
dichloromethane was added dropwise to the reaction vessel and allowed to stir for 2.5 hours. Hexanes were then added to the reaction vessel and the solution was filtered and concentrated. Next, Et₂O was added to the crude product and was again filtered and concentrated. The crude allylic chloride was then converted to the pyrophosphate following the same method described for 2.6a-f. Yield (89%). ¹H NMR (500 MHz, Deuterium Oxide) δ 7.93 – 6.96 (m, 10H), 5.27 (s, 1H), 4.34 (s, 2H), 4.20 (s, 2H), 2.80 (s, 4H), 2.35 (s, 2H), 1.53 (s,3H). ³¹P NMR (202 MHz, Deuterium Oxide) δ -9.89 (d, J = 14.0 Hz), -13.85 (d, J = 18.0 Hz). HRMS 506.1252 [M+2H]⁺, calculated 506.1246 (C₂₂H₂₆N₃O₇P₂).

**General procedure for *in vitro* biochemical substrate screening:**

All biochemical evaluations were performed in our collaborator Dr. Carol Fierke’s laboratory at the University of Michigan by Elia Wright. Preliminary evaluation of all pyrophosphate analogs were performed using GGPP analog (1, 5, or 10 µM), the peptide dansyl-GCVLL (5 µM), recombinant mammalian GGTase-I (50 nM), 50 nM HEPPSO (N-2-hydroxyethylpiperazine-Ν'-2-hydroxypropanesulfonic acid) pH 7.8, 5 mM tris(2carboxyethyl)phosphine (TCEP), and 5 mM MgCl₂ at 25°C in 96 well plates (Corning). Protein prenylation was determined by monitoring the dansylated peptide using a continuous spectrofluorometric assay and all assays were performed in triplicate. Upon prenylation of the peptide, the activity was measured by an increase in fluorescence intensity of the dansyl group (λₑₓ = 340 nm, λₑₘᵦ = 520 nm) in a POLARstar Galaxy plate reader. Peptide in assay buffer was utilized as a negative control and the baseline fluorescence of the peptide was subtracted from the reaction signal.
CHAPTER 3. SYNTHESIS & BIOCHEMICAL EVALUATION OF SATURATED GERANYLGERANYL PYROPHOSPHATE ANALOGS

3.1 Introduction

It is known that the first isoprene is critical for substrate activity. Addition of one methylene unit between the double bond of the first isoprene and the pyrophosphate moiety of FPP converts the native substrate of FTase into an inhibitor. It is still unclear whether or not the other isoprene units are required for substrate activity. In the past, there have been several inhibitors of GGTase-I that contain long saturated hydrocarbon chains with some of these inhibitors displaying submicromolar IC$_{50}$’s. Some of these inhibitors are bisubstrate inhibitors that contain components that mimic both the peptide substrate (Figure 3.1, blue) and the isoprenoid chain (Figure 3.1, red). Such compounds led us to question whether or not the β, γ, and ω isoprene units are essential for enzyme activity (Figure 3.2). To address the query, we synthesized a variety of pyrophosphate analogs in which one or more of the isoprene units were removed.

In order to determine if the α-isoprene is sufficient to produce substrate activity, the first set of analogs synthesized contained compounds in which the β, γ, and ω isoprene units were removed from GGPP and replaced with aliphatic chains (Figure 3.3). Next, analogs that contain only the α- and β-subunits were synthesized. This means that the ω-isoprene is missing in the case of FPP or that both the γ and ω-isoprene units are missing in GGPP. These analogs vary in length between that of FPP and GGPP and will determine if the first two isoprene units are sufficient for substrate activity.
Next, the ω-isoprene unit was re-installed to obtain compounds that lack the β and γ isoprene units. These compounds will examine the importance of the central isoprene units and whether or not they are required for substrate activity. Finally, we synthesized (2E,6E)-10,11,14,15-tetrahydrogeranylgeranyl pyrophosphate and (2E,6E,10E)-14,15-dihydrogeranylgeranyl pyrophosphate (Figure 3.3). The dihydro-GGPP analog lacks the ω double bond but retains the methylene unit at the 15 position (Figure 3.2). The tetrahydro-GGPP analog lacks both the γ and ω double bonds but retains the methylene units at the 11 and 15 positions (Figure 3.2). When compared to the saturated GGPP analogs and GGPP itself, these analogs will aid in determining the significance of the “isopropyl” motif.
Figure 3.1. Inhibitors of GGTase-I that contain saturated hydrocarbon moieties.\textsuperscript{119}
**Figure 3.2.** Conventional method of isoprene unit labeling.

<table>
<thead>
<tr>
<th>Farnesyl Pyrophosphate</th>
<th>Geranylgeranyl Pyrophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /></td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>12 Carbons in length</td>
<td>16 Carbons in length</td>
</tr>
<tr>
<td>15 Carbons overall</td>
<td>20 Carbons overall</td>
</tr>
</tbody>
</table>

**Figure 3.3.** Saturated GGPP analogs synthesized.

- ![Structure](image3) $n = 10 - 12$
- ![Structure](image4) $n = 5 - 8$
- ![Structure](image5) $n = 9 - 10$
- "Dihydro-GGPP"
- "Tetrahydro-GGPP"
3.2 Synthesis of Saturated Geranylgeranyl Pyrophosphate Analogs

The synthesis of the saturated GGPP analogs began with the compounds containing only the α-isoprene unit. The synthesis of these three analogs was simple and straightforward (Scheme 3.1). Briefly, commercially available alkynes 3.1 were subjected to Negishi’s ZACA reaction and quenched with paraformaldehyde to afford alcohols 3.2a-c in 50-58% yields. Next, these alcohols underwent Corey-Kim chlorination with NCS followed by pyrophosphorylation according to the procedure of Davisson et al. to produce the pyrophosphates 3.3a-c in moderate to good yields.112, 113

Next, we focused on the synthesis of analogs that contain only the α- and β-subunits (Scheme 3.2). To synthesize these compounds, commercially available alkynes 3.4 underwent Negishi’s ZACA reaction followed by and iodine quench to provided vinyl iodides 3.5a-d. Previously synthesized bromide 2.8 can then be converted into the organoborane and coupled to vinyl iodides 3.5a-d via a Suzuki coupling reaction to yield alcohols 3.6a-d.120 Following a THP deprotection with PPTS/EtOH and standard chlorination/pyrophosphorylation procedures,112, 113 diphosphates 3.7a-d were obtained in moderate to good yields.

We then turned our attention to the synthesis of analogs where the ω-isoprene unit was reinstalled to obtain compounds that lack the β- and γ-isoprene units (Scheme 3.3). In general, commercially available diol 3.7 was subjected to mono-iodination. With halo-alcohols 3.7 and 3.10 in hand, Swern oxidations afforded aldehydes 3.8 and 3.11, which then underwent Wittig reactions to install the ω-isoprene of 3.9 and 3.12. With these two alkenyl iodides in hand, we turned our attention to synthesizing vinyl iodide 3.14. This was accomplished by first generating the Schwartz’s reagent in situ following a method developed by Huang & Negishi.121 Following the addition of TBDMS-protected but-2-yn-1-ol, hydrozirconation-iodinolysis proceeds to yield vinyl iodide 3.14. Alkyl iodides 3.9 and 3.12 were then converted into their corresponding
organoboranes and coupled to vinyl iodide 3.14 via Suzuki coupling to yield vinyl alcohols 3.15a-b. Utilizing standard chlorination/pyrophosphorylation procedures, diphosphates 3.16a-d were afforded.

The synthesis of tetrahydro-GGPP began with the synthesis of diethyl phosphate 3.18, which was accomplished in a similar manner as 2.3 (Scheme 3.4). The remainder of this synthesis followed the methodology used to synthesize the aryl-modified analogs of Chapter 2. First, Grignard reagent 3.17 was generated from the corresponding bromide followed by the slow addition of diethyl phosphate 3.18 to the reaction lead to a $S_N2$ displacement of the phosphate group. Next, standard THP-deprotection yielded alcohol 3.20. Again, standard chlorination and pyrophosphorylation procedures were utilized to generate diphosphate 3.21.

The final compound of this series, dihydro-GGPP (3.26), was accomplished using a Cu(I)-mediated Grignard displacement of an allylic THP-ether. Briefly, alcohol 3.22 was protected as the THP-ether and then deacetylated using standard protocols. After Grignard reagent 3.24 was generated from the corresponding bromide, it was slowly added to a cooled solution of THP-ether 3.23 and Cu(I)Br to yield alcohol 3.25. It is crucial to keep this reaction at -10°C to avoid degradation of the organocuprate intermediate. Following standard chlorination/pyrophosphorylation procedures, diphosphate 3.26 was afforded in 28% yield.
Scheme 3.1. Synthesis of α-containing pyrophosphates. (a) i. Me$_3$Al, Cp$_2$ZrCl$_2$, DCM 0°C, 18 hr; ii. (CH$_2$O)$_n$, 3 hr; (b) NCS, DMS, DCM, 0°C to rt, 2.5 hr; (c) (NBu$_4$)$_3$HP$_2$O$_7$, ACN, 2.5 hr.

Scheme 3.2. Synthesis of α & β-containing pyrophosphates. (a) i. Me$_3$Al, Cp$_2$ZrCl$_2$, DCM, 0°C, 18 hr, then I$_2$, 3 hr, (b) i. t-BuLi, Et$_2$O, -78°C, ii. β-MeO-9-BBN, THF, -78°C warming to RT o/n, iii. K$_3$PO$_4$, PdCl$_2$(dppf), DMF, 85°C, 18 hr; (c) PPTS, MeOH, 60°C (Yields given for 2 steps); (d) NCS, DMS, DCM, 0°C to rt, 2.5 hr; (e) (NBu$_4$)$_3$HP$_2$O$_7$, ACN, 2.5 hr.
Scheme 3.3. Synthesis of α & ω-containing pyrophosphates. (a) PPh₃, Imidazole, I₂, DCM, 0°C; (b) (COCl)₂, DMSO, Et₃N, DCM, -78°C (28%); (c) i-PrPh₃I, n-BuLi, THF, -78°C; (d) i. Cp₂ZrCl₂, DIBAL, THF, 0°C, 0.5 hr; ii. 3.13, warm to rt, 1.5 hr; iii. I₂, THF, -78°C, 0.5 hr; (e) i. DIEA, TBDMSCl, DCM (95%); ii. DIBAL, Cp₂ZrCl₂, THF, 0°C (40%); (f) i. t-BuLi, Et₂O, -78°C, ii. β-MeO-9-BBN, THF, -78°C warming to RT o/n; iii. K₂PO₄, PdCl₂(dppf), DMF, 85°C, 18 hr; (g) TBAF, THF, 0°C; (h) NCS, DMS, DCM, 0°C to rt, 2.5 hr; (i) (NBu₃)₃, HP₂O₇, ACN, 2.5 hr.
Scheme 3.4. Synthesis of tetrahydro-GGPP. (a) R-MgX, THF, o/n (15%); (b) PPTS, EtOH, 70°C (70%); (c) NCS, DMS, DCM, 2.5 hr.; (d) (NBu₄)₂HP₂O₇, ACN, 3 hr (52% - 2 steps).

Scheme 3.5. Synthesis of dihydro-GGPP. (a) i. DHP, PPTS, DCM; (b) saturated K₂CO₃/MeOH, (74% - 2 steps); (c) 3.24, Cu(I)Br, THF, -10°C, 48 hr (17%); (d) NCS, DMS, DCM, 2.5 hr; (e) (NBu₄)₂HP₂O₇, ACN, 3 hr (28%).
3.3 Biochemical Evaluation of Saturated Geranylgeranyl Pyrophosphate Analogs

In the past, little has been done to investigate the isoprene requirements of GGTase-I. While it is known that the α-isoprene is required for substrate activity, it is unclear if the remaining three isoprenes are essential for enzyme recognition and activity. Therefore, our goal was to synthesize a library of compounds that was lacking the β, γ, or ω isoprene units or lacking a combination of isoprene units. These analogs were designed to range in length between FPP (12 carbons long) and GGPP (16 carbons long). The saturated GGPP analogs synthesized (3.3a-c, 3.7a-d, 3.16a-b, 3.21, & 3.24) were evaluated for their biochemical activity in an in vitro continuous spectrofluorometric assay with GGTase and the co-substrate CaaX-peptide dansyl-GCVLL. All biochemical assays were performed in our collaborator Dr. Carol Fierke’s laboratory at the University of Michigan by Elia Wright.

It appears that all of the saturated compounds synthesized display some degree of substrate activity. The first set of compounds containing only the α-isoprene unit (3.3a-c) revealed that GGTase-I can recognize and utilize these compounds as substrates (Figure 3.4); however, it is evident that the length of the aliphatic carbon chain plays a definite role in the level of enzyme activity. When the carbon chain length is 14 carbons (3.3a) we notice that the substrate activity is greatly diminished when compared to GGPP; however, when the carbon chain length is increased to 16 carbons (3.3c), the same length as GGPP, we see an increase in substrate activity although it is still much less than GGPP.

In order to determine if any substrate activity could be regained, we synthesized compounds that contained both the α- and β-isoprene units (3.7a-d). Indeed, replacing the β-subunit of 3.3a (14 carbons long) to generate 3.7b resulted in a moderate increase of substrate activity (Figure 3.5); however the same modification to 3.3c (16 carbons long) to generate 3.7d
only led to a minimal increase in substrate activity. Although decreasing the length of the carbon chain to 13 carbons seemed to hinder substrate activity, increasing the carbon chain from 14 to 16 carbons did not appear to greatly affect the substrate activity.

The next two analogs tested contained only the α- and ω-isoprene units (3.16a-b; Figure 3.6). These compounds were synthesized for two main reasons: (1) could we increase substrate activity when compared to compounds containing only α-isoprene units and (2) are the two central isoprene units necessary for substrate activity. In fact, replacing the ω-subunit of 3.3c to generate 3.16b resulted in an increase in activity. Similar to the α-isoprenoid analogs, we noticed that by decreasing the length of the carbon chain from 16 carbons (3.16b; the length of GGPP) to 15 carbons (3.16a) resulted in a significant decrease of substrate activity.

The final two compounds analyzed lacked the γ and/or ω isoprene double bonds but retained the methylene units (3.21 & 3.26). Interestingly, when both the γ and ω double bond are saturated the substrate activity greatly decreases (Figure 3.7). When compared to other saturated molecules of the same length (e.g. 3.3a and 3.7d), analog 3.21 is a poorer substrate. One possible explanation for this observation is that the compound has a chiral carbon at position 7 and was synthesized as a mixture of isomers. Thus, it is possible that only one isomer is active. A more likely explanation is molecular geometry. With the double bonds in place, the geometry of the molecule is planar; however, by removing the double bonds the molecular geometry changes from planar to tetrahedral. This change in geometry could lead to unfavorable interaction with the active site of the enzyme and ultimately reduce substrate ability. Analog 3.26 also displayed substrate activity, but is being retested due to an abnormality at 5µM analog.
Figure 3.4. Bar graphs of substrate activity represented in RFI of saturated GGPP analogs 3.3a-c versus GGPP (+ control) with 5 µM dansyl-GCVLL and 50 nM GGtase-I. Error bars represent mean ± SD (n = 3). *Values give are for 10 µM analog at time 1.5 hours.

Figure 3.5. Bar graphs of substrate activity represented in RFI of saturated GGPP analogs 3.7a-d versus GGPP (+ control) with 5 µM dansyl-GCVLL and 50 nM GGtase-I. Error bars represent mean ± SD (n = 3). Values give are for †5 µM or *10 µM analog at time 1.5 hours.
Figure 3.6. Bar graphs of substrate activity represented in RFI of saturated GGPP analogs 3.16a-b versus GGPP (+ control) with 5 µM dansyl-GCVLL and 50 nM GGTase-I. Error bars represent mean ± SD (n = 3). * Values give are for 10 µM analog at time 1.5 hours.

Figure 3.7. Bar graphs of substrate activity represented in RFI of saturated GGPP analogs 3.21 & 3.26 versus GGPP (+ control) with 5 µM dansyl-GCVLL and 50 nM GGTase-I. Error bars represent mean ± SD (n = 3). * Values give are for 10 µM analog at time 1.5 hours.
Figure 3.8. Monitoring continuous changes in fluorescence of saturated GGPP analogs versus GGPP (+ control) at various concentrations (1 µM, 5µM, & 10µM) with 5 µM dansyl-GCVLL and 50 nM GGTase-I. Experiments were performed in triplicate and data points represent the mean.
3.4 Conclusions

The goal of this chapter was to focus on the synthesis of a small library of saturated GGPP analogs in order to determine the isoprene requirements of the enzyme GGTase-I. While various types of FPP and GGPP analogs have been synthesized in the past (Figure 1.9), it was unknown if all of the isoprene units were required for enzyme recognition and activity.

This aim was centered on designing synthetic routes that would enable us to rapidly develop our small library of compounds. Although our laboratory has employed Negishi couplings in the past to generate FPP analogs, this reaction is highly sensitive to moisture and air. Therefore, we wished to develop alternative synthetic routes that utilized less sensitive coupling procedures such as Suzuki couplings.

Considering the number of commercially available alkynes, Negishi’s ZACA reaction was an appealing method for the facile generation of various isoprenoid units. Through the utilization of the ZACA reaction, we were able to synthesize the α-containing analogs 3.3a-c and the vinyl iodides 3.5a-d which underwent Suzuki couplings to produce the α/β-containing analogs 3.7a-d. In order to synthesize the α/ω-containing analogs, an alternative approach was needed. Thus, by utilizing Swern and Wittig reactions we could generate the alkyl halides 3.9 and 3.12 which were converted to organoboranes and subjected to Suzuki coupling to afford 3.16a-b.

The synthesis of the of the tetrahydro-GGPP analog 3.21 was accomplished using similar chemistry as discussed in Chapter 1; however, a different approach was needed to yield the dihydro-GGPP analog 3.24 due to an increase in S$_2$2’ product resulting from a decrease in the steric bulk of the Grignard reagent. Instead, a Cu(I)-mediated THP-ether displacement by a Grignard reagent was utilized.
Upon *in vitro* biochemical testing, it was revealed that all analogs displayed some degree of substrate activity. Generally, if we increase the number of carbons in the chain it results in greater substrate activity. Interestingly, it became evident that the α-isoprene unit is sufficient to warrant enzyme recognition and catalysis; however, increasing the carbon length from 14 to 16 greatly added to the analogs substrate ability. Replacing the β-isoprene unit seems to have the greatest effect on analogs that are shorter than GGPP (less than 16 carbons). For instance, replacing the β-isoprene of 3.3a (14 carbons long) to generate 3.7b had a much greater effect on substrate ability than the same modification of 3.3c (16 carbons long) to generate 3.7d.

When replacing the terminal isoprene to generate α/ω-containing analogs it appears that chain length is essential. Having just one carbon unit short of the length of GGPP is detrimental to the analog’s substrate ability (3.16a); however, with the proper chain length (3.16b), the data suggest that this modification is the most advantageous resulting in an analog comparable to GGPP. By examining the data in Figure 3.8, it appears that analog 3.16b is turned over more quickly than GGPP. That is to say, analog 3.16b reaches its fluorescence maximum more quickly than GGPP. Many of the other compounds seem to turn over more slowly than GGPP or have a lower affinity for the enzyme. Unfortunately, the analogs were tested at only three concentrations (1, 5, & 10 µM) and many of the reactions did not reach completion at 10 µM of analog. In order to determine accurate $k_{cat}$ and $K_m$ values, these analogs are currently being retested at more concentrations.

We have successfully synthesized a library of saturated GGPP compounds in which one or more of the isoprene units has been removed from the molecule. The preliminary biochemical evaluation revealed several intriguing results. At a later date, these compounds will be screened with an expansive library of dansyl-GCaaX peptides to determine if these analogs globally or selectively geranylgeranylate proteins. In addition, these compounds ranged in length between
GGPP and FPP; therefore, we will also be testing these compounds with FTase to determine their ability to be recognized and utilized by one enzyme over another.
3.5 Experimental Procedures Utilized for the Synthesis & Biochemical Evaluation of Saturated Geranylgeranyl Pyrophosphate Analogs

**General Experimental Procedures:** All reactions were performed with oven-dried or flame-dried glassware and under dry argon gas. All commercial reagents and solvents were used directly without subsequent purification. For the organometallic coupling reactions, anhydrous THF was freshly distilled from sodium and benzophenone. All other anhydrous solvents were purchased from Acros Organics as extra dry solvents and were bottled over molecular sieves. Reactions were monitored by thin layer chromatography and visualized with one or more of the following: UV light, iodine, vanillin solution, potassium permanganate solution, dinitrophenylhydrazine solution, and/or phosphomolybdic acid solution. All products were purified using flash chromatography silica gel 60 M purchased from Macherey-Nagel. All reactions involving either triphenyl phosphine or triphenyl phosphine oxide were first dry-loaded with sodium sulfated before column purification. **NOTE: Dry glassware is critical for the organometallic reactions in this publication. This was accomplished by taking oven-dried glassware (dried for at least 24 hr and then cooled under argon) and flame drying the round bottom flask under vacuum. The flask was then purged with argon and cooled to room temperature. This process was repeated 3 times to produce a completely water free environment.**

**Deoxygenated solvents are extremely crucial for successful organometallic reactions in this publication. In order to achieve completely deoxygenated solutions, the solvent was placed in a flame-dried round bottom and under vacuum was sonicated for 30 seconds and then purged with argon for 30 seconds. This process was repeated 3 times to yield completely deoxygenated solvents.**

All NMR spectra were taken either on a 300 MHz Bruker ARX300 or a 500 MHz Bruker DRX500 spectrometer. Low-resolution MS (EI/CI) were recorded with a
Hewlett Packard Engine and low-resolution MS (ESI) were taken on a Thermoquest LCQ. All high-resolution mass spectra were recorded on a FinniganMAT XL95.

**Representative procedure for the synthesis of vinyl alcohols 3.2a-c:**

(E)-3-methyltetradec-2-en-1-ol (3.2a):

\[
\text{Cp}_2\text{ZrCl}_2 (0.25 \text{ eq}, 0.38 \text{ mmol}) \text{ was dissolved in dichloromethane (1.5 mL) and the solution was cooled to 0°C, where AlMe}_3 \text{ solution (2.0 } M \text{ in heptanes, 2.5 eq, 4.5 mmol, 2.25 mL) was added dropwise. After stirring the reaction mixture at 0°C for 30 minutes, 1-tridecyne (1.0 eq, 1.5 mmol) was diluted in dichloromethane (1.0 mL) and added to the reaction mixture dropwise. The reaction mixture was allowed to slowly warm to room temperature and stirred an additional 12 hours. Next, the reaction mixture was cooled to 0°C, where } (\text{CH}_2\text{O})_n (5 \text{ eq, 4.5 mmol, 135 mg}) \text{ was added in several portions. The reaction continued to stir for 3 hours after which it was slowly poured into an ice-cold 10\% HCl(aq) solution. The solution was then filtered over a pad of Celite 545, extracted with 3×10 mL dichloromethane, washed with brine, dried with magnesium sulfated, filtered, and concentrated. Column chromatography (10\% Ethyl acetate in Hexanes) afforded 3.2 in 50\% yield.}^{1}\text{H NMR (300 MHz, CDCl}_3) \delta 5.41 – 5.30 (m, 1H), 4.11 (d, } J = 6.9 \text{ Hz, 2H), 2.01 – 1.91 (m, 2H), 1.62 (s, 3H), 1.23 (s, 18H), 0.84 (t, } J = 6.6 \text{ Hz, 3H); }^{13}\text{C NMR (75 MHz, Chloroform-}d) \delta 140.31, 123.26, 59.53, 39.76, 32.12, 29.88, 29.84, 29.82, 29.75, 29.56, 29.53, 27.89, 22.89, 16.34, 14.31. \text{ MS (EI) } m/z 226 [\text{M}^+]; \text{ MS (CI) } m/z 227 [\text{M}^+ + \text{H}].
(E)-3-methylpentadec-2-en-1-ol (3.2b):

Yield (58%); $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.49 – 5.24 (m, 1H), 4.13 (d, $J = 7.0$ Hz, 2H), 1.98 (t, $J = 7.5$ Hz, 2H), 1.64 (d, $J = 1.3$ Hz, 3H), 1.24 (d, $J = 4.1$ Hz, 20H), 0.86 (t, $J = 6.3$ Hz, 3H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 140.52, 123.25, 59.64, 39.79, 32.15, 29.94, 29.91, 29.87, 29.84, 29.77, 29.59, 29.55, 27.91, 22.92, 16.40, 14.36. MS (EI) $m/z$ 222 [M$^+$H$_2$O]; MS (CI) $m/z$ 241 [M$^+$+H].

(E)-3-methylhexadec-2-en-1-ol (3.2c):

Yield (56%); $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.38 (ddt, $J = 7.0$, 5.6, 1.5 Hz, 1H), 4.13 (d, $J = 7.0$ Hz, 2H), 1.98 (t, $J = 7.5$ Hz, 2H), 1.64 (d, $J = 1.3$ Hz, 3H), 1.24 (d, $J = 3.8$ Hz, 22H), 0.85 (t, $J = 6.3$ Hz, 3H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 140.53, 123.24, 59.65, 39.79, 32.15, 29.94, 29.91, 29.88, 29.84, 29.77, 29.59, 29.55, 27.91, 22.92, 16.40, 14.36. MS (EI) $m/z$ 236 [M$^+$H$_2$O]; MS (CI) $m/z$ 253 [M$^+$-H].

Representative procedure for the synthesis of vinyl iodides 3.5a-d:

(E)-1-iodo-2-methyloct-1-ene (3.5a):

$\text{Cp}_2\text{ZrCl}_2$ (0.25 eq, 0.75 mmol) was dissolved in dichloromethane (3 mL) and the solution was cooled to 0°C, where a $\text{Me}_3\text{Al}$ solution (2.0 $M$ in heptanes, 2.5eq, 9 mmol) was added dropwise. After stirring the reaction mixture at 0°C for 30 minutes, 1-octyne (1.0 eq, 3 mmol) was diluted in dichloromethane (1.5 mL) and added to the reaction mixture dropwise. The reaction mixture was allowed to slowly warm to room temperature and stirred an additional 12
hours. Next, the reaction mixture was cooled to 0°C, where I₂ (2.5 eq, 7.5 mmol) was added in several portions. The reaction continued to stir for 3 hours after which it was slowly poured into an ice-cold 10% HCl(aq) solution. The solution was then filtered over a pad of Celite 545, extracted with 3 × 20 mL dichloromethane, washed with saturated Na₂S₂O₃ × 2, washed with brine, dried with magnesium sulfate, filtered, and concentrated to afford 3.5a in 55% yield.

NMR: ¹H NMR (300 MHz, Chloroform-d) δ 5.97 – 5.56 (m, 1H), 2.17 (t, J = 7.5 Hz, 2H), 1.80 (s, 3H), 1.53 – 1.07 (m, 8H), 0.86 (t, J = 6.1 Hz, 3H). ¹³C NMR (75 MHz, Chloroform-d) δ 148.55, 74.53, 39.82, 31.82, 28.94, 27.89, 24.04, 22.79, 14.31.

(E)-1-iodo-2-methylnon-1-ene (3.5b):

Yield (63%); ¹H NMR (300 MHz, Chloroform-d) δ 5.83 (s, 1H), 2.17 (t, J = 7.5 Hz, 2H), 1.80 (s, 3H), 1.24 (s, 10H), 0.86 (dt, 3H). ¹³C NMR (75 MHz, Chloroform-d) δ 104.96, 74.52, 39.82, 31.99, 29.28, 29.23, 27.92, 24.04, 22.86, 14.32.

(E)-1-iodo-2-methyldec-1-ene (3.5c):

Yield (75%); ¹H NMR (300 MHz, CDCl₃) δ 5.85 – 5.81 (m, 1H), 2.22 – 2.11 (m, 2H), 1.80 (d, J = 1.0 Hz, 3H), 1.24 (s, 12H), 0.86 (t, J = 6.6 Hz, 3H). ¹³C NMR (75 MHz, Chloroform-d) δ 148.50, 74.54, 39.82, 32.08, 29.58, 29.45, 29.27, 27.92, 24.03, 22.89, 14.34.

(E)-1-iodo-2-methylundec-1-ene (3.5d):

Yield (81%); ¹H NMR (300 MHz, CDCl₃) δ 5.95 – 5.63 (m, 1H), 2.23 – 2.10 (m, 2H), 1.80 (s, 3H), 1.24 (s, 14H), 0.86 (t, J = 6.6 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 148.54, 74.53, 39.82, 32.10, 29.74, 29.62, 29.53, 29.27, 27.93, 24.04, 22.90, 14.35.
Representative Procedure for the Synthesis of Suzuki Couplings:

\[
\begin{align*}
\text{} & \text{O} \\
\text{THP} & \\
2-((2E,6E)-3,7\text{-dimethylhexadeca-2,6-dien-1-yl)}\text{oxy})\text{tetracyclo-2H-pyran:}
\end{align*}
\]

An oven-dried round-bottom flask containing powdered molecular sieves was charged with (E)-2-((5-bromo-3-methylpent-2-en-1-yl)oxy)tetrahydro-2H-pyran (stored over sieves; 2.0 eq, 4 mmol) which was dissolved in 13 mL of anhydrous Et₂O and cooled to -78°C. Next, t-BuLi (1.7M in pentane; 3.0 eq, 6.0 mmol, 3.53 mL) was slowly added to the reaction flask and stirred for 1 hour at -78°C. Afterward, β-MeO-9-BBN (1.0M in Hexanes; 3.8 eq, 7.6 mmol) was slowly added to the reaction vessel and the mixture was stirred for 16 hours slowly warming to room temperature.

In a scintillation vial, (E)-1-iodo-2-methyldec-1-ene (1.0 eq, 2.0 mmol) and Pd(dppf)Cl₂ (0.15 eq, 0.3 mmol) are dissolved in 7 mL of DMF. Next, K₃PO₄ (3 M in H2O, 3.0 eq, 6.0 mmol, 3 mL) is added to the vial and after deoxygenating the solvent, the vinyl-iodide solution is added to the round-bottom flask containing the newly formed organoborane. The reaction mixture is allowed to stir for an additional 16 hours at 85°C after which it is poured into a separatory funnel containing water and Et₂O and the aqueous layer was extracted 3 × 30 mL Et₂O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using 5% Diethyl ether/Hexanes as the mobile phase to afford THP-protected alcohol in 70% yield.

\[\text{\textsuperscript{1}H NMR (300 MHz, CDCl₃) } \delta \text{ 5.32 (t, } J = 7.0 \text{ Hz, 1H), 5.05 (t, } J = 6.7 \text{ Hz, 1H), 4.58 (t, } J = 3.2 \text{ Hz, 1H), 4.19 (dd, } J = 12.0, 6.4 \text{ Hz, 1H), 3.98 (dd, } J = 12.0, 7.4 \text{ Hz, 1H), 3.85 (td, } J = 8.7, 6.5, 2.6 \text{ Hz, 1H), 3.61 – 3.39 (m, 1H), 2.03 (dd, } J = 11.6, 6.0 \text{ Hz, 3H), 1.95 – 1.66 (m, 3H), 1.63 (s, 3H), 1.59 – 1.25 (m, 10H), 1.22 (s, 12H), 0.98 – 0.71 (m, 3H). \text{ \textsuperscript{13}C NMR (75 MHz, CDCl₃) } \delta \text{ 140.34,} \]
2-(((2E,6E)-3,7-dimethylpentadeca-2,6-dien-1-yl)oxy)tetrahydro-2H-pyran:

Yield (47%); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.39 – 5.27 (m, 1H), 5.05 (dd, $J = 7.1, 6.1$ Hz, 1H), 4.59 (t, $J = 3.4$ Hz, 1H), 4.20 (dd, $J = 11.9, 6.4$ Hz, 1H), 3.99 (dd, $J = 11.9, 7.3$ Hz, 1H), 3.86 (ddd, $J = 11.1, 7.2, 3.6$ Hz, 1H), 3.47 (dt, $J = 10.7, 5.3$ Hz, 1H), 2.05 (d, $J = 11.0$ Hz, 3H), 1.90 (d, $J = 7.9$ Hz, 2H), 1.86 – 1.66 (m, 2H), 1.64 (s, 3H), 1.52 (dd, $J = 10.0, 5.6$ Hz, 7H), 1.39 – 1.11 (m, 11H), 0.88 – 0.78 (m, 5H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 140.47, 135.79, 123.76, 120.71, 97.90, 63.79, 62.43, 39.87, 34.89, 32.10, 30.89, 29.72, 29.52, 29.48, 28.16, 26.42, 25.69, 22.87, 19.80, 16.58, 16.09, 14.31.

2-(((2E,6E)-3,7-dimethyltetradeca-2,6-dien-1-yl)oxy)tetrahydro-2H-pyran:

Yield (35%); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.32 (dd, $J = 10.1, 3.7$ Hz, 1H), 5.06 (dd, $J = 6.6, 5.8$ Hz, 1H), 4.59 (t, $J = 3.4$ Hz, 1H), 4.27 – 3.40 (m, 4H), 2.14 – 1.66 (m, 7H), 1.64 (s, 3H), 1.61 – 1.14 (m, 18H), 0.85 (dd, $J = 7.7, 5.7$ Hz, 4H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 140.44 (s), 135.78 (s), 123.76 (s), 120.73 (s), 97.90 (s), 63.79 (s), 62.42 (s), 39.86 (s), 32.07 (s), 30.90 (s), 29.44 (s), 28.16 (s), 26.42 (s), 25.70 (s), 22.87 (s), 19.81 (s), 16.58 (s), 16.08 (s), 14.30 (s).

2-(((2E,6E)-3,7-dimethyltrideca-2,6-dien-1-yl)oxy)tetrahydro-2H-pyran:

Yield (23 % based on NMR). Compound co-eluted with an impurity after column chromatography (5% Diethyl ether/Hexanes). Impure compound was taken on and deprotected in the next step.
Representative procedure for the synthesis of alcohols 3.6a-d:

(2E,6E)-3,7-dimethylhexadeca-2,6-dien-1-ol (3.6d):

In a scintillation vial, 2-(((2E,6E)-3,7-dimethylhexadeca-2,6-dien-1-yl)oxy)tetrahydro-2H-pyran (1.0 eq, 1.4 mmol) was dissolved in 7.5 mL of absolute EtOH and PPTS (0.1 eq, 0.14 mmol) was added to the vial. The reaction mixture was heated to 60°C and stirred for 12 hours. Next, the reaction was cooled to room temperature where it was poured into a separator funnel containing water and Et₂O and the aqueous layer was extracted 3 × 10 mL Et₂O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using 10% Ethyl Acetate/Hexanes as the mobile phase to afford 3.6d in 76% yield (mg). NMR: ¹H NMR (300 MHz, CDCl₃) δ 5.38 (t, J = 6.9 Hz, 1H), 5.06 (t, J = 6.7 Hz, 1H), 4.11 (d, J = 6.8 Hz, 2H), 2.13 – 1.97 (m, 3H), 1.92 (t, J = 7.5 Hz, 2H), 1.64 (s, 3H), 1.55 (s, 3H), 1.43 – 1.05 (m, 15H), 0.98 – 0.66 (m, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 139.85, 135.91, 123.66, 123.52, 59.50, 39.86, 39.77, 32.10, 29.81, 29.77, 29.55, 29.48, 28.17, 26.46, 22.89, 16.43, 16.06, 14.30. MS (EI) m/z 266 [M⁺]; MS (Cl) m/z 265 [M⁺-H].

(2E,6E)-3,7-dimethylpentadeca-2,6-dien-1-ol (3.6c):

Yield (56%): NMR: ¹H NMR (300 MHz, CDCl₃) δ 5.42 (td, J = 6.9, 1.2 Hz, 1H), 5.08 (dt, J = 6.7, 3.4 Hz, 1H), 4.15 (d, J = 6.9 Hz, 2H), 2.17 – 1.99 (m, 4H), 1.95 (t, J = 7.4 Hz, 2H), 1.68 (s, 3H), 1.58 (s, 3H), 1.42 (s, 1H), 1.40 – 1.29 (m, 2H), 1.26 (s, 10H), 0.88 (t, J = 6.7 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 140.06 (s), 135.98 (s), 123.56 (d, J = 14.2 Hz), 77.65 (s), 77.23 (s), 76.81 (s), 59.60 (s), 39.84 (d, J = 7.4 Hz), 32.12 (s), 29.87 – 29.37 (m), 28.19 (s), 26.46
(s), 22.89 (s), 16.48 (s), 16.11 (s), 14.34 (s). MS (EI) m/z 236 [M^+H_2O]; MS (Cl) m/z 235 [(M^+H)-H_2O].

(2E,6E)-3,7-dimethyltetradeca-2,6-dien-1-ol (3.6b):

Yield (66%); NMR: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.38 (td, $J = 6.9$, 1.2 Hz, 1H), 5.11 – 5.00 (m, 1H), 4.11 (d, $J = 6.9$ Hz, 2H), 2.14 – 1.96 (m, 4H), 1.92 (t, $J = 7.4$ Hz, 2H), 1.64 (s, 3H), 1.55 (s, 3H), 1.48 – 1.11 (m, 12H), 0.85 (dd, $J = 8.4$, 5.0 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 139.90 (s), 135.92 (s), 123.66 (s), 123.50 (s), 59.53 (s), 39.86 (s), 39.77 (s), 32.07 (s), 29.43 (s), 29.43 (s), 28.17 (s), 26.45 (s), 22.87 (s), 16.44 (s), 16.07 (s), 14.30 (s). MS (EI) m/z 238 [M^+]; MS (Cl) m/z 237 [M^+H].

(2E,6E)-3,7-dimethyltrideca-2,6-dien-1-ol (3.6a):

Yield (52%; 12% from coupling step). $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.42 (td, $J = 7.5$, 6.9, 1.2 Hz, 0H), 5.09 (t, $J = 6.5$ Hz, 0H), 4.15 (dd, $J = 7.1$, 2.9 Hz, 1H), 2.07 (ddt, $J = 13.7$, 9.1, 4.2 Hz, 1H), 1.95 (t, $J = 7.5$ Hz, 0H), 1.68 (s, 1H), 1.58 (s, 1H), 1.43 – 1.16 (m, 2H), 0.88 (t, $J = 6.7$ Hz, 1H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 140.08, 135.98, 123.67, 123.50, 59.63, 39.89, 39.80, 32.01, 29.17, 28.15, 26.47, 22.88, 16.48, 16.12, 14.34. MS (EI) m/z 224 [M^−H_2O]; MS (Cl) m/z 223 [M^−H].

10-iododecan-1-ol:

To an oven dried round bottom flask cooled under Argon is added decane-1,10-diol (1.3 eq, 39 mmol) dissolved in 50 mL of anhydrous dichloromethane. The reaction flask is cooled to 0°C where PPh$_3$ (1.0 eq, 30 mmol) followed by imidazole (1.5 eq, 45 mmol) are added to the
reaction vessel. After stirring at 0°C for 10 minutes, iodine (1.0 eq, 30 mmol) is added to the reaction portion wise; after complete addition, the reaction is stirred for 3.5 hours at room temperature. The reaction was then quenched with 10% NH₄Cl(aq) and the aqueous layer was extracted 3 × 100 mL of hexanes. The organic layers were combined, dried with sodium sulfate, and concentrated. The crude reaction product was purified column chromatography by dry-loading with sodium sulfate and using 30% Ethyl Acetate/Hexanes as the mobile phase to afford title compound in 45% yield. $^1$H NMR (300 MHz, CDCl₃) δ 3.61 (t, $J = 6.2$ Hz, 2H), 3.16 (t, $J = 7.0$ Hz, 2H), 1.85 – 1.71 (m, 2H), 1.52 (dd, $J = 13.7, 6.8$ Hz, 2H), 1.42 – 1.11 (m, 14H). $^{13}$C NMR (75 MHz, CDCl₃) δ 77.65 (s), 77.23 (s), 76.81 (s), 63.25 (s), 33.73 (s), 32.97 (s), 30.69 (s), 29.81 – 29.40 (m), 28.71 (s), 25.92 (s), 7.62 (s).

10-iododecanal (3.8):

An oven dried multi-neck round bottom flask is equipped with an oven dried addition funnel and cooled under Argon. The flask is charged with (COCl)$_2$ (1.2 eq, 16 mmol) dissolved in 16 mL of dichloromethane and cooled to -78°C. Next, DMSO (2.4 eq, 32 mmol) is dissolved in 2.2 mL of dichloromethane, added to the addition funnel, and slowly added to the reaction vessel over a 20 minute period at -78°C. The reaction is allowed to stir at this temperature for 30 minutes after which 11-iodoundecan-1-ol (1.0 eq, 13.34 mmol) diluted with 26 mL of dichloromethane is added slowly to the reaction mixture. The reaction is allowed to stir for an additional 1.5 hours at -78°C followed by the addition of Et$_3$N (5.0 eq, 66.7 mmol). After allowing the reaction to come to room temperature, it is quenched by adding 10% NH₄Cl(aq) and the aqueous layer was extracted 3 × 75 mL of DCM. The organic layers were combined, washed with brine, dried with magnesium sulfate, filtered, and concentrated. The crude reaction product was purified column chromatography using 5% Ethyl Acetate/Hexanes as the mobile phase to afford 3.8 in 81% yield. $^1$H NMR (300 MHz, CDCl₃) δ 9.67 (t, $J = 1.5$ Hz, 1H), 3.09 (t, $J = 7.0$ Hz, 1H), 1.52 (dd, $J = 13.7, 6.8$ Hz, 2H), 1.42 – 1.11 (m, 14H).
Hz, 2H), 2.33 (td, $J = 7.3, 1.7$ Hz, 2H), 1.71 (dd, $J = 14.4, 7.1$ Hz, 2H), 1.59 – 1.47 (m, 2H), 1.21 (s, 10H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 202.74, 43.88, 33.49, 30.44, 29.24, 29.19, 29.09, 28.45, 22.03, 7.43.

11-bromoundecanal (3.11):

Yield (75%); $^1$H NMR (300 MHz, CDCl$_3$) δ 9.74 (d, $J = 1.5$ Hz, 1H), 3.38 (t, $J = 6.8$ Hz, 2H), 2.40 (t, $J = 7.3$ Hz, 2H), 1.81 (dd, $J = 14.3, 7.2$ Hz, 2H), 1.68 – 1.53 (m, 2H), 1.38 (d, $J = 6.9$ Hz, 2H), 1.26 (s, 12H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 203.16 (s), 77.65 (s), 77.23 (s), 76.80 (s), 44.12 (s), 34.28 (s), 33.01 (s), 29.46 (t, $J = 8.0$ Hz), 28.93 (s), 28.35 (s), 22.26 (s).

**Representative procedure of the Wittig reaction:**

12-iodo-2-methyldec-2-ene (3.9):

An oven dried round bottom flask is charged with isopropyl triphenylphosphine iodide (1.5 eq, mmol) and THF. The flask is cooled to 0°C where n-BuLi (2.5 M in Hexanes, 1.5 eq, mmol) is added dropwise to give a red solution. The reaction mixture is stirred for 30 minutes at 0°C, after which 10-iododecanal (1.0 eq, mmol) dissolved in mL of THF is added dropwise to the reaction. The reaction is allowed to stir for an additional 4 hours at room temperature. Upon completion of the reaction, the reaction mixture is quenched by adding 10% NH$_4$Cl$_{aq}$ and the aqueous layer was extracted 3 × mL of Et$_2$O. The organic layers were combined, washed with brine, dried with magnesium sulfate, filtered, and concentrated. The crude reaction product was purified with column chromatography by dry-loading with sodium sulfate and using hexanes as the mobile phase to afford 3.9 in 58% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 5.13 – 5.04 (m, 1H), 3.15 (t, $J = 7.1$ Hz, 2H), 1.92 (t, $J = 6.5$ Hz, 2H), 1.78 (dd, $J = 14.5, 7.1$ Hz, 2H), 1.66 (d, $J = 0.9$
Hz, 3H), 1.57 (s, 3H), 1.42 – 1.31 (m, 2H), 1.26 (s, 11H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 131.19, 125.03, 33.74, 30.69, 30.05, 29.66, 29.59, 29.46, 28.73, 28.20, 25.92, 17.85, 7.35.

Br

13-bromo-2-methyltridec-2-ene (3.10):

Yield (63%); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.14 – 5.04 (m, 1H), 3.39 (t, $J$ = 6.9 Hz, 2H), 1.92 (t, $J$ = 6.6 Hz, 2H), 1.88 – 1.77 (m, 2H), 1.66 (d, $J$ = 0.9 Hz, 3H), 1.58 (s, 3H), 1.53 (s, 1H), 1.46 – 1.34 (m, 2H), 1.25 (s, 14H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 131.37, 125.13, 34.32, 33.06, 30.11, 29.75, 29.66, 29.54, 29.00, 28.40, 28.26, 25.96, 17.89.

OTBS

(but-2-yn-1-yloxy)(tert-butyl)dimethylsilane:

To a round bottom flask is added but-2-yn-1-ol (1.0 eq, 12 mmol) dissolved in DCM. Next, DIEA (1.3 eq, 15.6 mmol) followed by TBDMSCl (1.3 eq, 15.6 mmol) is added to the reaction vessel and stirred for 6 hours at room temperature. Upon completion of the reaction, the reaction mixture is quenched by adding 10% NH$_4$Cl(aq) and the aqueous layer was extracted 3 × 50 mL of Et$_2$O. The organic layers were combined, washed with brine, dried with magnesium sulfate, filtered, and concentrated. The crude reaction product was purified column chromatography using 2% ethyl acetate/hexanes as the mobile phase to afford the title compound in 95% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.23 (q, $J$ = 2.3 Hz, 1H), 1.79 (t, $J$ = 2.4 Hz, 1H), 0.87 (s, 5H), 0.07 (s, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 81.02, 77.92, 52.14, 26.05, 18.54, 3.77, -5.00.
To an oven dried round bottom flask, which was also flame dried under vacuum and cooled with Argon three times, is added Cp₂ZrCl₂ (1.5 eq, 17 mmol) in anhydrous THF (39 mL) and cooled to 0°C. DIBAL (1.0 M in Toluene, 1.5 eq, 17 mmol) is then slowly added to afford a milky yellow solution which is stirred 30 minutes at 0°C. Next, (but-2-yn-1-yloxy)(tert-butyl)dimethylsilane dissolved in THF (6 mL) is slowly added and the reaction mixture is allowed to warm to room temperature where it is stirred until a homogeneous solution results (~1 hour). Finally, the reaction is cooled to -78°C and I₂ dissolved in THF (17 mL) is added slowly and the mixture is allowed to stir for an additional 30 minutes at -78°C. The reaction mixture is quenched by pouring into a beaker containing 10% HCl (aq) and the aqueous layer was extracted 3 × 50 mL of Et₂O. The organic layers were combined, washed with saturated Na₂S₂O₃, saturated NaHCO₃, and brine. The organic layer is dried with magnesium sulfate, filtered, and concentrated. The crude reaction product was purified column chromatography using 2% ethyl acetate/hexanes as the mobile phase to afford 3.14 in 40% yield. ¹H NMR (300 MHz, Chloroform-d) δ 6.26 (td, J = 6.3, 1.5 Hz, 1H), 4.09 (d, J = 6.5 Hz, 3H), 2.38 (d, J = 1.4 Hz, 4H), 0.86 (s, 12H), 0.04 (s, 7H). ¹³C NMR (75 MHz, Chloroform-d) δ 140.70, 96.11, 60.77, 28.22, 26.00, 18.44, -5.07.
in 86% yield. $^1$H NMR (300 MHz, Chloroform-d) δ 5.27 (t, $J = 6.4$ Hz, 1H), 5.14 – 5.03 (m, 1H), 4.17 (d, $J = 6.3$ Hz, 2H), 1.94 (q, $J = 8.0$ Hz, 5H), 1.66 (s, 4H), 1.57 (s, 10H), 1.24 (s, 15H), 0.88 (s, 15H), 0.05 (s, 8H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 137.52, 131.29, 125.16, 124.33, 60.56, 39.77, 30.13, 29.85, 29.82, 29.78, 29.57, 28.28, 27.90, 26.25, 26.20, 25.95, 17.88, 16.47, -4.81.

(E)-tert-butyl((3,15-dimethylhexadeca-2,14-dien-1-yl)oxy)dimethylsilane:

13-bromo-2-methyltridec-2-ene and 12-iodo-2-methyldec-2-ene were coupled using the same procedure as 2-(((2E,6E)-3,7-dimethylhexadeca-2,6-dien-1-yl)oxy)tetrahydro-2H-pyran. The crude reaction product was purified by column chromatography using 5% ethyl acetate/hexanes as the mobile phase to afford the title compound in 66% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 5.29 (tq, $J = 6.4$, 5.1, 1.3 Hz, 1H), 5.11 (tq, $J = 7.3$, 5.8, 2.9, 1.5 Hz, 1H), 4.19 (dd, $J = 6.4$, 0.9 Hz, 2H), 1.96 (q, $J = 8.2$ Hz, 4H), 1.69 (d, $J = 1.4$ Hz, 3H), 1.60 (dd, $J = 2.8$, 1.3 Hz, 6H), 1.27 (d, $J = 2.7$ Hz, 16H), 0.91 (s, 9H), 0.07 (s, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 137.51, 131.27, 125.16, 124.34, 60.55, 39.77, 30.14, 29.88, 29.82, 29.78, 29.59, 29.56, 28.28, 27.90, 26.24, 25.94, 18.65, 17.87, 16.46, -4.82.

Representative procedure for TBDMS deprotection:

(E)-3,14-dimethylpentadeca-2,13-dien-1-ol (3.15a):

In a scintillation vial equipped with a magnetic stirrer was added (E)-tert-butyl((E)-3,14-dimethylpentadeca-2,13-dien-1-yl)oxy)dimethylsilane (1.0 eq, 1.32 mmol) in THF (2 mL) and cooled to 0°C. Next, TBAF (1.5 eq, 1.97 mmol) was added to the vial and the reaction mixture
was allowed to stir for 2 hours coming to room temperature. The reaction mixture was quenched by adding 10% NH₄Cl (aq) and the aqueous layer was extracted 3 × mL of Et₂O. The organic layers were combined, washed with brine, dried with magnesium sulfate, filtered, and concentrated. The crude reaction product was purified by column chromatography using hexanes as the mobile phase to afford 3.15 in 26% yield. ¹H NMR (300 MHz, CDCl₃) δ 5.37 (t, J = 7.0 Hz, 1H), 5.09 (t, J = 7.4 Hz, 1H), 4.12 (d, J = 7.0 Hz, 2H), 2.04 – 1.85 (m, 4H), 1.64 (d, J = 7.0 Hz, 6H), 1.57 (s, 3H), 1.24 (s, 14H). ¹³C NMR (75 MHz, Chloroform-d) δ 140.39, 131.30, 125.13, 123.25, 59.57, 39.76, 30.10, 29.79, 29.79, 29.74, 29.55, 29.52, 28.25, 27.89, 25.92, 17.85, 16.36. MS (EI) m/z 234 [M⁺-H₂O].

(E)-3,15-dimethylhexadeca-2,14-dien-1-ol (3.15b):

Yield (64%); ¹H NMR (300 MHz, CDCl₃) δ 5.45 – 5.29 (m, 1H), 5.14 – 4.99 (m, 1H), 4.12 (d, J = 7.1 Hz, 2H), 2.02 – 1.86 (m, 4H), 1.66 (s, 3H), 1.63 (s, 3H), 1.57 (s, 3H), 1.23 (s, 16H). ¹³C NMR (75 MHz, Chloroform-d) δ 140.38, 131.29, 125.14, 123.26, 59.57, 39.77, 30.11, 29.86, 29.82, 29.80, 29.75, 29.56, 29.52, 28.25, 27.90, 25.93, 17.85, 16.36. MS (EI) m/z 248 [M⁺-H₂O].

(2E,6E)-2,6-dimethyl-8-((tetrahydro-2H-pyran-2-yl)oxy)octa-2,6-dien-1-yl diethyl phosphate (3.18):

To an Erlenmeyer flask charged with t-BuOOH (2.0 eq, 49 mmol, 70% in water) and 65 mL of CH₂Cl₂ is added MgSO₄. The solution was then filtered into a round bottom flask equipped with a magnetic stir bar. Next, SeO₂ (0.12 eq, 2.94 mmol) and salicylic acid (0.1 eq, 2.5 mmol) were added to the reaction mixture. While the solution stirred, THP-protected geraniol
(1.0 eq, 24.5 mmol) dissolved in 10 mL of CH₂Cl₂ was added dropwise to the reaction vessel. The reaction mixture was allowed to stir for 12 hours. The CH₂Cl₂ was removed and the resulting residue was resuspended in Et₂O. Next, 10% NaOH was added to solution, the organic layer was removed, and the aqueous layer was extracted 3 × Et₂O (40 mL). The organic layers are combined, washed with brine, dried with MgSO₄, filtered, and concentrated. In an oven-dried round bottom flask, the crude reaction product was diluted with 80 mL of ethanol and NaBH₄ (1.0 eq, 24.5 mmol) was added in several portions to the reaction vessel. The reaction was allowed to stir for 1 hour and then diluted with 200 mL of water. The aqueous layer was extracted 3 × Et₂O (40 mL) and the organic layers were combined, washed with brine, dried with MgSO₄, filtered, and concentrated. The crude reaction product was purified by column chromatography using 20% Ethyl Acetate/Hexanes as the mobile phase to afford (2E,6E)-2,6-dimethyl-8-((tetrahydro-2H-pyran-2-yl)oxy)octa-2,6-dien-1-ol in 27% yield.

To a separate round bottom flask equipped with a magnetic stir bar was added (2E,6E)-2,6-dimethyl-8-((tetrahydro-2H-pyran-2-yl)oxy)octa-2,6-dien-1-ol (1.0 eq, 5.15 mmol) in 10 mL of dichloromethane. Next, pyridine (2.5 eq, 12.9 mmol) was added to the reaction vessel and the mixture was cooled to 0°C, where diethyl chlorophosphate (1.5 eq, 7.7 mmol) was added dropwise to the reaction. The reaction was allowed to warm to room temperature and stirred for 12 hours. Next, 10% NaOH was added to solution, the organic layer was removed, and the aqueous layer was extracted 3 × EtOAc (20 mL). The organic layers are combined, washed with brine, dried with MgSO₄, filtered, and concentrated. The crude reaction product was purified by column chromatography using 80% Ethyl Acetate/Hexanes as the mobile phase to afford diethyl phosphosphate 3.18 in 76% yield. ¹H NMR (300 MHz, Chloroform-d) δ 5.44 (t, J = 6.8 Hz, 1H), 5.31 (t, J = 6.7 Hz, 1H), 4.68 – 4.50 (m, 1H), 4.34 (d, J = 7.0 Hz, 2H), 4.18 (dd, 1H), 4.05 (dp, 4H), 3.98 (dd, J = 7.4 Hz, 1H), 3.89 – 3.77 (m, 1H), 3.55 – 3.37 (m, 1H), 2.13 (q, J = 7.7, 7.3 Hz, 2H), 2.02 (dd, J = 9.4, 6.2 Hz, 2H), 1.86 – 1.66 (m, 1H), 1.63 (s, 6H), 1.59 – 1.40 (m, 5H), 1.34 –
1.22 (m, 6H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 139.67, 130.87, 129.52, 121.12, 98.06, 73.31 (d, $J = 5.5$ Hz), 63.79 (d, $J = 5.6$ Hz). 63.78, 62.47, 39.02, 30.85, 26.15, 25.63, 19.78, 16.58, 16.35, 16.26, 13.74. $^{31}$P NMR (202 MHz, Chloroform-d) δ -0.32.

(2E,6E)-3,7,11,15-tetramethylhexadeca-2,6-dien-1-ol (3.20):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added magnesium powder (18 eq, 20 mmol) and 6 mL of anhydrous Et$_2$O and an iodide chip was added to the round bottom and the mixture was stirred for 5 minutes. Next, 1-bromo-3,7-dimethyloctane (4.5 eq, 4.5 mmol) in 2 mL of Et$_2$O was added dropwise to the reaction vessel over a 10 minute period and the reaction mixture was stirred for 4 hours. After the allotted time, the reaction mixture was cooled to 0°C where diethyl phosphate 3.18 (1.0 eq, 1.0 mmol) dissolved in 6 mL of THF was added dropwise to the mixture and the reaction was allowed to stir for 12 hours. The reaction was quenched with 10% NH$_4$Cl, the organic layer was removed, and the aqueous layer was extracted 3 × Et$_2$O (5 mL). The organic layers are combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was purified by column chromatography using 3% Ethyl Acetate/Hexanes as the mobile phase to afford 3.19 in 15% yield.

In a scintillation vial equipped with a magnetic stir bar, 3.19 (1.0 eq, 0.155 mmol) was dissolved in 5.0 mL of absolute EtOH and PPTS (0.1 eq, 0.016 mmol) was added to the vial. The reaction mixture was heated to 75°C and stirred for 12 hours. Next, the reaction was cooled to room temperature where it was poured into a separator funnel containing water and Et$_2$O and the aqueous layer was extracted 3 × 5 mL Et$_2$O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using 10% Ethyl Acetate/Hexanes as the mobile phase.
to afford 3.20 in 70% yield. $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.39 (t, $J = 7.4$ Hz, 1H), 5.07 (t, $J = 6.2$ Hz, 1H), 4.12 (d, $J = 6.9$ Hz, 2H), 2.17 – 1.97 (m, 4H), 1.91 (t, $J = 7.3$ Hz, 2H), 1.65 (s, 3H), 1.56 (s, 3H), 1.55 – 1.42 (m, 2H), 1.29 – 0.98 (m, 10H), 0.84 (d, $J = 6.5$ Hz, 6H), 0.81 (d, $J = 5.5$ Hz, 3H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 140.00, 135.96, 123.71, 123.49, 59.56, 40.17, 39.79, 39.54, 37.48, 36.85, 32.86, 28.17, 26.48, 25.58, 25.01, 22.92, 22.83, 19.91, 16.47, 16.10. MS (EI) $m/z$ 294 [M$^+$] and 276 [M$^+$-H$_2$O]; MS (CI) 293 [M-H$^+$] and 295 [M+H$^+$].

(2E,6E,10E)-12-hydroxy-3,7,11-trimethyldeca-2,6,10-trien-1-yl acetate (3.22):

Compound 3.21 was synthesized using the same method as compound 2.2 with the exception that acetyl-protected farnesol was utilized instead of THP-protected farnesol. Yield (28%). $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.37 – 5.16 (m, 2H), 5.13 – 4.85 (m, 1H), 4.50 (d, $J = 7.1$ Hz, 2H), 3.89 (s, 2H), 2.10 – 1.72 (m, 13H), 1.62 (s, 3H), 1.57 (s, 3H), 1.52 (s, 3H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 171.30, 142.24, 135.17, 134.82, 125.75, 123.89, 118.34, 68.77, 61.47, 39.51, 39.34, 26.24, 26.17, 21.07, 16.49, 16.05, 13.71.

(2E,6E,10E)-3,7,11-trimethyl-12-((tetrahydro-2H-pyran-2-yl)oxy)deca-2,6,10-trien-1-ol (3.23):

To a round bottom flask equipped with a magnetic stir bar was added 3.21 (1.0 eq, 5.62 mmol) and 30 mL of dichloromethane. Next, 3,4-dihydro-2H-pyran (2.0 eq, 11.24 mmol) followed by pyridinium $p$-toluenesulfonate (0.15 eq, 0.84 mmol) was added to the reaction flask and the reaction was allowed to stir for 12 hours. Saturated NaHCO$_3$(aq) was added to the reaction flask and organic layer was isolated. The aqueous layer was further extracted 3 × DCM (25 mL) and the organic layers were combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. In a round bottom flask, the crude product was redissolved in a saturated solution
of K$_2$CO$_3$ in MeOH (30 mL) and stirred for 12 hours. After the allotted time, water (150 mL) and Et$_2$O (30 mL) were added to the reaction mixture and the organic layer was isolated. The aqueous layer was further extracted $3 \times$ Et$_2$O (25 mL) and the organic layers were combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was purified by column chromatography using 15% Ethyl Acetate/Hexanes as the mobile phase to afford 3.22 in 74% yield. $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.40 (t, $J$ = 6.9 Hz, 2H), 5.10 (t, $J$ = 6.4 Hz, 1H), 4.64 – 4.56 (m, 1H), 4.14 (d, $J$ = 6.9 Hz, 2H), 4.08 (d, $J$ = 11.6 Hz, 1H), 3.94 – 3.78 (m, 2H), 3.59 – 3.40 (m, 1H), 2.07 (dp, $J$ = 27.1, 7.4 Hz, 8H), 1.92 – 1.78 (m, 1H), 1.66 (s, 3H), 1.64 (s, 3H), 1.59 (s, 3H), 1.65 – 1.46 (m, 6H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 139.55, 135.11, 131.90, 127.98, 124.16, 123.59, 97.48, 73.07, 62.28, 59.46, 39.61, 39.34, 30.77, 26.37, 26.36, 25.61, 19.63, 16.39, 16.09, 14.19.

(2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10-trien-1-ol (3.25):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added magnesium powder (14.5 eq, 14.5 mmol) and 5 mL of anhydrous THF and an iodide chip was added to the round bottom and the mixture was stirred for 5 minutes. Next, 1-bromo-3-methylbutane (10 eq, 10 mmol) in 3 mL of Et$_2$O was added dropwise to the reaction vessel over a 10 minute period and the reaction mixture was stirred for 4 hours to produce Grignard 3.24.

In a separate oven-dried round bottom flask was added THP-ether 3.23 (1.0 eq, 1 mmol) and dissolved in 5 mL of anhydrous THF and the solution was cooled to -35°C. Next, Cu(I)Br (1.5 eq, 1.5 mmol) was added in one portion followed by the dropwise addition of the newly formed Grignard reagent. The reaction was stirred for 1 hour at -35°C and allowed to warm to -10°C where it was stirred for 48 hours. The reaction mixture is quenched with 10% NH$_4$Cl$_{aq}$ and the aqueous layer was extracted $3 \times$ 15 mL of Et$_2$O. The organic layers were combined, washed
with brine, dried with magnesium sulfate, filtered, and concentrated. The crude reaction product was purified column chromatography using 15% ethyl acetate/hexanes as the mobile phase to afford 3.25 in 17% yield. $^1$H NMR (300 MHz, Chloroform-d) δ 5.39 (t, $J = 6.9$ Hz, 1H), 5.07 (q, $J = 6.8, 6.3$ Hz, 2H), 4.12 (d, $J = 6.9$ Hz, 2H), 1.98 (ddt, $J = 31.0, 15.2, 6.8$ Hz, 10H), 1.65 (s, 3H), 1.58 (s, 3H), 1.55 (s, 3H), 1.55 – 1.40 (m, 1H), 1.42 – 1.27 (m, 3H), 1.16 – 1.01 (m, 2H), 0.84 (d, $J = 6.6$ Hz, 6H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 40.10, 39.92, 39.75, 38.79, 28.09, 26.77, 26.51, 25.94, 22.85, 16.47, 16.20, 16.08. MS (EI) m/z 274 [M-H$_2$O]; MS (CI) m/z 293 [M-H]$^+$. 

**Representative procedure for the synthesis of pyrophosphates:**

(E)-3-methyltetradec-2-en-1-yl diphosphate (3.3a):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added NCS (2.5 eq, 0.44 mmol) in 1 mL of CH$_2$Cl$_2$ and cooled to -30°C where dimethyl sulfide (2.5 eq, 0.44 mmol) was added dropwise to the reaction. Following the addition, the mixture is then placed in a 0°C ice bath and stirred for 5 minutes before being re-cooled back to -30°C. Next, alcohol 3.2a (1 eq, 0.22 mmol) is dissolved in CH$_2$Cl$_2$ (0.44 mL) and added dropwise to the reaction mixture. The mixture is then placed in a 0°C ice bath and stirred for 2.5 hours coming to room temperature. After the allotted time, brine is added to the reaction mixture and the organic layer was extracted. The aqueous layer was further extracted 3 × 5 mL CH$_2$Cl$_2$ and the organic layers were combined, dried with magnesium sulfate, and concentrated. The crude reaction product was used immediately in the following step.

To another oven-dried round bottom flask equipped with a magnetic stir bar was added tris (tetrabutylammonium) hydrogen pyrophosphate (3.0eq, 0.66 mmol) dissolved in 3 mL of
acetonitrile. Next, a solution of crude allylic chloride dissolve in 2 mL of acetonitrile was added dropwise to the reaction vessel. The reaction was stirred for 3 hours at room temperature and then the solvent was removed by rotary evaporation at 34°C. The residue was the dissolve in a minimal amount of ion exchange NH₃HCO₃ buffer (700mg NH₃HCO₃, 1 L of deionized H₂O, 20 mL of isopropanol) and the resulting solution was passed through a Dowex AG 50 × 8 ion exchange column (2 × 8 cm) using the NH₃HCO₃ buffer as an eluent and 25 mL was collected in a flask. The resulting solution was lyophilized for 3-5 hours. The resulting residue was then redissolved in deionized watered and purified by cellulose flash column chromatography (3 × 15 cm) using isopropanol:deionized H₂O:acetonitrile: NH₃HCO₃ buffer (500 mL : 250 mL : 250 mL : 4 g) as the eluent. In a beaker was collected 40 mL of eluent, then twenty-four 2.5 mL fractions were collected. Typically, fractions 12-18 were collected and the organic solvents were removed by rotary evaporation at 34°C. The resulting solution was then lyophilized to afford pyrophosphate (3.3a) as a white fluffy solid in 57% yield. ¹H NMR (500 MHz, Chloroform-d) δ 5.44 – 5.23 (m, 1H), 4.34 (s, 2H), 1.92 (s, 2H), 1.62 (d, J = 24.3 Hz, 3H), 1.20 (s, 18H), 0.98 – 0.70 (m, 3H). ³¹P NMR (202 MHz, Deuterium Oxide) δ -9.96 (d, J = 15.9 Hz), -13.61 (d, J = 22.1 Hz). HRMS 385.1548 [M+2H]⁺, calculated 385.1545 (C₁₅H₃₁O₇P₂).

(E)-3-methylpentadec-2-en-1-yl diphosphate (3.3b):

Yield (64%). ¹H NMR (300 MHz, Deuterium Oxide) δ 5.43 (t, 1H), 4.44 (s, 2H), 2.03 (s, 2H), 1.68 (s, 3H), 1.25 (s, 20H), 0.83 (t, J = 5.8 Hz, 3H). ³¹P NMR (122 MHz, Chloroform-d) δ -10.43, -14.05. HRMS 399.1701 [M+2H]⁺, calculated 399.1702 (C₁₆H₃₃O₇P₂).
(E)-3-methylhexadec-2-en-1-yl diphosphate (3.3c):

Yield (83%). $^1$H NMR (500 MHz, Deuterium Oxide) δ 5.37 (s, 1H), 4.39 (s, 2H), 2.04 – 1.93 (m, 2H), 1.65 (s, 3H), 1.25 (d, $J = 7.4$ Hz, 22H), 0.91 (t, $J = 7.4$ Hz, 3H). $^{31}$P NMR (202 MHz, Deuterium Oxide) δ -9.99 (d, $J = 19.2$ Hz), -13.70 (d, $J = 20.9$ Hz). HRMS 413.1852 [M+2H$^+]$, calculated 413.1858 (C$_{17}$H$_{35}$O$_7$P$_2$).

(2E,6E)-3,7-dimethyltrideca-2,6-dien-1-yl diphosphate (3.6a):

Yield (91%). $^1$H NMR (500 MHz, Deuterium Oxide) δ 5.38 (t, $J = 6.9$ Hz, 1H), 5.10 (d, $J = 6.4$ Hz, 1H), 4.40 (d, $J = 7.3$ Hz, 2H), 2.16 – 2.02 (m, 2H), 2.03 – 1.94 (m, 2H), 1.89 (t, $J = 7.8$ Hz, 2H), 1.66 (s, 3H), 1.54 (s, 3H), 1.21 (s, 8H), 0.82 (t, $J = 6.9$ Hz, 3H). $^{31}$P NMR (202 MHz, Deuterium Oxide) δ -10.01, -13.74 (d, $J = 15.5$ Hz). HRMS 383.1391 [M+2H$^+]$, calculated 383.1389 (C$_{15}$H$_{29}$O$_7$P$_2$).

(2E,6E)-3,7-dimethyltetradeca-2,6-dien-1-yl diphosphate (3.6b):

Yield (49%). $^1$H NMR (500 MHz, Deuterium Oxide) δ 5.52 – 5.32 (m, 1H), 5.08 (d, $J = 7.1$ Hz, 1H), 4.41 (s, 2H), 2.05 (s, 2H), 2.02 – 1.94 (m, 2H), 1.91 (d, $J = 8.0$ Hz, 2H), 1.68 (d, $J = 16.2$ Hz, 3H), 1.60 – 1.49 (m, 3H), 1.24 (s, 10H), 0.84 (d, $J = 6.9$ Hz, 3H). $^{31}$P NMR (202 MHz, Deuterium Oxide) δ -11.27 (d, $J = 14.8$ Hz), -13.98 (d, $J = 15.3$ Hz). HRMS 397.1547 [M+2H$^+]$, calculated 397.1545 (C$_{16}$H$_{31}$O$_7$P$_2$).
(2E,6E)-3,7-dimethylpentadeca-2,6-dien-1-yl diphosphate (3.6c):

Yield (58%). $^1$H NMR (500 MHz, Deuterium Oxide) δ 5.38 (d, $J = 5.9$ Hz, 1H), 5.23 – 4.99 (m, 1H), 4.67 – 4.36 (m, 2H), 2.09 – 2.01 (m, 2H), 2.00 – 1.94 (m, 2H), 1.94 – 1.83 (m, 2H), 1.65 (s, 3H), 1.54 (s, 3H), 1.24 (s, 12H), 0.84 (t, $J = 6.4$ Hz, 3H). $^{31}$P NMR (202 MHz, Deuterium Oxide) δ -12.53 (d, $J = 19.6$ Hz), -16.19 (d, $J = 17.9$ Hz). HRMS 411.1698 [M+2H]$^+$, calculated 411.1702 (C$_{17}$H$_{33}$O$_7$P$_2$).

(2E,6E)-3,7-dimethylhexadeca-2,6-dien-1-yl diphosphate (3.6d):

Yield (70%). $^1$H NMR (300 MHz, Deuterium Oxide) δ 5.39 (s, 1H), 5.09 (s, 1H), 4.43 (s, 2H), 1.93 (t, $J = 19.3$ Hz, 6H), 1.70 (d, $J = 10.3$ Hz, 3H), 1.56 (d, $J = 7.1$ Hz, 3H), 1.26 (d, $J = 8.2$ Hz, 14H), 0.88 (t, $J = 5.9$ Hz, 3H). $^{31}$P NMR (122 MHz, Chloroform-d) δ -15.06, -16.83. HRMS 425.1860 [M+2H]$^+$, calculated 425.1858 (C$_{18}$H$_{35}$O$_7$P$_2$).

(E)-3,14-dimethylpentadeca-2,13-dien-1-yl diphosphate (3.16a):

Yield (86%). $^1$H NMR (500 MHz, Chloroform-d) δ 5.37 (s, 1H), 5.03 (s, 1H), 4.41 (s, 2H), 1.90 (s, 4H), 1.69 – 1.45 (m, 9H), 1.24 (s, 14H). $^{31}$P NMR (202 MHz, Deuterium Oxide) δ -10.09 (d, $J = 19.2$ Hz), -13.78 (d, $J = 25.9$ Hz). HRMS 411.1707 [M+2H]$^+$, calculated 411.1702 (C$_{17}$H$_{33}$O$_7$P$_2$).
(E)-3,15-dimethylhexadeca-2,14-dien-1-yl diphosphate (3.16b):

Yield (69%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 5.38 (s, 1H), 5.06 (s, 1H), 4.42 (s, 2H), 1.96 (d, $J = 37.9$ Hz, 4H), 1.67 (s, 3H), 1.61 (s, 3H), 1.53 (s, 3H), 1.27 (s, 16H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -14.26 (d, $J = 18.7$ Hz), -16.53 (d, $J = 17.6$ Hz). HRMS 425.1859 [M+2H]$^+$, calculated 425.1858 (C$_{18}$H$_{35}$O$_7$P$_2$).

(2E,6E)-3,7,11,15-tetramethylhexadeca-2,6-dien-1-yl diphosphate (3.21):

Yield (52%). $^1$H NMR (500 MHz, Chloroform-d) $\delta$ 5.40 (s, 1H), 5.10 (s, 1H), 4.41 (s, 2H), 2.06 (s, 2H), 1.98 (s, 2H), 1.91 (s, 2H), 1.68 (s, 3H), 1.57 (s, 3H), 1.25 (s, 10H), 0.85 (s, 3H), 0.84 (s, 6H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -9.88 (d $J = 16.2$ Hz), -13.59 (d, $J = 18.3$ Hz). HRMS 453.2174 [M+2H]$^+$, calculated 453.2171 (C$_{20}$H$_{39}$O$_7$P$_2$).

(2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10-trien-1-yl diphosphate (3.24):

Yield (28%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 5.42 (s, 1H), 5.05 (s, 2H), 4.43 (s, 2H), 2.13 – 1.76 (m, 10H), 1.68 (s, 3H), 1.65 – 1.39 (m, 7H), 1.32 (s, 3H), 1.14 – 1.01 (m, 2H), 0.82 (s, 6H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -10.16, -14.05. HRMS 451.2022 [M+2H]$^+$, calculated 451.2014 (C$_{20}$H$_{39}$O$_7$P$_2$).

**General procedure for in vitro biochemical substrate screening:**

All biochemical evaluations were performed in our collaborator Dr. Carol Fierke’s laboratory at the University of Michigan by Elia Wright. Preliminary evaluation of all pyrophosphate analogs were performed using GGPP analog (1, 5, or 10 µM), the peptide dansyl-
GCVLL (5 µM), recombinant mammalian GGTase-I (50 nM), 50 nM HEPPSO pH 7.8, 5 mM tris(2carboxyethyl)phosphine (TCEP), and 5 mM MgCl₂ at 25°C in 96 well plates (Corning). Protein prenylation was determined by monitoring the dansylated peptide using a continuous spectrofluorometric assay and all assays were performed in triplicate. Upon prenylation of the peptide, the activity was measured by an increase in fluorescence intensity of the dansyl group (λ<sub>ex</sub> = 340 nm, λ<sub>em</sub> = 520 nm) in a POLARstar Galaxy plate reader. Peptide in assay buffer was utilized as a negative control and the baseline fluorescence of the peptide was subtracted from the reaction signal.
CHAPTER 4. SYNTHESIS & BIOCHEMICAL EVALUATION OF FRAME-SHIFTED GERANYLGERANYL PYROPHOSPHATE ANALOGS

4.1 Introduction

Isoprenoids are found in almost all life forms and are the largest and most structurally diverse class of natural products.\textsuperscript{123, 124} As such, they are responsible for a multitude of biochemical functions including their use as hormones (e.g. steroids, gibberellins, and abscisic acid) and roles in cell membrane structure (e.g. cholesterol), electron transfer (e.g. quinones), and photosynthesis (e.g. carotenoids).\textsuperscript{125} As precursors to a myriad of lipid moieties, isoprenoids are important biosynthetic intermediates that lead to the production of sterols, triterpenes (e.g. squalene), carotenoids, and hopanoids.\textsuperscript{125} Isoprenoids can also serve as lipid anchors for proteins and carbohydrates.\textsuperscript{4, 126} Perhaps the most interesting and complex group of isoprenoid biosynthetic products is the vast set of cyclic terpene natural products such as monoterpenes, sesquiterpenes, and diterpenes.\textsuperscript{124, 127} Due to the extensive diversity of isoprenoid natural products, it is not surprising that many promising and effective pharmaceuticals such as Taxol (cancer), artemisinin (malaria), vinblastine (cancer), and prostratin (HIV) have been discovered.\textsuperscript{125} Not only are cyclic isoprenoids of importance to the pharmaceutical industry, but they are also of great interest in the materials, chemical, and fuel industries.\textsuperscript{128} Therefore, there is significant interest in generating novel isoprenoid diphosphate analogs to use as chemical tools to further explore these multifarious processes.
Our lab has a long-standing interest in the design and synthesis of novel FPP and GGPP analogs as chemical tools to explore the enzyme specificity and requirements of FTase. Recently, Dr. Andrew Placzek developed a method for the preparation of a small library of frame-shifted FPP analogs (Figure 4.1). These analogs were designed to increase and/or decrease the carbon spacers of the FPP backbone in order to examine the relevance of the length and flexibility of the isoprenoid chain and how this pertains to FTase activity. Preliminary evaluation revealed that four of the eight FPP frame-shifted analogs are substrates of FTase (2,2,1,10OPP; 1,2,1-OPP; 1,3,1-OPP, 3,1,1-OPP; Figure 4.1, Blue solid box). The numbering scheme refers to the number of carbon spacers between the double bonds or between the first isoprene double bond and the pyrophosphate group. While three of these compounds have an overall comparable length to that of FPP, the surprising result was the ability of 2,2,1,10OPP (an analog that is only one CH$_2$ unit shorter than GGPP) to act as a substrate of FTase. Moreover, it appears that conformationally restricting the last isoprene unit leads to very poor or non-substrates. With this information in hand, we decided to construct 1,2,2,10OPP (4.21). This analog is of same overall length as 2,2,1,10OPP and also incorporates the conformationally restrictive 1E,4E-pentadiene structural motif; thus, it would be interesting to see how these features translate into biochemical activity in FTase and GGTase-I binding. Additionally, in the case of 3,1,1-OPP and 2,2,1,1-OPP, it appeared that deletion of a methylene group from between the α and β isoprenoids led to good substrates with comparable $k_{cat}/K_M$ to FPP; however, the $k_{cat}$ and $K_M$ values were ~10-fold lower than FPP.

Of the four analogs that did not behave as substrates, a preliminary inhibitory potency assay revealed that one analog, homofarnesyl diphosphate (2,2,2-OPP), was an inhibitor of FTase with an IC$_{50}$ below 1 µM (Figure 4.1, Purple dashed box). This inhibitory activity is believed to be attributed to the lower nucleophilicity at the now non-allylic C$_1$ (the C attached to the –OPP). To see if this holds true in GGTase-I binding as well, we synthesized a nonallylic GGPP analog, homogeranylgeranyl pyrophosphate (2,2,2,2-OPP; 4.32).
With the preliminary data in hand, our goal was to expand upon this theme and develop novel frame-shifted analogs in an effort to explore the enzyme specificity and requirements of GGTase-I versus FTase. The target compounds were 3,3,1-OPP; 2,3,1-OPP; 1,2,2,1-OPP; 2,2,2,2-OPP; 3,2,1-OPP; 4,2,1-OPP; 5,2,1-OPP; and 6,2,1-OPP (Figure 4.2). Unlike the previously synthesized frame-shifted analogs, these analogs are much more flexible and vary greatly in length between FPP and GGPP. Additionally, 6,2,1-OPP is essentially the same as GGPP with the exception that the third (γ) isoprene unit has been removed. We believe that increased flexibility may aid in binding ability.
Figure 4.1. Frame-shifted analog numbering scheme & previously synthesized FPP analogs. (A) General numbering scheme. (B) Previously synthesized frame-shifted FPP analogs by Andrew Plazcek. Green box indicates substrates; red box indicates inhibitor. Green circle indicates methylene unit added; Red square represents methylene unit removed; Blue solid box represents substrates; Purple dashed box represents inhibitor; TC = total carbons; \( l \) = carbon chain length.
Figure 4.2. Newly synthesized frame-shifted analogs. Green circle indicates methylene unit added; Red square represents methylene unit removed; Blue solid box represents substrates; Purple dashed box represents inhibitor; TC = total carbons; ℓ = carbon chain length.
4.2 Synthesis of Frame-Shifted Geranylgeranyl Pyrophosphate Analogs

The synthesis began with the preparation of 3,3,10-OPP (Scheme 4.1). To begin, 4-pentyn-1-ol (4.1) was transformed into the corresponding iodide (4.2) which underwent Negishi’s ZACA reaction and quenched with paraformaldehyde to afford iodo-alcohol 4.3. Similarly, 5-hexyn-1-ol (4.5) underwent a ZACA reaction and was quenched with iodide to yield the vinyl-iodide 4.6. To install the last isoprene unit, 4.6 was subjected to Swern oxidation conditions followed by a Wittig reaction to generate vinyl-iodide 4.8. Following THP-protection of alcohol 4.3, alkyl iodide 4.4 could be converted into the organoborane and then coupled with vinyl-iodide 4.8 under Suzuki cross-coupling conditions to afford 3,3,10-OTHP (4.9), which was then deprotected to yield 4.10. Pyrophosphorylation was accomplished in a similar manner as described by Davisson et al. to produce 4.11 (3,3,1-OPP).

To synthesize 2,3,1-OH (Scheme 4.2), we first needed to synthesize bishomogeranyl iodide, 4.14. This could be accomplished by employing a method described by Kuwajima & Doi and later used in our laboratory. Briefly, LDA was added to an equimolar amount of ethyl acetate in the presence of Cu(I)I at -110°C. The solution was then allowed to slowly warm to -30°C at which point geranyl bromide (4.12) was added to the reaction to give ester 4.13 in 34% yield. The ester was then subjected to DIBAL reduction followed by iodination to afford bishomogeranyl iodide 4.15. Utilizing Suzuki coupling, vinyl-iodide 3.14 and alkyl-iodide 4.15 were coupled. Following deprotection of the TBS-group with TBAF, 4.16 (2,3,1-OH) was produced in 38% yield. Subsequent chlorination and pyrophosphorylation resulted in compound 4.17.

The strategy for the synthesis of 1,2,2,1-OH was based on the displacement of allylic diethyl phosphates with Grignard reagents utilized by Snyder and colleagues (Scheme 4.3).
Displacement of the phosphate group by Grignard 4.18 followed by THP-deportation produced 4.20 (1,2,2,1-OH). Subsequent chlorination and pyrophosphorylation resulted in compound 4.21.

In the synthesis of 3,2,1-OH (Scheme 4.4), iodide 4.25 was obtained by first converting the THP ether of farnesol to epoxide 4.22 via a bromohydrin intermediate. Epoxide 4.22 was then converted to aldehyde 4.23 using periodic acid which was later reduced to alcohol 4.24 via a borohydride reduction. Following iodination, iodide 4.22 was obtained and subjected to THP-deprotection to afford iodo-alcohol 4.26. Employing a method originally developed by Derguini-Boumechal & Linstrumelle, vinyl-Grignard 4.18 can be used to substitute the alkyl iodide of 4.26 when done in the presence of Cu(I)I to produce 3,2,1,0-OH (4.27). Subsequent chlorination and pyrophosphorylation resulted in compound 4.28.

One compound targeted for synthesis was a GGPP analog containing a nonallylic diphosphate (4.32). Previously, homofarnesyl pyrophosphate (2,2,2-OPP) was synthesized in our lab as part of an FTase screen. The significant decrease in nucleophilicity at C1 of the homoallylic diphosphate lead to the hypothesis that 2,2,2-OPP would behave as a non-substrate. In fact, homofarnesyl pyrophosphate displayed inhibitory activity with an IC50 below 1µM. Thus, we hypothesis that homogeranylgeranyl pyrophosphate will behave in a similar manner as the farnesyl derivative. In order to synthesized homogeranylgeranyl pyrophosphonate (2,2,2,2-OPP, 4.32), we utilized a synthetic route similar to the one our laboratory used to synthesized geranylgeraniol (Scheme 4.5). Briefly, farnesyl chloride (4.29) is subjected to the TMS-propynyl anion, displacing the chloride. Following TMS-deprotection with TBAF, alkyne 4.30 can undergo a ZACA reaction which is subsequently quenched with oxirane to produce homogeranylgeraniol (3.31). Attempts to chlorinate alcohol 4.31 via NCS lead to poor yields which may be contributed to the less reactive homoallylic position. To avoid this problem, the alcohol was first converted into the mesylate and subsequently pyrophosphorylated to yield compound 4.32.
With the above alcohols in hand, we turned to synthesizing the remaining three alcohols (Scheme 4.6). Commercially available bromo-alkylesters (4.33) were easily converted into the corresponding iodoalkenes (4.34a-c) with a series of Finkelstein, DIBAL reduction, and Wittig reactions. In order to complete the unique transformation of 4.34 to 4.37, we employed a strategy utilized previously in our laboratory which was first developed by Wenkert and colleagues and later used by Kocienski and colleagues.\textsuperscript{75, 134-136} In order to transform the carbon-oxygen bonds into carbon-carbon bonds, this method relies upon the nickel-catalyzed ring opening of dihydrofurans (or dihydropyrans) with Grignard reagents to produces stereo-defined trisubstituted alkenes. First, iodides 4.34a-c underwent alkylation with 5-lithio-2,3-dihydrofuran (4.36) using a modified procedure of Placzek and coworkers.\textsuperscript{75} We found that increasing the equivalents of 4.36 from 0.95 to 3.0 equivalents resulted in higher yields when applied to our compounds. The newly prepared alkylated dihydrofuran was immediately reacted with MeMgBr in the presence of NiCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2} to produce alcohols 4.37a-c. The corresponding iodides (4.48a-c) were converted into their organoborane derivatives. Suzuki cross-coupling of these organoboranes with vinyl iodide 3.14 followed by TBS-deprotection with TBAF afforded alcohols 4.39a-c. Subsequent chlorination and pyrophosphorylation produced compounds 4.40a-c.
Scheme 4.1. Synthesis of 3,3,1-OPP. (a) I₂, PPh₃, Imidazole, DCM, 0°C, 4 hr; (b) Me₃Al, Cp₂ZrCl₂, DCM, 0°C, 18 hr then (CH₂O)₅, 3 hr (52%; 2 steps); (c) PPTS, DHP, DCM (86%); (d) Me₃Al, Cp₂ZrCl₂, DMC 0°C, 18 hr then I₂, 3 hr (25%); (e) (COCl)₂, DMSO, DCM, -78°C, 1hr then Et₃N (67%); (f) i-PrPPh₃I, n-BuLi, THF, -78°C, 4 hr (65%); (g) i. t-BuLi, Et₂O, -78°C, ii. β-MeO-9-BBN, THF, -78°C warming to RT o/n, iii. K₃PO₄, PdCl₂(dpff), DMF, 18 hr; (h) PPTS, MeOH, 60°C (25%-2 Steps); (i) NCS, DMS, DCM, 0°C to rt, 2.5 hr; (j) (NB₄)₃HP₂O₇, ACN, 2.5 hr (70% - 2 steps).

Scheme 4.2. Synthesis of 2,3,1-OPP. (a) Ethyl Acetate, Cu(I), LDA, THF, -110°C (34%) (b) DIBAL, THF, 0°C; (c) I₂, PPh₃, Imidazole, DCM, 0°C (68% - 2 Steps); (d) i. t-BuLi, Et₂O, -78°C, ii. β-MeO-9-BBN, THF, -78°C warming to RT o/n, iii. K₃PO₄, PdCl₂(dpff), DMF, 85°C, 18 hr; (e) TBAF, THF, 0°C (56% - 2 Steps); (f) NCS, DMS, DCM, 0°C to rt, 2.5 hr; (g) (NB₄)₃HP₂O₇, ACN, 2.5 hr (43% - 2 steps).
Scheme 4.3. Synthesis of 1,2,2,1-OPP. (a) 4.18, THF, o/n (52%); (b) PPTS, EtOH, 70°C (71%); (c) NCS, DMS, DCM, 0°C to rt, 2.5 hr; (d) (NBu₄)₂HP₂O₇, ACN, 2.5 hr (69% - 2 steps).

Scheme 4.4. Synthesis of 3,2,1-OPP. (a) i. NBS, THF:H₂O (2:1); ii. K₂CO₃, MeOH (46% - 2 Steps); (b) i.H₂O₂, THF, Et₂O; (c) NaBH₄, EtOH; (d) PPh₃, I₂, imidazole, DCM (55%-3 steps); (e) PPTS, EtOH, 65°C (68%); (f) 4.18, Cu(I)I, THF, -30°C (29%); (g) NCS, DMS, DCM, 0°C to rt, 2.5 hr; (h) (NBu₄)₂HP₂O₇, ACN, 2.5 hr (73% - 2 steps).
Scheme 4.5. Synthesis of 2,2,2,2-OPP. (a) i. TMS-propyne, n-BuLi, THF, -78°C; ii. TBAF, THF, 0°C (73%); (b) i. Cp₂ZrCl₂, Me₃Al, DCM, 0°C to r.t., 16 hr; ii. n-BuLi, 0.5 hr, -78°C; iii. oxirane, 4 hr, -78°C to r.t. (18%); (c) MsCl, DMAP, DCM, 0°C to rt, 2.5 hr; (d) (NBu₄)₂HP₂O₇, ACN, 2.5 hr (71% - 2 steps).

Scheme 4.6. Synthesis of 4,2,1-OPP, 5,2,1-OPP, & 6,2,1-OPP. (a) i. KI, Acetone, Reflux; ii. DIBAL, DCM, -78°C; iii. i-PrPPh₃I, n-BuLi, THF, 0°C; (b) t-BuLi, Et₂O, -78°C; (c) i. 4.35, THF, -78°C to r.t., 16 hr; ii. NiCl₂(PPh₃)₂, MeMgBr, PhH, 75°C; (d) Imidazole, PPh₃, I₂, 0°C; (e) i. t-BuLi, Et₂O, -78°C; ii. β-MeO-9-BBN, THF, -78°C to r.t., 16 hr; iii. 3.14, K₃PO₄, PdCl₂(dppf), DMF, 85°C, 16 hr; (f) TBAF, THF, 0°C; (g) NCS, DMS, DCM, 0°C to rt, 2.5 hr; (h) (NBu₄)₂HP₂O₇, ACN, 2.5 hr.
4.3 Biochemical Evaluation of Frame-Modified Geranylgeranyl Pyrophosphate Analogs

Previously, Dr. Andrew Placzek of our laboratory synthesized a small library of frame-shifted FPP analogs. Upon evaluation of these analogs, it was revealed that four behaved as substrates while one was shown to be an inhibitor of FTase. Based on these results, our goal was to synthesize a small library of similar compounds ranging in length between FPP and GGPP. These frame-shifted analogs (4.11, 4.17, 4.21, 4.28, 4.32, 4.40a-c) were evaluated for their biochemical activity in an in vitro continuous spectrofluorometric assay with GGTase and the co-substrate CaaX-peptide dansyl-GCVLL. All biochemical assays were performed in our collaborator Dr. Carol Fierke’s laboratory at the University of Michigan by Elia Wright.

Upon in vitro evaluation, it was evident that seven of the eight frame-shifted analogs (4.11, 4.17, 4.21, 4.28, 4.40a-c) were substrates of GGTase-I. While the data in Figure 4.3 may not appear to indicate that 4.11 and 4.17 are substrates, an endpoint assay monitored 1 µM analog, 5 µM peptide, and 50 nM GGTase-I for 3 hours and revealed these analogs do get turned over by the enzyme, albeit very slowly (Figure 4.5); however, further investigation of these analogs as potential inhibitors should be considered.

Once again, the chain length of our analogs plays a crucial role in determining substrate activity; however, by comparing analogs of 13 carbons in chain length (4.17 and 4.28), an additional feature seems to hinder substrate activity (Figure 4.3 & 4.4). Compounds 4.17 and 4.28 differ only in the position of the β-isoprene unit; by adding an extra methylene group between the α- and β-isoprene units, substrate activity is decreased. This trend is also apparent when comparing analogs of 14 carbons in chain length (4.11 and 4.40a; Figures 4.3-4.5). One possible reason for this observation could be that extending the β-isoprene into the binding pocket of GGTase could result in unfavorable interactions between the analog and the enzyme and/or peptide substrate; however, further structural studies are needed. Hence, the β-isoprene unit is
not required for substrate recognition and activation as demonstrated in Chapter 3; however, if it is present, its location/orientation in the molecule appears to be crucial.

When comparing compounds of 15 carbons in length (4.21 and 4.40b), it becomes apparent that chain length plays a much larger role in determining substrate activity rather than flexibility (Figures 4.3-4.5). The only difference between these two analogs is that 4.40b lacks the γ-isoprene unit of 4.21; however, both analogs have very similar substrate abilities. Thus, the lack of flexibility in the ω-isoprene of 4.21 seems not to be an important factor. The similarity between these two compounds also indicates that the γ-isoprene has no added benefit to the substrate activity. Additionally, chain length of molecules containing the ω-isoprene is critical for substrate activity.

By examining the length of analogs 4.28 and 4.40a-b (Figure 4.4), it appears that increasing the length of the carbon chain from 13 to 15 carbons has minimal effects on substrate activity. Thus, having three isoprene units attenuates the effects of increased chain length when the number of carbons is less than GGPP; however, when the carbon length is increased to that of GGPP (4.40c), we notice a significant jump in substrate ability. In fact, analog 4.40c exhibited substrate activity comparable to GGPP. Analog 4.40c also reveals that the γ-isoprene unit of GGPP is not needed for enzyme recognition.

Not surprisingly, our homo-GGPP analog (4.32) displayed no substrate activity. This analog was originally synthesized to determine if it would act as an inhibitor of GGTase-I. Reactions with 1 µM or 5 µM analog with 10 µM GGPP displayed no increase in fluorescence (Figure 4.6). A preliminary inhibitor assay (Figure 4.6) suggests that analog 4.32 is an inhibitor of GGTase; a more extensive evaluation is currently underway.
Figure 4.3. Bar graphs of substrate activity represented in RFI of 4.11, 4.17, 4.21, 4.32, & 1,2,2,1-OPP versus GGPP (+ control) with 5 μM dansyl-GCVLL and 50 nM GGTase-I. Error bars represent mean ± SD (n = 3). *Values shown are for 10 μM analog at 1.5 hours.

Figure 4.4. Bar graphs of substrate activity represented in RFI of 4.28 & 4.40a-c versus GGPP (+ control) with 5 μM dansyl-GCVLL and 50 nM GGTase-I. Error bars represent mean ± SD (n = 3). *Values shown are for 10 μM analog at 1.5 hours.
Figure 4.5. Monitoring continuous changes in fluorescence of frame-shifted GGPP analogs versus GGPP (+ control) at various concentrations (1 µM, 5µM, & 10µM) with 5 µM dansyl-GCVLL and 50 nM GGase-I. Experiments were performed in triplicate and data points represent the mean. Endpoint assays of 4.11 & 4.17 are also included.
**Figure 4.6.** Preliminary inhibitor assay results for 4.32 with 50 nM GGTase-I. Raw fluorescence was not subtracted from background. Reactions with inhibitor were not above background peptide fluorescence. Experiment was performed in triplicate and data represents the mean.
4.4 Conclusions

The goal of this chapter was to focus on the synthesis of a small library of frame-shifted GGPP analogs in order to determine the isoprene requirements of the enzyme GGTase-I. Previously, our lab synthesized a library of frame-shifted FPP analogs that displayed interesting result in vivo. Therefore, our laboratory aimed to expand this library to include analogs in length between FPP and GGPP.

This aim was centered on designing synthetic routes that would enable us to generate our small library of eight compounds to use as chemical tools to probe the tolerance of the GGTase-I binding pocket. Unfortunately, no one synthetic route could be utilized for all frame-shifted compounds. The synthesis of 4.11 was dependent upon the availability of the alkynyl-alcohols 4.1 and 4.5 (Scheme 4.1). This route could not be utilized for the other analogs for multiple reasons. First, various attempts to oxidize iodo-alcohols shorter than 4.6 resulted in low yields or unstable aldehydes. Second, longer alkynyl-alcohols of 4.5 needed in the synthesis of 4.40a-c were not commercially available. Therefore, in order to synthesize 4.17 we utilized a method originally developed by Kuwajima & Doi and was later used in our laboratory to generate bishomogeraniol.129 The synthesis of 4.21 was accomplished in a similar manner as employed in Chapter 2 to generate the aryl-modified analogs. To generate 4.28, we employed a method developed by Derguini-Boumechal & Linstrumell to substitute an alkyl iodide with a vinyl-Grignard reagent.131 This method was later used by Andrew Placzek in the synthesis of the frame-shifted FPP analogs.75 Homogeranylgeraniol could easily be synthesized utilizing the ZACA reaction. By simply substituting oxirane for paraformaldehyde we could generate a homoallylic alcohol which was then converted into 4.32. The remaining analogs 4.40a-c were synthesized using a modified method as employed by Andrew Placzek to generate the frame-shifted FPP analogs.75 This method was originally developed by Wenkert and colleagues and was
used to synthesize the intermediates (4.37a-c). The original method called for 0.95 equivalents of dihydrofuran 4.35; however, this produced very low yields (< 20%). By increasing the equivalents to 3 resulted in much higher yielding transformations.

The biochemical evaluation of these compounds revealed several interesting trends. The first observation was that by increasing or decreasing the number of carbons between the α- and β-isoprenes by one methylene result in a significant decrease in substrate activity. These analogs could be selective substrates; thus, while these analogs are poor co-substrates with Dansyl-GCVLL, they could potentially be great co-substrates with other CaaX sequences. Alternatively, these modifications could hinder product release from the enzyme, which is dependent on the movement of the isoprene chain into an exit groove; however, further structural analyses are needed. The second observation is that the length of the carbon chain appears to be more important than flexibility (4.21 vs 4.39b). Moreover, when the analog has three or more isoprene units and two carbons between the α- and β-isoprene units, increasing the carbon chain length from 13 to 15 carbons has a minimal effect; however, increasing the length to 16 carbons (4.39c) has a substantial effect on enzyme activity. Additionally, the γ-isoprene appears to be irrelevant; by removing this isoprene unit we generated an analog that displays substrate activity comparable to GGPP. The preliminary data suggests the homoallylic analog (4.32) is an inhibitor of GGTase-I. Further analysis is underway to determine the IC₅₀ value of this analog.

We have successfully generated a small library of frame-shifted GGPP analogs designed to probe the GGPP binding pocket of GGTase-I. Data shown in Figure 4.5 indicates our frame-shifted molecules are either turned over more slowly than GGPP or display a lower affinity for the enzyme than GGPP. Unfortunately, the analogs were tested at only three concentrations (1, 5, & 10 µM) and none of the reactions reached completion at 5 or 10 µM of analog in the time they were monitored. In order to determine accurate $k_{cat}$ and $K_m$ values, these analogs are currently being retested at more concentrations and longer reaction times. Our preliminary results are very
promising and we anticipate doing more extensive *in vitro* screening with a large library of dansyl-GCaaX sequences to determine the selectivity of our analogs. Additionally, we also expect to test the analogs that demonstrated poor or no substrate activity as inhibitors. These compounds range in length between GGPP and FPP; therefore, at a later date, we will also be testing these compounds with FTase to determine their ability to be recognized and utilized by one enzyme over another.
4.5 Experimental Procedures Utilized for the Synthesis & Biochemical Evaluation of Frame-Shifted Geranylgeranyl Pyrophosphate Analogs

**General Experimental Procedures:** All reactions were performed with oven-dried or flame-dried glassware and under dry argon gas. All commercial reagents and solvents were used directly without subsequent purification. For the organometallic coupling reactions, anhydrous THF was freshly distilled from sodium and benzophenone. All other anhydrous solvents were purchased from Acros Organics as extra dry solvents and were bottled over molecular sieves. Reactions were monitored by thin layer chromatography and visualized with one or more of the following: UV light, iodine, vanillin solution, potassium permanganate solution, dinitrophenylhydrazine solution, and/or phosphomolybdic acid solution. All products were purified using flash chromatography silica gel 60 M purchased from Macherey-Nagel. All reactions involving either triphenyl phosphine or triphenyl phosphine oxide were first dry-loaded with sodium sulfated before column purification. NOTE: *Dry glassware is critical for the organometallic reactions in this publication. This was accomplished by taking oven-dried glassware (dried for at least 24 hr and then cooled under argon) and flame drying the round bottom flask under vacuum. The flask was then purged with argon and cooled to room temperature. This process was repeated 3 times to produce a completely water free environment.*  
**Deoxygenated solvents are extremely crucial for successful organometallic reactions in this publication. In order to achieve completely deoxygenated solutions, the solvent was placed in a flame-dried round bottom and under vacuum was sonicated for 30 seconds and then purged with argon for 30 seconds. This process was repeated 3 times to yield completely deoxygenated solvents.** All NMR spectra were taken either on a 300 MHz Bruker ARX300 or a 500 MHz Bruker DRX500 spectrometer. Low-resolution MS (EI/Cl) were recorded with a
Hewlett Packard Engine and low-resolution MS (ESI) were taken on a Thermoquest LCQ. All high-resolution mass spectra were recorded on a FinniganMAT XL95.

(E)-6-ido-3-methylhex-2-en-1-ol (4.3):

To a round bottom flask was added pent-4-yn-1-ol (1 eq, 8 mmol), PPh$_3$ (1.3 eq, 10.4 mmol) and imidazole (1.7 eq, 13.6 mmol) dissolved in dichloromethane (10 mL). Next, the solution was cooled to 0°C with and ice-bath and I$_2$ (1.3 eq, 10.4 mmol) was added to the reaction flask. The reaction was allowed to stir 0.5 hr at 0°C then allowed to warm to room temperature and stirred for an additional 1.5 hr. Upon completion of the reaction, 10% Na$_2$S$_2$O$_3$ was added to the flask and stirred for 20 minutes. The organic layer was isolated and the aqueous layer was extracted 1 × DCM (10 mL). Due to the volatility of 5-iodopent-1-yne, it was used in the next step with purification and was not concentrated.

Cp$_2$ZrCl$_2$ (0.25 eq, 2.0 mmol) was dissolved in dichloromethane (8.0 mL) and the solution was cooled to 0°C, where AlMe$_3$ solution (2.0 M in heptanes, 2.5 eq, 24 mmol, 12.0 mL) was added dropwise. After stirring the reaction mixture at 0°C for 30 minutes, 5-iodopent-1-yne (1.0 eq, 8.0 mmol) was diluted in dichloromethane (20 mL) and added to the reaction mixture dropwise. The reaction mixture was allowed to slowly warm to room temperature and stirred an additional 12 hours. Next, the reaction mixture was cooled to 0°C, where (CH$_3$O)$_n$ (5 eq, 40.0 mmol) was added in several portions. The reaction continued to stir for 3 hours after which it was slowly poured into an ice-cold 10% HCl$_{aq}$ solution. The solution was then filtered over a pad of Celite 545, extracted with 3 × 30 mL dichloromethane, washed with brine, dried with magnesium sulfated, filtered, and concentrated. Column chromatography (30% Ethyl acetate in Hexanes) afforded 4.3a in 52% yield over 2-steps. $^1$H NMR: (300 MHz, CDCl$_3$) δ 5.44 (ddd, $J = 8.1, 5.7, 1.3$ Hz, 1H), 4.13 (d, $J = 6.8$ Hz, 2H), 3.14 (t, $J = 6.9$ Hz, 2H), 2.11 (t, $J = 7.3$ Hz, 2H),
1.98 – 1.87 (m, 2H), 1.65 (s, 3H).  

\[ ^{13}C \text{NMR (75 MHz, CDCl}_3\] ` δ 137.81 (s), 124.84 (s), 59.50 (s), 40.12 (s), 31.48 (s), 16.41 (s), 6.51 (s).

\[ \text{(E)-2-((6-iodo-3-methylhex-2-en-1-yl)oxy)tetrahydro-2H-pyran (4.4)} \]:

A round bottom flask was charged with (E)-6-iodo-3-methylhex-2-en-1-ol (1.0 eq, 4.13 mmol) dissolved in DCM (10 mL). Next, 3,4-dihydro-2H-pyran (3.0 eq, 12.40 mmol) and pyridinium p-toluenesulfonate (0.1 eq, 0.413 eq) were added to the reaction flask. The reaction was stirred at room temperature for 1 hour and then concentrated. The residue was loaded directly onto a silica column and column chromatography (7.5% Ethyl acetate in Hexanes) afforded 4.4a in 86% yield. 

\[ ^{1}H \text{NMR (300 MHz, Chloroform-d)} \] ` δ 5.32 (t, \( J = 6.3 \) Hz, 1H), 4.53 (s, 1H), 4.04 (ddd, \( J = 64.9, 11.9, 6.9 \) Hz, 2H), 3.79 (dd, \( J = 12.8, 5.6 \) Hz, 1H), 3.68 – 3.32 (m, 1H), 3.08 (t, \( J = 6.9 \) Hz, 2H), 2.05 (t, \( J = 7.3 \) Hz, 2H), 1.86 (p, \( J = 7.0 \) Hz, 2H), 1.81 – 1.69 (m, 1H), 1.59 (s, 3H), 1.54 – 1.37 (m, 5H). \n
\[ ^{13}C \text{NMR (75 MHz, Chloroform-d)} \] ` δ 137.95, 122.02, 97.85, 63.52, 62.28, 40.02, 31.33, 30.70, 25.50, 19.62, 16.37, 6.45.

\[ \text{(E)-6-iodo-5-methylhex-5-en-1-ol (4.6)} \]:

\[ \text{Cp}_2\text{ZrCl}_2 \] (0.25 eq, 4.0 mmol) was dissolved in dichloromethane (16.0 mL) and the solution was cooled to 0°C, where AlMe\(_3\) solution (2.0 M in heptanes, 2.5 eq, 48 mmol, 24.0 mL) was added dropwise. After stirring the reaction mixture at 0°C for 30 minutes, 5-hexyn-1-ol (1.0 eq, 16.0 mmol) was diluted in dichloromethane (8 mL) and added to the reaction mixture dropwise. The reaction mixture was allowed to slowly warm to room temperature and stirred an additional 12 hours. Next, the reaction mixture was cooled to 0°C, where I\(_2\) (3.5 eq, 56.0 mmol) was added in several portions. The reaction continued to stir for 3 hours after which it was slowly poured into an ice-cold 10% HCl\(_{aq}\) solution. The solution was then filtered over a pad of
Celite 545, extracted with $3 \times 50$ mL dichloromethane, washed with brine, dried with magnesium sulfated, filtered, and concentrated. Column chromatography (15% Ethyl acetate in Hexanes) afforded 4.6 in 25% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.86 (dd, $J = 2.2, 1.1$ Hz, 1H), 3.63 (dd, $J = 7.2, 5.0$ Hz, 2H), 2.24 – 2.17 (m, 2H), 1.81 (d, $J = 1.0$ Hz, 3H), 1.54 – 1.47 (m, 5H). $^{13}$C NMR (75 MHz, Chloroform-d) $\delta$ 148.00, 75.01, 62.83, 39.46, 32.23, 24.06, 23.96.

(E)-6-iodo-5-methylhex-5-enal (4.7):

An oven-dried multi-neck round bottom flask and addition funnel are assembled while hot and cooled down under argon. The flask is then charged with (COCl)$_2$ (1.2 eq, 4.84 mmol) dissolved in 5 mL of anhydrous DCM. The flask is then cooled down to -78°C and DMSO (2.4 eq, 9.67 mmol) diluted in 2 mL of anhydrous DCM is added dropwise to the reaction mixture over the course of 20 minutes via the addition funnel and stirred for an additional 30 minutes at -78°C. Next, (E)-6-iodo-5-methylhex-5-en-1-ol (1.0 eq, 4.03 mmol) is dissolved in 8 mL of anhydrous DCM and slowly added to the reaction mixture via the addition funnel. Upon complete addition of the alcohol, the reaction is stirred for an additional 1.5 hr at -78°C. Finally, Et$_3$N (5.0 eq, 20.15 mmol) is added to the reaction; the mixture is removed from the cooling bath and is allowed to stir until it warms to room temperature. The reaction is then quenched by the addition of 10% NH$_4$Cl and extracted 3 $\times$ 50 mL DCM. The organic layers were combined washed brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography (30% Et$_2$O/Hexanes) to afford 4.7 in 67% yield. $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 9.69 (q, $J = 1.2$ Hz, 1H), 5.84 (t, 1H), 2.36 (tt, $J = 7.2, 1.2$ Hz, 2H), 2.17 (t, $J = 7.5$ Hz, 2H), 1.75 (t, $J = 1.1$ Hz, 3H), 1.74 – 1.67 (m, 2H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 201.83 (s), 146.97 (s), 75.72 (s), 42.87 (s), 38.64 (s), 23.71 (s), 19.95 (s).
(E)-1-iodo-2,7-dimethylocta-1,6-diene (4.8):

To an oven-dried round bottom flask is added (i-Pr)Ph$_3$PI (1.3 eq, 3.51 mmol) dissolved in 17.5 mL of anhydrous THF. The mixture is cooled to 0°C where n-BuLi (2.5 M in hexanes, 1.3 eq, 3.51 mmol) is added dropwise. The resulting orange solution is stirred for 1 hr at 0°C. Next, E)-1-iodo-2,7-dimethylocta-1,6-diene (1.0 eq, 2.7 mmol) is diluted in 1.2 mL of anhydrous THF and added dropwise to the reaction mixture, which is allowed to stir for 4 hours coming to room temperature. Upon completion of the reaction, saturated NH$_4$Cl(aq) is added to the reaction vessel and stirred for 15 minutes. Following extraction with 3 × 50 mL hexanes, the organic layers were combined, washed with brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography (100% Hexanes) to afford 4.8 in 65% yield. $^1$H NMR (300 MHz, Chloroform-d) δ 5.84 (dd, $J = 2.2$, 1.1 Hz, 1H), 5.06 (t, $J = 7.2$ Hz, 1H), 2.18 (t, 2H), 1.93 (q, $J = 7.3$ Hz, 2H), 1.80 (d, $J = 1.0$ Hz, 3H), 1.67 (d, $J = 0.9$ Hz, 3H), 1.57 (s, 3H), 1.50 – 1.39 (m, 2H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 148.21 (s), 132.13 (s), 124.11 (s), 74.75 (s), 39.28 (s), 28.01 (s), 27.50 (s), 25.94 (s), 24.01 (s), 17.95 (s).

2-(((2E,7E)-3,8,13-trimethyltetradeca-2,7,12-trien-1-yl)oxy)tetrahydro-2H-pyran (4.9):

An oven-dried round-bottom flask containing powdered molecular sieves was charged with (E)-2-(((6-iodo-3-methylhex-2-en-1-yl)oxy)tetrahydro-2H-pyran (stored over sieves; 2.11 eq, 3.55 mmol) which was dissolved in 3.5 mL of anhydrous Et$_2$O and cooled to -78°C. Next, t-BuLi (1.7 M in pentane; 4.22 eq, 7.1 mmol) was slowly added to the reaction flask and stirred for 1 hour at -78°C. Afterward, β-MeO-9-BBN (1.0M in Hexanes; 4 eq, 6.72 mmol) was slowly added
to the reaction vessel and the mixture was stirred for 16 hours slowly warming to room
temperature.

In a scintillation vial, (E)-1-iodo-2,7-dimethylocta-1,6-diene (1.0 eq, 1.68 mmol) and
Pd(dppf)Cl$_2$ (0.1 eq, 0.17 mmol) are dissolved in 8 mL of DMF. Next, K$_3$PO$_4$ (3.0 eq, 5.0 mmol)
is added to the vial and after deoxygenating the solvent, the vinyl-iodide solution is added to the
round-bottom flask containing the newly formed organoborane. The reaction mixture is allowed
to stir for an additional 16 hours at 85°C after which it is poured into a separatory funnel
containing water and Et$_2$O and the aqueous layer was extracted 3 × 50 mL Et$_2$O. The organic
layers were combined and washed with water then brine, dried with magnesium sulfate, and
concentrated. The crude reaction product was purified by column chromatography (5%
Et$_2$O/Hexanes) to afford **4.9** in 38% yield. $^1$H NMR (300 MHz, Chloroform-d) $\delta$ 5.32 (t, $J = 7.0$
Hz, 1H), 5.08 (t, $J = 7.1$ Hz, 2H), 4.60 (t, $J = 3.5$ Hz, 1H), 4.10 (ddd, $J = 62.6, 11.9, 6.8$ Hz, 2H),
3.86 (td, $J = 7.6, 3.8$ Hz, 1H), 3.47 (dd, $J = 11.0, 5.1$ Hz, 1H), 2.06 – 1.85 (m, 8H), 1.66 (s, 3H),
1.63 (s, 3H), 1.56 (s, 3H), 1.54 (s, 3H), 1.53 – 1.28 (m, 10H).

(2E,7E)-3,8,13-trimethyltetradeca-2,7,12-trien-1-ol (**4.10**):

In a scintillation vial, 2-(((2E,7E)-3,8,13-trimethyltetradeca-2,7,12-trien-1-
yl)oxy)tetrahydro-2H-pyran (1.0 eq, 0.63 mmol) was dissolved in 5.0 mL of absolute EtOH and
PPTS (0.1 eq, 0.07 mmol) was added to the vial. The reaction mixture was heated to 60°C and
stirred for 12 hours. Next, the reaction was cooled to room temperature where it was poured into
a separator funnel containing water and Et$_2$O and the aqueous layer was extracted 3 × 5 mL Et$_2$O.
The organic layers were combined and washed with water then brine, dried with magnesium
sulfate, and concentrated. The crude reaction product was purified by column chromatography
using 10% Ethyl Acetate/Hexanes as the mobile phase to afford **4.10** in 67% yield. $^1$H NMR
(300 MHz, Chloroform-d) δ 5.38 (dd, J = 7.9, 6.2 Hz, 1H), 5.13 – 5.01 (m, 2H), 4.12 (d, J = 6.8 Hz, 2H), 1.95 (ddd, J = 13.1, 9.4, 6.0 Hz, 8H), 1.66 (s, 3H), 1.64 (s, 3H), 1.57 (s, 3H), 1.55 (s, 3H), 1.41 (ddt, J = 15.0, 12.8, 7.5 Hz, 4H).  

\(^{13}\)C NMR (75 MHz, Chloroform-d) δ 140.25, 135.63, 131.53, 124.89, 124.37, 123.42, 59.60, 39.53, 39.34, 28.38, 28.09, 27.84, 27.71, 25.94, 17.89, 16.39, 16.15.  

MS (EI) \(m/z\) 250; MS (CI) \(m/z\) 249.

(E)-ethyl 5,9-dimethyldeca-4,8-dienoate (4.13):

A round bottom flask charged with Cu(I)I (4.0 eq, 12 mmol) and placed in a drying oven for 1.5 hr, after which it is cooled to room temperature under argon. Anhydrous EtOAc (2.05 eq, 24.6 mmol, 2.42 mL) and anhydrous THF (90 mL) are added to the round bottom flask, which is then cooled to -107°C utilizing an isoctane/N\(_2\)liq bath. To this mixture is added LDA (2.0 M in heptanes/THF/ethyl benzene, 2.05 eq, 24.6 mmol) dropwise. The reaction is allowed to slowly warm to -30°C over 2 hr, at which point geranyl bromide (1.0 eq, 12 mmol) is dissolved in 15 mL of THF and added slowly to the reaction mixture. Upon complete addition of geranyl bromide, the reaction is stirred an additional 1 hr at -30°C, allowed to warm to 0°C and quenched with saturated NH\(_4\)Cl (80 mL). The aqueous layer was extracted with 30 mL Et\(_2\)O × 3, the organic layers were pooled together, washed with brine, dried with MgSO\(_4\), filtered and concentrated. Purification by flash chromatography using 3% EtOAc/Hexanes as the eluent afforded the title compound in 34% yield.  

\(^1\)H NMR (300 MHz, Chloroform-d) δ 5.13 – 5.01 (m, 2H), 4.10 (q, J = 7.1 Hz, 2H), 2.29 (d, J = 3.1 Hz, 4H), 2.10 – 1.88 (m, 4H), 1.65 (s, 3H), 1.59 (s, 3H), 1.57 (s, 3H), 1.23 (t, J = 7.2 Hz, 3H).  

\(^{13}\)C NMR (75 MHz, Chloroform-d) δ 191.48, 136.71, 131.54, 124.30, 122.45, 60.35, 39.78, 34.69, 26.76, 25.82, 23.71, 17.82, 16.13, 14.40.
(E)-5,9-dimethyldeca-4,8-dien-1-ol (4.14):

To an oven-dried round bottom flask is added (E)-ethyl 5,9-dimethyldeca-4,8-dienoate (1.0 eq, 4.034 mmol) dissolved in 15 mL of toluene which is then cooled to -78°C. DIBAL (1.0 M in toluene, 4.0 eq) was added dropwise to the reaction mixture and allowed to stir for 4 hours. The reaction mixtures was then slowly poured into an ice-cold 10% HCl(aq) solution and then filtered over a pad of Celite 545. The aqueous layer was extracted with 30 mL Et₂O × 3, the organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. Purification by flash chromatography using 15% EtOAc/Hexanes as the eluent afforded the title compound in 38% yield (Yield given is for 2-steps from geranyl bromide). ¹H NMR (300 MHz, Chloroform-d) δ 5.09 (t, J = 7.6 Hz, 1H), 5.03 (t, J = 7.0 Hz, 1H), 3.56 (t, J = 6.6 Hz, 2H), 2.36 (s, 1H), 2.08 – 1.87 (m, 6H), 1.63 (s, 3H), 1.61 – 1.49 (m, 8H). ¹³C NMR (75 MHz, Chloroform-d) δ 135.76, 131.46, 124.38, 123.92, 62.57, 39.84, 32.82, 26.77, 25.79, 24.36, 17.77, 16.05.

(Z)-10-iodo-2,6-dimethyldeca-2,6-diene (4.15):

To a round bottom flask was added (E)-5,9-dimethyldeca-4,8-dien-1-ol (1 eq, 4.6 mmol), PPh₃ (1.3 eq, 6.0 mmol) and imidazole (1.7 eq, 7.8 mmol) dissolved in dichloromethane (15 mL). Next, the solution was cooled to 0°C with and ice-bath and I₂ (1.3 eq, 6.0 mmol) was added to the reaction flask. The reaction was allowed to stir 0.5 hr at 0°C then allowed to warm to room temperature and stirred for an additional 1.5 hr. Upon completion of the reaction, 10% Na₂S₂O₃ was added to the flask and stirred for 20 minutes. The organic layer was isolated and the aqueous layer was extracted 3 × DCM (10 mL). The organic layers were pooled together, washed with
brine, dried with MgSO4, filtered and concentrated. Purification by flash chromatography using hexanes as the eluent afforded the title compound in 68% yield. \(^1\)H NMR (300 MHz, Chloroform-d) \(\delta\) 5.04 (td, \(J = 7.4, 6.7, 3.9\) Hz, 2H), 3.16 (t, \(J = 6.9\) Hz, 2H), 2.14 – 1.92 (m, 6H), 1.84 (p, \(J = 7.0\) Hz, 2H), 1.66 (s, 3H), 1.61 (s, 3H), 1.58 (s, 3H). \(^13\)C NMR (75 MHz, Chloroform-d) \(\delta\) 136.94, 131.64, 124.38, 122.46, 39.91, 33.81, 28.79, 26.77, 25.94, 17.92, 16.42, 7.06.

(2E,7E)-3,8,12-trimethyltrideca-2,7,11-trien-1-ol (4.16):

An oven-dried round-bottom flask containing powdered molecular sieves was charged with (Z)-10-iodo-2,6-dimethyldeca-2,6-diene (stored over sieves; 2.0 eq) which was dissolved in 4.6 mL of anhydrous Et\(_2\)O and cooled to -78°C. Next, \(t\)-BuLi (1.7M in pentane; 3.0 eq, 2.06 mmol) was slowly added to the reaction flask and stirred for 1 hour at -78°C. Afterward, \(\beta\)-MeO-9-BBN (1.0M in Hexanes; 3.8 eq, 2.6 mmol) was slowly added to the reaction vessel and the mixture was stirred for 16 hours slowly warming to room temperature.

In a scintillation vial, (E)-tert-butyl((3-iodobut-2-en-1-yl)oxy)dimethylsilane (1.0 eq, 0.69 mmol) and Pd(dppf)Cl\(_2\) (0.15 eq, 0.10 mmol) are dissolved in 6.6 mL of DMF. Next, K\(_3\)PO\(_4\) (3.0 eq, 2.06 mmol) is added to the vial and after deoxygenating the solvent, the vinyl-iodide solution is added to the round-bottom flask containing the newly formed organoborane. The reaction mixture is allowed to stir for an additional 16 hours at 85°C after which it is poured into a separatory funnel containing water and Et\(_2\)O and the aqueous layer was extracted 3 × 50 mL Et\(_2\)O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was run through a silica column (3% EtOAc/Hexanes) to remove most of the impurities and any oxidizing species.
In a scintillation vial, the semi-crude product was dissolved in 5.0 mL of THF, cooled to 0°C, and TBAF (1.0 M in THF, 2.0 eq, 1.15 mmol) was added to the vial. The reaction was allowed to stir for 4 hours and then quenched with 10% NH$_4$Cl(aq). Next, the organic layer was removed, and the aqueous layer was extracted 3 × Et$_2$O (10 mL). The organic layers are combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was purified by column chromatography using 15% Ethyl Acetate/Hexanes as the mobile phase to afford 4.16 in 56% yield. $^1$H NMR (300 MHz, Chloroform-d) δ 5.35 (t, $J = 7.0$ Hz, 1H), 5.07 (q, $J = 7.3$ Hz, 2H), 4.09 (d, $J = 6.9$ Hz, 2H), 1.95 (h, $J = 7.0$ Hz, 8H), 1.64 (s, 3H), 1.62 (s, 3H), 1.56 (s, 3H), 1.55 (s, 3H), 1.42 (p, $J = 7.5$ Hz, 2H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 140.15, 135.40, 131.47, 124.53, 124.46, 123.43, 59.55, 39.92, 39.28, 28.03, 27.67, 26.87, 25.89, 17.87, 16.37, 16.19. MS (EI) m/z 236; MS (CI) m/z 235.

2-(((2E,6E,10E)-3,7,11,14-tetramethylpentadeca-2,6,10,13-tetraen-1-yl)oxy)tetrahydro-2H-pyran (4.19):

In an oven-dried round bottom flask is added 2.3 (1.0 eq, 0.5 mmol) dissolved in 2.5 mL of anhydrous THF. The mixture is cooled to 0°C where 2-methyl-1-propenylmagnesium bromide (0.5 M in THF, 10 eq, 5 mmol) is added dropwise. The mixture is allowed to stir for 12 hours and quenched by the addition of 10% NH$_4$Cl(aq) solution. The mixture was then poured into a separator funnel and the aqueous layer was extracted 3 × 10 mL Et$_2$O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using 3% Ethyl Acetate/Hexanes as the mobile phase to afford 4.19 in 52% yield. $^1$H NMR (300 MHz, Chloroform-d) δ 5.40 – 5.30 (m, 1H), 5.19 – 4.97 (m, 3H), 4.60 (dd, $J = 4.3$, 2.7 Hz, 1H), 4.21 (dd, $J = 11.9$, 6.4 Hz, 1H), 4.00 (dd, $J = 11.9$, 7.4 Hz, 1H), 3.95 – 3.79 (m, 1H), 3.59 – 3.44 (m,
1H), 2.61 (d, J = 7.5 Hz, 2H), 2.22 – 1.88 (m, 8H), 1.69 (d, J = 1.5 Hz, 3H), 1.66 (d, J = 1.3 Hz, 4H), 1.61 – 1.46 (m, 14H).  \(^{13}\)C NMR (75 MHz, Chloroform-d) δ 140.49, 135.42, 134.74, 132.57, 124.22, 124.10, 122.86, 120.73, 97.96, 63.84, 62.47, 39.91, 39.85, 38.43, 30.92, 26.87, 26.50, 26.01, 25.71, 19.83, 17.86, 16.64, 16.36, 16.23.

(2E,6E,10E)-3,7,11,14-tetramethylpentadeca-2,6,10,13-tetraen-1-ol (4.20):

In a scintillation vial, 1,2,2,10-OTHP (1.0 eq, 0.258 mmol) was dissolved in 5.0 mL of absolute EtOH and PPTS (0.1 eq, 0.026 mmol) was added to the vial. The reaction mixture was heated to 75°C and stirred for 12 hours. Next, the reaction was cooled to room temperature where it was poured into a separator funnel containing water and Et₂O and the aqueous layer was extracted 3 × 5 mL Et₂O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using 10% Ethyl Acetate/Hexanes as the mobile phase to afford 4.20 in 71% yield. \(^1\)H NMR (300 MHz, Chloroform-d) δ 5.48 – 5.36 (m, 1H), 5.22 – 5.04 (m, 3H), 4.12 (d, J = 7.0 Hz, 2H), 2.61 (d, J = 7.4 Hz, 2H), 2.19 – 1.86 (m, 9H), 1.68 (s, 3H), 1.65 (s, 3H), 1.59 (s, 3H), 1.57 (s, 3H), 1.54 (s, 3H). \(^{13}\)C NMR (75 MHz, Chloroform-d) δ 139.94, 135.53, 134.76, 132.58, 124.17, 123.97, 123.49, 122.84, 59.55, 39.88, 39.74, 38.40, 26.84, 26.49, 25.99, 17.84, 16.47, 16.35, 16.19. MS (El) m/z 258 [M⁺–H₂O]; MS (Cl) m/z 259 [M⁺+H–H₂O].

2-(((2E,6E)-9-(3,3-dimethyloxiran-2-yl)-3,7-dimethylnona-2,6-dien-1-yl)oxy)tetrahydro-2H-pyran (4.22):

A suspension of THP-protected farnesol (1.0 eq, 5.55 mmol) in 50 mL THF/H₂O (2:1) was added to a round bottom flask and cooled to 0°C. THF was carefully added dropwise to the
mixture to discharge the turbidity. Next, NBS (1.1 eq, 6.1 mmol) was added and the reaction mixture was allowed to warm to room temperature. After 3 hr of stirring, the THF is evaporated and the aqueous layer is extracted 3 × 20 mL hexanes. The organic layers were pooled, washed with brine, dried with MgSO₄, and concentrated. The bromohydrin was taken on cured in the following step by dissolving it in MeOH (70 mL) and adding K₂CO₃ (2.0 eq, 11.1 mmol) to the mixture. The reaction was allowed to stir for 1 hr then the MeOH was removed. Water (50 mL) and Et₂O (20 mL) were added to the concentrated solution. The Et₂O layer was collected and the aqueous layer was further extracted with 20 mL Et₂O × 2, the organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. Purification by flash chromatography using 10% EtOAc/Hexanes as the eluent afforded the title compound in 46% yield. ¹H NMR (300 MHz, Chloroform-d) δ 5.31 (t, J = 6.3 Hz, 1H), 5.11 (t, J = 6.2 Hz, 1H), 4.58 (t, J = 3.4 Hz, 1H), 4.08 (ddd, J = 66.2, 11.9, 6.9 Hz, 2H), 3.85 (ddd, J = 11.1, 7.3, 3.4 Hz, 1H), 3.46 (dt, J = 10.5, 4.8 Hz, 1H), 2.65 (t, J = 6.2 Hz, 1H), 2.03 (dq, J = 13.6, 7.1, 5.8 Hz, 6H), 1.95 – 1.39 (m, 14H), 1.25 (s, 3H), 1.21 (s, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 140.24 (s), 134.49 (s), 124.69 (s), 120.81 (s), 97.97 (s), 64.34 (s), 63.80 (s), 62.44 (s), 58.50 (s), 39.70 (s), 36.46 (s), 30.87 (s), 27.59 (s), 26.43 (s), 25.66 (s), 25.07 (s), 19.79 (s), 18.92 (s), 16.60 (s), 16.18 (s).

4E,8E)-4,8-dimethyl-10-((tetrahydro-2H-pyran-2-yl)oxy)deca-4,8-dien-1-ol (4.25):

To an oven-dried round bottom flask is added H₂IO₆ (1.2 eq, 3.05 mmol) in 2.5 mL of THF and cooled to 0°C where 4.22 (1.0 eq, 2.54 mmol) in 13 mL of Et₂O was added rapidly to the reaction flask. The reaction was allowed to stir for 30 minutes and quenched by the addition of 40 mL of saturated Na₂S₂O₃(aq). The Et₂O layer was collected and the aqueous layer was further extracted with 20 mL Et₂O × 3, the organic layers were pooled together, washed with brine, dried with MgSO₄, filtered, concentrated, and taken on crude.
To an oven-dried round bottom flask was added 4.23 (1.0 eq, 2.54 mmol) in 30 mL of absolute EtOH and cooled to 0°C. Next, NaBH₄ (2.0 eq, 5.1 mmol) was added to the reaction vessel in several portions and the reaction was allowed to stir for 1 hour. The reaction was then quenched by the addition of 50 mL of water and extracted with 20 mL Et₂O × 3, the organic layers were pooled together, washed with brine, dried with MgSO₄, filtered, concentrated, and again taken on crude.

To a round bottom flask was added alcohol 4.24 (1.0 eq, 2.54 mmol), PPh₃ (1.3 eq, 3.3 mmol) and imidazole (1.7 eq, 4.23 mmol) dissolved in dichloromethane (10 mL). Next, the solution was cooled to 0°C with an ice-bath and I₂ (1.3 eq, 3.3 mmol) was added to the reaction flask. The reaction was allowed to stir 0.5 hr at 0°C then allowed to warm to room temperature and stirred for an additional 1.5 hr. Upon completion of the reaction, 10% Na₂S₂O₃ was added to the flask and stirred for 20 minutes. The organic layer was isolated and the aqueous layer was extracted 3 × DCM (10 mL). The organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. Purification by flash chromatography using 5% EtOAc/hexanes as the eluent afforded the title compound in 55% yield over 3 steps. 

1H NMR (300 MHz, Chloroform-d) δ 5.37 – 5.29 (m, 1H), 5.13 (t, J = 6.2 Hz, 1H), 4.59 (t, J = 3.5 Hz, 1H), 4.10 (ddd, J = 66.0, 11.9, 6.9 Hz, 2H), 3.91 – 3.79 (m, 1H), 3.58 – 3.39 (m, 1H), 3.10 (t, J = 6.9 Hz, 2H), 2.16 – 1.67 (m, 10H), 1.64 (s, 3H), 1.60 – 1.43 (m, 7H). 13C NMR (75 MHz, CDCl₃) δ 140.15 (s), 133.28 (s), 125.61 (s), 120.92 (s), 97.98 (s), 63.81 (s), 62.46 (s), 40.15 (s), 39.66 (s), 31.67 (s), 30.89 (s), 26.36 (s), 25.67 (s), 19.80 (s), 16.57 (s), 16.01 (s), 6.91 (s).

(2E,6E)-10-iodo-3,7-dimethyldeca-2,6-dien-1-ol (4.26):

In a scintillation vial, 2-(((2E,6E)-10-iodo-3,7-dimethyldeca-2,6-dien-1-yl)oxy)tetrahydro-2H-pyran (1.0 eq, 0.88 mmol) was dissolved in 5.0 mL of absolute EtOH and
PPTS (0.1 eq, 0.09 mmol) was added to the vial. The reaction mixture was heated to 75°C and stirred for 12 hours. Next, the reaction was cooled to room temperature where it was poured into a separator funnel containing water and Et₂O and the aqueous layer was extracted 3 × 5 mL Et₂O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography using 10% Ethyl Acetate/Hexanes as the mobile phase to afford 4.26 in 68% yield. 

$^1$H NMR (300 MHz, Chloroform-d) δ 5.37 (t, $J = 6.9$ Hz, 1H), 5.13 (t, $J = 6.7$ Hz, 1H), 4.11 (d, $J = 7.0$ Hz, 2H), 3.10 (t, $J = 6.9$ Hz, 2H), 2.04 (dd, $J = 13.7$, 6.2 Hz, 6H), 1.92 – 1.77 (m, 2H), 1.63 (s, 3H), 1.55 (s, 3H). 

$^{13}$C NMR (75 MHz, Chloroform-d) δ 139.49, 133.32, 125.51, 123.71, 59.49, 40.06, 39.55, 31.57, 26.33, 16.40, 15.98, 6.95.

(2E,6E)-3,7,12-trimethyltrideca-2,6,11-trien-1-ol (4.27):

To an oven-dried round bottom flask is added (2E,6E)-10-iodo-3,7-dimethyldeca-2,6-dien-1-ol (1.0 eq, 0.6 mmol) and dissolved in 2.2 mL of anhydrous THF. Next, Cu(I)I (0.5 eq, 0.3 mmol) is added to the flask and the mixture is cooled to -40°C, where 2-methyl-1-propenylmagnesium bromide (0.5 M in THF, 5.0 eq, 3.0 mmol, 6 mL) is slowly added dropwise to the reaction. The reaction is rapidly warmed to 0°C and stirred for 9 hr. Warming to room temperature, the reaction is allowed to stir for an additional 9 hr then slowly poured into beaker containing 30 mL of 10% NH₄Cl (aq) and 10 mL of Et₂O. The organic layer was isolated and the aqueous layer was extracted 3 × Et₂O (10 mL). The organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. Purification by flash chromatography using 25% EtOAc/hexanes as the eluent afforded the title compound in 29% yield. 

$^1$H NMR (300 MHz, Chloroform-d) δ 5.44 – 5.33 (m, 1H), 5.22 – 4.94 (m, 2H), 4.12 (d, $J = 6.9$ Hz, 2H), 2.15 – 1.97 (m, 4H), 1.91 (dt, $J = 13.7$, 7.3 Hz, 4H), 1.66 (d, $J = 2.8$ Hz, 6H), 1.56 (s, 6H), 1.38 (p, $J =$
(5E,9E)-6,10,14-trimethylpentadeca-5,9,13-trien-1-yne (4.30):

An oven-dried round bottom flask is cooled under argon and charged with TMS-propyne (1.3 eq, 6.5 mmol) and 8 mL of anhydrous THF then cooled to -78°C. Next, n-BuLi (2.5 M in hexanes, 1.3 eq, 6.5 mmol) is added dropwise. The reaction mixture is allowed to stir for 1.5 hr, after which farnesyl chloride (1.0 eq, 5 mmol) is dissolved in THF (2 mL) and added slowly to the reaction mixture at -78°C. The reaction is allowed to stir 16 hr and is then quenched by the addition of 10% NH₄Cl(aq). The organic layer was isolated and the aqueous layer was extracted 3 × Et₂O (25 mL). The organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. The crude TMS-protected alkyne was used directly in the next step without purification.

In a round bottom flask was added trimethyl((5E,9E)-6,10,14-trimethylpentadeca-5,9,13-trien-1-yn-1-yl)silane (1.0 eq, 5 mmol) and 20 mL of THF and cooled to 0°C. Next, TBAF (1.0 M in THF, 1.5 eq, 7.5 mmol) is added to the reaction flask and allowed to stir for 2 hours. Upon completion of the reaction, 30 mL of 10% NH₄Cl(aq) and 15 mL Et₂O are added and the organic layer was isolated. The aqueous layer was further extracted 3 × Et₂O (20 mL). The organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. The crude reaction product was purified by column chromatography using 1% Ethyl Acetate/Hexanes as the mobile phase to afford 4.30 in 73% yield (yield given is for 2 steps).

1H NMR (300 MHz, Chloroform-d) δ 5.15 (t, J = 7.0 Hz, 1H), 5.08 (q, J = 6.4 Hz, 2H), 2.26 – 2.12 (m, 4H), 2.12 – 1.93 (m, 8H), 1.92 (d, J = 2.4 Hz, 1H), 1.66 (s, 3H), 1.61 (s, 3H), 1.58 (s, 6H).
$^{13}$C NMR (75 MHz, Chloroform-d) δ 136.90, 135.20, 131.44, 124.57, 124.26, 122.65, 84.71, 68.30, 39.92, 39.83, 27.39, 26.95, 26.70, 25.91, 19.12, 17.88, 16.32, 16.21.

(3E,7E,11E)-4,8,12,16-tetramethylheptadeca-3,7,11,15-tetraen-1-ol (4.31):

Cp$_2$ZrCl$_2$ (0.25 eq, 0.81 mmol) was dissolved in dichloromethane (3.5 mL) and the solution was cooled to 0°C, where Me$_3$Al solution (2.0 M in heptanes, 2.5 eq, 8.1 mmol) was added dropwise. After stirring the reaction mixture at 0°C for 30 minutes, (5E,9E)-6,10,14-trimethylpentadeca-5,9,13-trien-1-yne (1.0 eq, 3.22 mmol) was diluted in dichloromethane (1.6 mL) and added to the reaction mixture dropwise. The reaction mixture was allowed to slowly warm to room temperature and stirred an additional 12 hours. Next, the reaction mixture was cooled to -78°C and n-BuLi (2.5 M in hexanes, 1.5 eq, 4.8 mmol) was added dropwise and the reaction was stirred for 1 hr. After 1 hr, ethylene oxide (1.4 M in toluene, 3.0 eq, 9.7 mmol) was slowly added dropwise to the reaction at -78°C. The reaction continued to stir for 5 hours, slowly warming to room temperature. The reaction mixture was then slowly poured into an ice-cold 10% HCl$_{(aq)}$ solution. The solution was then filtered over a pad of Celite 545, extracted with 3 × 50 mL dichloromethane, washed with brine, dried with magnesium sulfated, filtered, and concentrated. Column chromatography (15% acetone/hexanes) afforded 4.31 in 41% yield. $^1$H NMR (300 MHz, Chloroform-d) δ 5.09 (q, $J = 7.7$ Hz, 4H), 3.59 (t, $J = 6.5$ Hz, 2H), 2.26 (q, $J = 6.8$ Hz, 2H), 2.14 – 1.86 (m, 12H), 1.65 (s, 3H), 1.62 (s, 3H), 1.57 (s, 9H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 139.12, 135.49, 135.13, 131.47, 124.57, 124.37, 124.17, 120.03, 62.60, 40.01, 39.93, 39.90, 31.68, 26.95, 26.82, 26.69, 25.91, 17.89, 16.42, 16.23, 16.20. MS (El) $m/z$ 304; MS (CI) $m/z$ 305.
Representative procedure for the synthesis of alkyl-iodides 4.34a-c:

7-iodo-2-methylhept-2-ene (4.34a):

To a round bottom flask was added ethyl 5-bromovalerate (1.0 eq, 20 mmol) and acetone (30 mL). Next, KI (2.0 eq, 40 mmol) was to the flask and the reaction was refluxed at 65°C for 20 hr. The acetone was removed and the crude iodide was used directly without purification.

To an oven-dried round bottom flask cooled under argon was added ethyl 5-iodovalerate (1.0 eq, 20 mmol) dissolved in 100 mL of anhydrous toluene. The solution was then cooled to -78°C where DIBAL (1.1 eq, 22 mmol) was added dropwise. The reaction was allowed to stir for 5 hours at -78°C and then was poured into an ice-cold 10% HCl(aq) solution. The mixture was then filtered over a pad of Celite 545, extracted with 3 × 50 mL Et₂O, washed with brine, dried with magnesium sulfated, filtered, and concentrated. The crude aldehyde was used directly without purification.

To an oven-dried round bottom flask is added (i-Pr)Ph₃P (1.1 eq, 22 mmol) dissolved in 80 mL of anhydrous THF. The mixture is cooled to 0°C where n-BuLi (2.5 M in hexanes, 1.1 eq, 22 mmol) is added dropwise. The resulting orange solution is stirred for 1 hr at 0°C. Next, 5-iodopentanal (1.0 eq, 20 mmol) is diluted in 9 mL of anhydrous THF and added dropwise to the reaction mixture, which is allowed to stir for 16 hours coming to room temperature. Upon completion of the reaction, saturated NH₄Cl(aq) is added to the reaction vessel and stirred for 15 minutes. Following extraction with 3 × 50 mL hexanes, the organic layers were combined, washed with brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography (100% Hexanes) to afford 4.34a in 64% yield. ¹H NMR (300 MHz, Chloroform-d) δ 5.07 (tt, J = 8.7, 5.8, 1.4 Hz, 1H), 3.16 (t, J = 7.1 Hz, 2H), 1.96 (q,
2H), 1.80 (p, J = 14.6, 7.1 Hz, 2H), 1.66 (s, 3H), 1.57 (s, 3H), 1.40 (p, J = 7.4 Hz, 2H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 132.17, 124.06, 33.32, 30.87, 27.08, 25.92, 17.90, 7.35.

8-iodo-2-methyloct-2-ene (4.34b):

Yield (30%). $^1$H NMR (300 MHz, Chloroform-d) δ 5.09 (dddt, J = 7.2, 5.8, 2.9, 1.5 Hz, 1H), 3.18 (t, J = 7.1 Hz, 2H), 1.98 (q, J = 6.4, 6.0 Hz, 2H), 1.82 (p, J = 7.1 Hz, 2H), 1.68 (s, 3H), 1.60 (s, 3H), 1.47 – 1.27 (m, 4H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 131.79, 124.52, 33.71, 30.37, 28.97, 27.97, 25.94, 17.91, 7.42.

9-iodo-2-methylnon-2-ene (4.34c):

Yield (47%). $^1$H NMR (300 MHz, Chloroform-d) δ 5.12 – 4.82 (m, 1H), 3.16 (t, J = 7.0 Hz, 2H), 1.94 (q, J = 6.8 Hz, 2H), 1.80 (p, J = 7.0 Hz, 2H), 1.66 (s, 3H), 1.57 (s, 3H), 1.43 – 1.23 (m, 6H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 131.54, 124.77, 33.75, 30.62, 29.82, 28.37, 28.08, 25.94, 17.89, 7.49.

**Representative procedure for the synthesis of homo-allylic alcohols 4.37 a-c:**

(E)-4,12-dimethyltrideca-3,11-dien-1-ol (4.37c):

To an oven-dried round bottom flask is added powdered molecular sieves and is cooled under argon. The round bottom was then flamed-dried under vacuum and cooled under argon; this process was repeated 3 times. Next, 3 mL of anhydrous THF was added to the round bottom flask which was sonicated under vacuum for 30 seconds and then the atmosphere was replaced with argon for 30 seconds; this process was also repeated 3 times. Next, 2,3-dihydrofuran (stored over molecular sieves, 3.0 eq, 18 mmol) was added to the reaction vessel and the solution was
cooled to -78°C where t-BuLi (1.7 M in heptanes, 3.0 eq, 18 mmol) was slowly added dropwise over 5 – 10 minutes. The reaction was stirred at -78°C for 10 minutes then placed in a 0°C ice bath and continued to stir for 1 hr. Next, the reaction mixture was cooled back -78°C and 9-iodo-2-methylnon-2-ene (1.0 eq, 5.7 mmol) in 2.5 mL of anhydrous THF (also sonicated as described above) was added to the reaction vessel. The reaction was stirred for 20 hr slowly coming to room temperature and then poured into a beaker containing ice-cold 20 mL of saturated NH₄Cl(aq) and 2 mL of NH₄OH. The mixture was allowed to stir for 20 minutes and then extracted with 3 × 20 mL Et₂O, the organic layers were combined, washed with brine, dried with magnesium sulfate, concentrated (at room temperature), and used immediately in the next step.

To an oven-dried round bottom flask is added NiCl₂(PPh₃)₂ (0.05 eq, 0.3 mmol) dissolved in anhydrous benzene (17 mL). The round bottom flask was sonicated under vacuum for 30 seconds and then the atmosphere was replaced with argon for 30 seconds; this process was repeated 3 times. Next, MeMgBr (3.0 M in Et₂O, 2.9 eq, 17.4 mmol) was added slowly to the Ni-catalyst solution and stirred for 30 minutes at room temperature. Next, a solution of the newly alkylated-furan (1.0 eq, 5.7 mmol) was dissolved in benzene (15 mL) and deoxygenated by sonication as previously described. After deoxygenation, the alkylated-furan solution was added slowly to the Ni-catalyst solution and the reaction was refluxed at 80°C for 12 hours. The reaction is then cooled to room temperature and poured into a beaker containing 250 mL of saturated NH₄Cl(aq) and 100 mL of Et₂O. The organic layer was isolated and the aqueous layer was extracted 3 × Et₂O (50 mL). The organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. Purification by flash chromatography using 20% EtOAc/hexanes as the eluent afforded the title compound in 69% yield. 

¹H NMR (300 MHz, Chloroform-d) δ 5.18 – 4.94 (m, 2H), 3.59 (t, J = 6.5 Hz, 2H), 2.26 (q, J = 6.8 Hz, 2H), 1.95 (q, J = 7.4 Hz, 4H), 1.66 (s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 1.31 – 1.14 (m, 8H). 

¹³C NMR (75 MHz,
Chloroform-d) δ 139.46, 131.37, 125.06, 119.65, 62.70, 40.02, 31.68, 30.05, 29.45, 29.41, 28.22, 28.13, 25.93, 17.86, 16.34.

\[
\text{(E)-4,11-dimethylundeca-3,10-dien-1-ol (4.37b):}
\]

Yield (72%). \(^1\)H NMR (300 MHz, Chloroform-d) δ 5.08 (ddt, \(J = 7.1, 2.9, 1.5\) Hz, 2H), 3.58 (t, 2H), 2.25 (q, \(J = 6.6\) Hz, 2H), 1.95 (h, \(J = 7.1\) Hz, 4H), 1.66 (s, 3H), 1.60 (s, 3H), 1.56 (s, 3H), 1.43 – 1.17 (m, 6H). \(^{13}\)C NMR (75 MHz, Chloroform-d) δ 139.36, 131.40, 125.00, 119.69, 62.66, 39.98, 31.67, 29.94, 29.16, 28.05, 25.91, 17.84, 16.30.

\[
\text{(E)-4,10-dimethylundeca-3,9-dien-1-ol (4.37a):}
\]

Yield (33%). \(^1\)H NMR (300 MHz, Chloroform-d) δ 5.08 (q, \(J = 6.3, 5.5\) Hz, 2H), 3.58 (t, \(J = 6.6\) Hz, 2H), 2.25 (q, \(J = 6.6\) Hz, 2H), 1.95 (dt, \(J = 13.2, 6.7\) Hz, 4H), 1.65 (s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 1.45 – 1.19 (m, 4H). \(^{13}\)C NMR (75 MHz, Chloroform-d) δ 139.29, 131.48, 124.89, 119.73, 62.67, 39.89, 31.66, 29.69, 28.08, 27.74, 25.91, 17.85, 16.28.

**Representative procedure for the synthesis of homo-allylic iodides 4.38a-c:**

\[
\text{(E)-11-iodo-2,8-dimethylundeca-2,8-diene (4.38a):}
\]

To a round bottom flask was added 4.37a (1 eq, 1.93 mmol), PPh\(_3\) (1.3 eq, 2.51 mmol) and imidazole (1.7 eq, 3.28 mmol) dissolved in dichloromethane (14 mL). Next, the solution was cooled to 0°C with an ice-bath and I\(_2\) (1.3 eq, 2.51 mmol) was added to the reaction flask. The reaction was allowed to stir 0.5 hr at 0°C then allowed to warm to room temperature and stirred for an additional 1.5 hr. Upon completion of the reaction, 10% Na\(_2\)S\(_2\)O\(_3\) was added to the flask and stirred for 20 minutes. The organic layer was isolated and the aqueous layer was extracted 3
× DCM (mL). The organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. Purification by flash chromatography using hexanes as the eluent afforded the title compound in 61% yield. \( ^1\)H NMR (300 MHz, Chloroform-d) \( \delta \) 5.08 (dtt, \( J = 8.3, 5.8, 1.3 \) Hz, 2H), 3.09 (t, \( J = 7.4 \) Hz, 2H), 2.56 (q, \( J = 7.3 \) Hz, 2H), 2.08 – 1.85 (m, 4H), 1.67 (d, 3H), 1.58 (s, 6H), 1.47 – 0.98 (m, 4H). \( ^{13}\)C NMR (75 MHz, Chloroform-d) \( \delta \) 138.63, 131.48, 124.93, 122.94, 39.71, 32.60, 29.65, 28.10, 27.60, 25.96, 17.91, 16.39, 6.42.

(E)-12-iodo-2,9-dimethylundeca-2,9-diene (4.38b):

Yield (82%). \( ^1\)H NMR (300 MHz, Chloroform-d) \( \delta \) 5.22 – 4.68 (m, 2H), 3.09 (t, \( J = 7.4 \) Hz, 2H), 2.56 (q, \( J = 7.3 \) Hz, 2H), 1.95 (q, 4H), 1.67 (s, 3H), 1.57 (s, 3H), 1.45 – 1.13 (m, 6H). \( ^{13}\)C NMR (75 MHz, Chloroform-d) \( \delta \) 138.67, 131.39, 125.04, 122.91, 39.79, 32.58, 29.96, 29.11, 28.22, 27.88, 25.96, 17.90, 16.39, 6.43.

(E)-13-iodo-2,10-dimethyltrideca-2,10-diene (4.38c):

Yield (76%). \( ^1\)H NMR (300 MHz, Chloroform-d) \( \delta \) 5.16 – 4.98 (m, 2H), 3.09 (t, \( J = 7.4 \) Hz, 2H), 2.56 (q, \( J = 7.3 \) Hz, 2H), 2.04 – 1.85 (m, 4H), 1.66 (s, 3H), 1.57 (s, 6H), 1.45 – 1.13 (m, 8H). \( ^{13}\)C NMR (75 MHz, Chloroform-d) \( \delta \) 138.68, 131.34, 125.08, 122.87, 39.81, 32.58, 30.07, 29.41, 29.37, 28.24, 27.93, 25.95, 17.89, 16.40, 6.42.
Representative procedure for the synthesis of frame-shifted alcohols 4.39a-c:

\[ (2E,6E)-3,7,13\text{-trimethyltetradeca-2,6,12-trien-1-ol (4.39a)}: \]

An oven-dried round-bottom flask containing powdered molecular sieves was charged with (E)-11-iodo-2,8-dimethylundeca-2,8-diene (stored over sieves; 2.0 eq, 1.38 mmol) which was dissolved in 5 mL of anhydrous Et\(_2\)O and cooled to -78°C. Next, t-BuLi (1.7 M in pentane; 3.0 eq, 2.06 mmol) was slowly added to the reaction flask and stirred for 1 hour at -78°C. Afterward, β-MeO-9-BBN (1.0M in Hexanes; 3.8 eq, 2.62 mmol) was slowly added to the reaction vessel followed by 2 mL of THF and the mixture was stirred for 16 hours slowly warming to room temperature.

In a scintillation vial, (E)-tert-butyl((3-iodobut-2-en-1-yl)oxy)dimethylsilane (1.0 eq, 0.69 mmol) and Pd(dppf)Cl\(_2\) (0.15 eq, 0.10 mmol) are dissolved in 3mL of DMF. Next, K\(_3\)PO\(_4\) (3.0 eq, 2.06 mmol) is added to the vial and after deoxygenating the solvent, the vinyl-iodide solution is added to the round-bottom flask containing the newly formed organoborane. The reaction mixture is allowed to stir for an additional 16 hours at 85°C after which it is poured into a separatory funnel containing water and Et\(_2\)O and the aqueous layer was extracted 3 × 5 mL Et\(_2\)O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was run through a silica column (1% EtO\(_2\)/Hexanes) to remove most of the impurities and any oxidizing species.

In a scintillation vial, the semi-crude product was dissolved in 2.0 mL of THF, cooled to 0°C, and TBAF (1.0 M in THF, 2.4 eq, 1.42 mmol) was added to the vial. The reaction was allowed to stir for 4 hours and then quenched with 10% NH\(_4\)Cl\(_{(aq)}\). Next, the organic layer was removed, and the aqueous layer was extracted 3 × Et\(_2\)O (5 mL). The organic layers are combined, washed with brine, dried with MgSO\(_4\), filtered, and concentrated. The crude reaction
product was purified by column chromatography using 20% Ethyl Acetate/Hexanes as the mobile phase to afford **4.39a** in 46% yield. $^1$H NMR (300 MHz, Chloroform-d) δ 5.45 – 5.30 (m, 1H), 5.08 (tdq, J = 7.0, 4.2, 1.4 Hz, 2H), 4.12 (d, J = 6.9 Hz, 2H), 2.19 – 1.76 (m, 8H), 1.66 (dd, J = 2.5, 1.3 Hz, 6H), 1.57 (s, 3H), 1.56 (s, 3H), 1.43 – 1.15 (m, 4H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 139.99, 135.83, 131.44, 125.01, 123.79, 123.52, 59.60, 39.76, 39.72, 29.65, 28.12, 27.79, 26.45, 25.94, 17.88, 16.47, 16.07. MS (EI) m/z 250; MS (CI) m/z 249.

(2E,6E)-3,7,14-trimethylpentadeca-2,6,13-trien-1-ol (**4.39b**):

Yield (30% - 2 Steps). $^1$H NMR (300 MHz, Chloroform-d) δ 5.39 (tq, J = 7.0, 1.3 Hz, 1H), 5.15 – 5.00 (m, 2H), 4.13 (d, J = 7.0 Hz, 2H), 2.16 – 1.97 (m, 4H), 1.92 (t, J = 6.7 Hz, 4H), 1.66 (s, 6H), 1.57 (s, 3H), 1.56 (s, 3H), 1.40 – 1.13 (m, 6H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 140.04, 135.92, 131.38, 125.08, 123.72, 123.50, 59.62, 39.86, 39.79, 29.98, 29.16, 28.22, 28.11, 26.47, 25.94, 17.87, 16.48, 16.11. MS (EI) m/z 246 [M$^+$-H$_2$O]; MS (CI) m/z 247 [M$^+$+H-H$_2$O].

(2E,6E)-3,7,15-trimethylhexadeca-2,6,14-trien-1-ol (**4.39c**):

Yield (25% - 2 steps). $^1$H NMR (300 MHz, Chloroform-d) δ 5.39 (tq, J = 6.9, 1.4 Hz, 1H), 5.16 – 5.01 (m, 2H), 4.12 (d, J = 6.9 Hz, 2H), 2.04 (s, 4H), 1.92 (t, J = 7.1 Hz, 4H), 1.65 (s, 6H), 1.57 (s, 3H), 1.55 (s, 3H), 1.45 – 1.06 (m, 8H). $^{13}$C NMR (75 MHz, Chloroform-d) δ 139.94, 135.90, 131.32, 125.09, 123.67, 123.50, 59.54, 39.86, 39.77, 30.06, 29.43, 29.39, 28.23, 28.14, 26.45, 25.92, 17.85, 16.45, 16.09. MS (EI) m/z 278; MS (CI) m/z 277.
Representative procedure for the synthesis of pyrophosphates 4.11, 4.17, 4.21, 4.28, 4.40a-c:

(2E,7E)-3,8,13-trimethyltetradeca-2,7,12-trien-1-yl diphosphate (4.11):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added NCS (2.5 eq, 0.25 mmol) in 0.5 mL of CH$_2$Cl$_2$ and cooled to -30°C where dimethyl sulfide (2.5 eq, 0.25 mmol) was added dropwise to the reaction. Following the addition, the mixture is then placed in a 0°C ice bath and stirred for 5 minutes before being re-cooled back to -30°C. Next, alcohol 4.9 (1 eq, 0.10 mmol) is dissolved in CH$_2$Cl$_2$ (0.2 mL) and added dropwise to the reaction mixture. The mixture is then placed in a 0°C ice bath and stirred for 2.5 hours coming to room temperature. After the allotted time, brine is added to the reaction mixture and the organic layer was extracted. The aqueous layer was further extracted 3 × 5 mL CH$_2$Cl$_2$ and the organic layers were combined, dried with magnesium sulfate, and concentrated. The crude reaction product was used immediately in the following step.

To another oven-dried round bottom flask equipped with a magnetic stir bar was added tris (tetrabutylammonium) hydrogen pyrophosphate (3.0 eq, 0.3 mmol) dissolved in 1.3 mL of acetonitrile. Next, a solution of crude allylic chloride dissolve in 0.9 mL acetonitrile was added dropwise to the reaction vessel. The reaction was stirred for 3 hours at room temperature and then the solvent was removed by rotary evaporation at 34°C. The residue was dissolved in a minimal amount of ion exchange NH$_3$HCO$_3$ buffer (700 mg NH$_3$HCO$_3$, 1 L of deionized H$_2$O, 20 mL of isopropanol) and the resulting solution was passed through a Dowex AG 50 × 8 ion exchange column (2 × 8 cm) using the NH$_3$HCO$_3$ buffer as an eluent and 25 mL was collected in a flask. The resulting solution was lyophilized for 3-5 hours. The resulting residue was then redissolved in deionized watered and purified by cellulose flash column chromatography (3 × 15 cm) using isopropanol:deionized H$_2$O:acetonitrile: NH$_3$HCO$_3$ buffer (500 mL: 250 mL: 250 mL
4 g) as the eluent. In a beaker was collected 40 mL of eluent, then twenty-four 2.5 mL fractions were collected. Typically, fractions 12-18 were collected and the organic solvents were removed by rotary evaporation at 34°C. The resulting solution was then lyophilized to afford pyrophosphate (4.11) as a white fluffy solid in 70% yield. \(^1\)H NMR (300 MHz, Deuterium Oxide) \(\delta\) 5.40 (s, 1H), 5.11 (d, \(J = 7.4\) Hz, 2H), 4.42 (d, \(J = 7.6\) Hz, 2H), 2.08 – 1.79 (m, 8H), 1.65 (d, \(J = 3.9\) Hz, 3H), 1.62 (t, \(J = 3.0\) Hz, 3H), 1.52 (d, \(J = 6.9\) Hz, 6H), 1.47 – 1.28 (m, 4H). \(^{31}\)P NMR (122 MHz, D2O) \(\delta\) -10.16, -13.91. HRMS 409.1548 [M+2H]\(^0\), calculated 409.1545 (C\(_{17}\)H\(_{31}\)O\(_7\)P\(_2\)).

(2E,7E)-3,8,12-trimethyltrideca-2,7,11-trien-1-yl diphosphate (4.17):

Yield (43%). \(^1\)H NMR (300 MHz, Deuterium Oxide) \(\delta\) 5.52 – 5.36 (m, 1H), 5.22 – 5.12 (m, 1H), 5.09 – 4.98 (m, 1H), 4.44 (d, \(J = 7.0\) Hz, 2H), 1.97 (td, \(J = 20.7, 19.5, 10.3\) Hz, 8H), 1.70 – 1.52 (m, 12H), 1.36 – 1.28 (m, 2H). \(^{31}\)P NMR (122 MHz, Deuterium Oxide) \(\delta\) -10.22 (d, \(J = 19.5\) Hz), -14.05 (d, \(J = 18.7\) Hz). HRMS 395.1389 [M+2H]\(^0\), calculated 395.1389 (C\(_{16}\)H\(_{29}\)O\(_7\)P\(_2\)).

(2E,6E,10E)-3,7,11,14-tetramethylpentadeca-2,6,10,13-tetraen-1-yl diphosphate (4.21):

Yield (69%). \(^1\)H NMR (500 MHz, Deuterium Oxide) \(\delta\) 5.38 (s, 1H), 5.17 – 4.97 (m, 3H), 4.43 (s, 2H), 2.66 – 2.51 (m, 2H), 2.02 (d, \(J = 13.3\) Hz, 6H), 1.91 (s, 2H), 1.82 – 1.46 (m, 15H). \(^{31}\)P NMR (122 MHz, Deuterium Oxide) \(\delta\) -12.73 (d, \(J = 15.4\) Hz), -14.42 (d, \(J = 12.9\) Hz). HRMS 435.1705 [M+2H]\(^0\), calculated 435.1702 (C\(_{19}\)H\(_{33}\)O\(_7\)P\(_2\)).

(2E,6E)-3,7,12-trimethyltrideca-2,6,11-trien-1-yl diphosphate (4.28):
Yield (73%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 5.40 (s, 1H), 5.13 (d, $J = 12.2$ Hz, 2H), 4.41 (s, 2H), 2.08 (d, $J = 12.2$ Hz, 2H), 2.07 – 2.01 (m, 2H), 1.91 (q, $J = 8.8$, 7.5 Hz, 4H), 1.67 (s, 3H), 1.63 (d, $J = 7.5$ Hz, 3H), 1.55 (s, 6H), 1.37 (dd, $J = 14.8$, 7.4 Hz, 2H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -10.19 (d, $J = 20.9$ Hz), -13.99 (d, $J = 22.1$ Hz). HRMS 395.1389 [M+2H]$^-$, calculated 395.1389 (C$_{16}$H$_{29}$O$_7$P$_2$).

(2E,6E)-3,7,13-trimethyltetradeca-2,6,12-trien-1-yl diphosphate (4.40a):

Yield (60%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 5.38 (s, 1H), 5.08 (d, $J = 7.3$ Hz, 2H), 4.40 (s, 2H), 2.04 (s, 2H), 1.97 (s, 2H), 1.90 (s, 4H), 1.66 (d, $J = 12.4$ Hz, 3H), 1.59 (d, $J = 5.9$ Hz, 3H), 1.51 (d, $J = 8.8$ Hz, 6H), 1.22 (s, 4H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -9.86 (d, $J = 14.3$ Hz), -13.78 (d, $J = 12.2$ Hz). HRMS 409.1543 [M+2H]$^-$, calculated 409.1545 (C$_{17}$H$_{31}$O$_7$P$_2$).

(2E,6E)-3,7,14-trimethylpentadeca-2,6,13-trien-1-yl diphosphate (4.40b):

Yield (59%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 5.40 (s, 1H), 5.10 (s, 2H), 4.42 (s, 2H), 2.07 (s, 2H), 1.99 (s, 2H), 1.91 (s, 4H), 1.64 – 1.57 (m, 12H), 1.26 – 1.17 (m, 6H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -9.93 (d, $J = 37.6$ Hz), -13.92 (d, $J = 23.5$ Hz). HRMS 423.1705 [M+2H]$^-$, calculated 423.1702 (C$_{18}$H$_{33}$O$_7$P$_2$).

(2E,6E)-3,7,15-trimethylhexadeca-2,6,14-trien-1-yl diphosphate (4.40c):

Yield (94%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 5.37 (d, $J = 8.6$ Hz, 1H), 5.05 (dd, $J = 13.0$, 6.5 Hz, 2H), 4.40 (s, 2H), 1.93 (d, $J = 29.2$ Hz, 8H), 1.66 (s, 3H), 1.60 (s, 3H), 1.54
(s, 3H), 1.52 (s, 3H), 1.25 (s, 8H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -9.81 (d, $J = 15.6$ Hz), -13.66 (d, $J = 13.2$ Hz). HRMS 437.1866 [M+2H], calculated 437.1858 (C$_{19}$H$_{35}$O$_7$P$_2$).

(3E,7E,11E)-4,8,12,16-tetramethylheptadeca-3,7,11,15-tetraen-1-yl diphosphate (4.32):

To an oven-dried round bottom flask equipped with a magnetic stir bar were added methanesulfonyl chloride (1.3 eq, 0.14 mmol), DMAP (1.5 eq, 0.16 mmol) and 0.6 mL of dichloromethane. The mixture was cooled to 0°C and a solution of alcohol 4.31 in 0.2 mL of dichloromethane was added dropwise to the reaction vessel and allowed to stir for 3 hours. Hexanes were then added to the reaction vessel and the solution was filtered and concentrated. Next, Et$_2$O was added to the crude product and was again filtered and concentrated. The crude allylic chloride was then converted to pyrophosphate 4.32 following the same method described for 4.11. Yield (71%). $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 5.06 (ddt, $J = 26.0$, 13.5, 7.3 Hz, 4H), 3.82 (d, $J = 7.3$ Hz, 2H), 2.33 (t, $J = 9.8$ Hz, 2H), 2.18 – 1.82 (m, 12H), 1.61 (s, 3H), 1.59 (s, 3H), 1.53 (s, 3H), 1.51 (s, 6H). $^{31}$P NMR (202 MHz, Deuterium Oxide) $\delta$ -12.10 (d, $J = 16.2$ Hz), -14.13 (d, $J = 19.0$ Hz). HRMS 463.2017 [M+2H], calculated 463.2015 (C$_{21}$H$_{37}$O$_7$P$_2$).

**General procedure for in vitro biochemical substrate screening:**

All biochemical evaluations were performed in our collaborator Dr. Carol Fierke’s laboratory at the University of Michigan by Elia Wright. Preliminary evaluation of all pyrophosphate analogs were performed using GGPP analog (1, 5, or 10 µM), the peptide dansyl-GCVLL (5 µM), recombinant mammalian GGTase-I (50 nM), 50 nM HEPPSO pH 7.8, 5 mM tris(2(carboxyethyl)phosphine (TCEP), and 5 mM MgCl$_2$ at 25°C in 96 well plates (Corning). Protein prenylation was determined by monitoring the dansylated peptide using a continuous spectrofluorometric assay and all assays were performed in triplicate. Upon prenylation of the
peptide, the activity was measured by an increase in fluorescence intensity of the dansyl group ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 520$ nm) in a POLARstar Galaxy plate reader. Peptide in assay buffer was utilized as a negative control and the baseline fluorescence of the peptide was subtracted from the reaction signal.

**General procedure for in vitro GGTase-I inhibitor screening:**

The same assay as described above was used with the exception that 1 or 5 µM of GGPP analog was added to every well in addition to 10 µM GGPP. All readings were recorded in the same manner.
CHAPTER 5. SYNTHESIS & BIOCHEMICAL EVALUATION OF ALKYNYL-TAGGED GERANYLGERANYL PYROPHOSPHATE ANALOGS

5.1 Introduction

Our laboratory has successfully utilized several alkynyl-FPP analogs as chemical tools to identify farnesylated proteins in cells (Figure 5.1A). These alkynyl substrates can be used not only to identify farnesylated proteins, but also as a method to evaluate the analog-induced protein selectivity in cells through proteomic studies. While much work has been done to understand the reaction mechanisms of PTases, a great deal remains unknown about their in vivo function. The proteome is believed to be 100-1000 fold more complex than the genome, which predicts ~30,000 genes. Thus, identification of prenylated proteins is difficult and the total number of prenylated proteins remains unknown. There are several techniques available to confirm prenylation of cellular proteins. Unfortunately, these methods suffer from a number of setbacks such as the need for large samples and heavy sample modification. The greatest pitfall of these methods is that they do not provide researchers with information about the modification of individual proteins. An approach that can afford direct identification of a protein’s modification is radio-labeling proteins by incorporation of tritiated mevalonate or tritiated alcohols. However, this approach is not readily adaptable to purification and MS analysis. Another setback is that tritium is a weak emitter resulting in extremely slow detection via autoradiography (3 weeks – 2 months). Therefore, a more direct and high throughput method would aid in uncovering the proteome’s mysteries.
To avoid these dilemmas, our laboratory has developed a “tagging-via-substrate” method that attempts to determine the extent of protein farnesylation \textit{in vivo}. This involves exposing the cells to an alkynyl-based FPP substrate that can be subsequently tagged with azido-biotin via click chemistry. \cite{78, 115, 137, 142} The biotinylated proteins can then be detected and identified via mass spectroscopy and western blotting (\textbf{Figure 5.1C}). Click chemistry has many advantages such as being able to take place in aqueous media (e.g. the cell lysate) and it needs only mild reducing conditions in the presence of catalytic Cu(I). These alkynyl-FPP analogs have provided us with valuable information about what proteins can be farnesylated and have proven to be valuable biochemical tools. This tagging-via-substrate method combined with MS analysis technique was developed and greatly refined in our laboratory by Dr. Jiao Song. By using these alkynyl-FPP analogs, Dr. Song was able to identify over 190 farnesylated proteins which is by far the most extensive list to date.

Thus, if the alkynyl-GGPP analogs show similar biochemical profiles to their FPP counterparts, then we would be able to develop chemical tools analogous to our \textit{in vivo} FPP-tags (\textbf{Figure 5.1B}). Therefore, we envisioned a similar alkynyl-based-tagging approach for geranylgeranylated proteins. To better understand how the alkynyl-GGPP analogs might bind to GGTase-I, we overlaid these analogs with GGPP (\textbf{Figure 5.2}).

The corresponding alcohols of the terminal alkynyl-FPP tags (\textbf{Figure 5.1B, left}) have been previously synthesized by Charron \textit{et al.}; however, the pyrophosphates were not evaluated nor were they used in a robust screen such as our laboratory’s. \cite{143} While effective in our screens, the terminal alkynes were less efficient than the 7-substituted alkynyl-tags of FPP (\textbf{Figure 5.1A, right}). \cite{137} Therefore, the terminal alkynyl-GGPP tags were synthesized to compare their efficiency to the 7-substituted alkynyl-GGPP tags (\textbf{Figure 5.1B}). Although 7-substituted GGPP analogs have been synthesized previously in the Gibbs laboratory and are able to act as substrates
of GGTase-I,\textsuperscript{144} 7-substituted alkynyl-analogs had yet to be successfully synthesized and investigated.

The final class of tags to be synthesized is alkynyl-triazoles (Figure 5.1B). Evidence suggests that triazole containing analogs can act as substrates in the PTases (unpublished data). As mentioned previously, the $\omega$-isoprene unit of GGPP is oriented nearly perpendicular to the rest of the molecule. Triazoles could prove to be useful linkers in the synthesis of GGPP analogs by potentially allowing for the proper orientation of the alkynyl-tail within GGTase. It is our goal that these compounds can be utilized to determine the extent of protein geranylgeranylation \textit{in vivo}. 
Figure 5.1. Potential alkynyl pyrophosphate cellular probes for proteomic studies. (A) Currently utilized alkynyl FPP analogs as chemical tools to confirm alternative farnesylation in cells. (B) Proposed alkynyl GGPP analogs to be synthesized and tested as substrates with GGTase-I. (C) Utilizing alkynyl-based FPP/GGPP derivatives to tag and identify prenylated proteins.
Figure 5.2. Structural overlay of alkynyl-GGPP analogs with GGPP. (A) Compound 5.7 (Cyan) & GGPP (Green). (B) Compound 5.6 (Magenta) & GGPP (Green). (C) Overlay of 5.6, 5.7, & GGPP zoomed in to the terminal isoprene unit. (D) Compound 5.14 (Cyan) & GGPP (Green). (E) Compound 5.11 (Magenta) & GGPP (Green). (F) Overlay of 5.11, 5.14, & GGPP zoomed in to the terminal isoprene unit. (G) Compound 5.25 & GGPP. (PDB: 1N4P)
5.2 Synthesis of Alkynyl-Tagged Geranylgeranyl Pyrophosphate Analogs

The synthesis of the alkynyl analogs began with the synthesis of the two terminal alkynes 5.6 and 5.7 according to the procedures of Charron and colleagues. Briefly, alcohol 2.2 underwent a NBS bromination to yield bromide 5.1. Due to the instability of allylic bromides, bromide 5.1 was quickly purified using a very short silica column and used immediately in the following steps. Allylic bromide 5.1 was then subjected to a mild copper catalyzed coupling with TMS-acetylene as described by Bieber et al. to yield the protected product 5.2. Although reported as a single isomer by Charron et al., in our hands a mixture of the desired S_N2 product and the S_N2' byproduct was obtained (7.7:2.3) and could not be separated with standard column chromatography. Thus, 5.2 was taken on as a mixture of isomers and underwent THP-deprotection with PPTS in ethanol followed by TMS-removal with TBAF at 0°C to produce alcohol 5.3. To obtain the second terminal alkyne 5.5, the aforementioned allylic bromide 5.1 underwent a displacement reaction with the TMS-propyne anion to yield the protected product 5.4. Following the same deprotection procedure used for the conversion of 5.2 to 5.3, 5.4 was converted into the free alcohol 5.5. Allylic alcohols 5.3 and 5.5 were then chlorinated via Corey-Kim chlorination protocols and converted into pyrophosphates 5.6 and 5.7, respectively, using the method developed by Davisson et al.

The second set of alkynyl-GGPP analogs to be synthesized was the triazole containing analogs 5.11 and 5.14. To assemble these compounds we relied on the vastly used Cu-modified 1,3-dipolar Huisgen cycloaddition (“Click” chemistry) between terminal azides and alkynes to generate 1,4-disubstituted 1,2,3-triazoles. This method has been widely employed in our laboratory to generate vast libraries of both GGTase-I and Icmt inhibitors. To begin the synthesis, previously synthesized iodide 4.26 was “clicked” with either di-alkyne 5.8 or 5.12. The triazole products obtained were first deprotected with PPTS to generate the free allylic
alcohols and further deprotected with TBAF to acquire the free terminal alkynes 5.10 and 5.13. In the past, our laboratory has had difficulties halogenating triazole-containing compounds. These difficulties have included low yields due to unwanted halogenations of the triazole-ring. Thus, in order to convert the allylic alcohols into pyrophosphates we first made the corresponding mesylates of 5.10 and 5.13. The crude mesylates were used immediately in the pyrophosphorylation reaction developed by Davisson et al. to afford analogs 5.11 and 5.14.\textsuperscript{112,113}

The synthesis of 7-propargyl GGPP (5.25) was accomplished in a similar manner as described by Placzek for the synthesis of 7-propargyl FPP. First, cyclopropyl methyl ketone (5.15) was transformed into homoprenyl iodide 5.16 using the method of Biernacki and colleges.\textsuperscript{31,147,148} With 5.16 in hand, we then could utilize a strategy first developed by Wenkert et al. and later used in our laboratory for the synthesis of both frame-shifted FPP analogs.\textsuperscript{134,149} This unique transformation is based on the transforming carbon-oxygen bonds into carbon-carbon bonds and was previously discussed in Chapter 4 and utilized for the synthesis of the frame-shifted GGPP analogs. Compound 5.16 was first alkylated with 5-lithio-2,3-dihydrofuran according to the modified procedure discussed in Chapter 4. Immediately reacting the alkylated dihydrofuran with NiCl$_2$(PPH$_3$)$_2$ and MeMgBr afforded homogerianol (5.17) in 61% yield.

Homogeraniol was then iodinated using standard Appel reaction conditions to generate homoallylic iodide 5.18 which underwent a subsequent lithium-halogen exchange in the presence of t-BuLi. Following the addition of CuCN to the reaction, organocupurate 5.19 was generated. Following a modified procedure of Placzek et al., addition of 5-lithio-2,3-dihydrofuran (4.36) to organocupurate 5.19 lead to a 1,2-metallate rearrangement to produce the higher order alkenylcuprate 5.20 which was coupled with TMS-propargyl bromide to generated 5.22.\textsuperscript{31,150-152} Homoallylic alcohol 5.22 was then iodinated in the same manner as 5.17 to produce iodide 5.23 which was then converted into the organoborane and coupled with vinyl-iodide 3.14 via a Suzuki reaction.\textsuperscript{120} After removal of the TMS and TBDMS groups with TBAF to generate 5.24, the
allylic alcohol was converted into the pyrophosphate (5.25) using the same procedure utilized to generate diphosphates 5.6 and 5.7. It is important to note that only a small quantity of 5.24 was obtained; thus, the pyrophosphorylation was performed on a small scale and only a crude $^{31}$P NMR could be obtained for this product.
Scheme 5.1. Synthesis of terminal alkynyl-GGPP analogs. (a) NBS, DMS, DCM, -40°C (crude purification) (a) i. TMS-acetylene, K₂CO₃, Na₂SO₃, Cu(I), DMF (27%-2 steps includes bromination) (c) i. PPTS, EtOH, 50°C, o/n; ii. TBAF, THF (21%-2 Steps) (d) i. TMS-propyne, n-BuLi, THF, -78°C, 12 hr (49% - 2 steps includes bromination) (e) i. PPTS, EtOH, 50°C, o/n; ii. TBAF, THF (53%-2 Steps); (f) NCS, DMS, DCM -30°C to r.t., 2.5 hr; (g) (NBu₄)₃HP₂O₇, ACN, 3 hr.
Scheme 5.2. Synthesis of triazole-containing alkynyl-GGPP analogs. (a) NaN₃, Na Ascorbate, CuSO₄•5•H₂O, DMF, then 5.6 or 5.9; (b) i. PPTS, EtOH, 50°C, o/n; (c) TBAF, THF; (d) MsCl, DMAP, DCM, 0°C to r.t., 2.5 hr; (e) (NBu₄)₃HP₂O₇, ACN, 3 hr.
Scheme 5.3. Synthesis of 7-propargyl GGPP. (a) i. MeMgl, Et₂O, 0°C, 1 hr; ii. 6M H₂SO₄(aq), 30 min, 0°C (68%); (b) t-BuLi, Et₂O, -78°C; (c) i. 5.16, THF, -78°C to r.t., 16 hr; ii. NiCl₂(PPh₃)₂, MeMgBr, PhH, 75°C (61%); (d) Imidazole, PPh₃, I₂, 0°C (61%); (e) i. t-BuLi, Et₂O, -78°C to 0°C then THF; ii) CuCN, Me₂S, Et₂O, -78°C to 0°C; (f) 4.36; (g) PBu₃, Et₂O, 5.21, 0°C to r.t.(8%); (h) Imidazole, PPh₃, I₂, 0°C (77%); (i) i. t-BuLi, Et₂O, -78°C, ii. β-MeO-9-BBN, THF, -78°C warming to r.t, o/n; iii. 3.14, K₂PO₄, PdCl₂(dppf), DMF, 85°C, 18 hr; (j) TBAF, THF, 0°C; (k) i. NCS, DMS, DCM, -30°C to r.t., 2.5 hr; ii. (NBu₄)₂HP₂O₇, ACN, 3 hr (71%).
5.3 Biochemical Evaluation of Alkynyl-Tagged Geranylgeranyl Pyrophosphate Analogs

Previously, Dr. Amanda Kryziak and Dr. Andrew Placzek of our laboratory synthesized a small library of alkynyl-FPP analogs (Figure 5.1A).\(^{31, 78, 80}\) Upon evaluation of these analogs, it was determined that these analogs are substrates of FTase and can farnesylate a wide range of dansyl-GCaaX peptides. Dr. Jiao Song then utilized these alkynes in proteomic studies to identify ~192 farnesylated proteins via mass spectrometry.\(^{137}\)

Based on these results, our goal was to synthesize a small library of alkynyl-GGPP analogs. These alkynyl analogs (5.6, 5.7, 5.11, 5.14, 5.25; Figure 5.1B) were evaluated for their biochemical activity in an \textit{in vitro} continuous spectrofluorometric assay with GGTase and the co-substrate CaaX-peptide dansyl-GCVLL. All biochemical assays were performed in our collaborator Dr. Carol Fierke’s laboratory at the University of Michigan by Elia Wright.

Of the alkynes tested, only the terminal alkynes 5.7 displayed moderate substrate activity. At 1.5 hours with 10 \(\mu M\) analog, we observed 28 \(\pm\) 0.004\% RFI when compared to GGPP (Figure 5.3 and 5.4). Terminal alkyne 5.6 also displayed substrate activity; however, it was a weaker substrate than 5.7. Although analogs 5.11, 5.14, and 5.25 do not appear to be substrates based on the bar graph in Figure 5.3, an endpoint point analysis indicates that these analogs may indeed be substrates, albeit very slow substrates (Figure 5.5). It is important to note that due to small amounts of material, only a very crude \(^{31}\)P NMR could be obtained for 5.25. Therefore, it could be beneficial if this compound is remade, fully characterized, and retested. The saturated alkynyl compounds have yet to be tested.
Figure 5.3. Bar graphs of substrate activity represented in RFI of alkynyl-GGPP analogs versus GGPP (+ control) with 5 µM dansyl-GCVLL and 50 nM GGTase-I. Error bars represent mean ± SD (n = 3). *Values shown are for 10 µM analog at time 1.5 hours.
Figure 5.4. Monitoring continuous changes in fluorescence of alkynyl-GGPP analogs versus GGPP (+ control) at various concentrations (1 µM, 5µM, & 10µM) with 5 µM dansyl-GCIVLL and 50 nM GGTase-I. Experiments were performed in triplicate and data points represent the mean.
**Figure 5.5.** Endpoint assay results for slow alkynyl-GGPP analogs with 5µM dansyl-GCVLL and 50 nM GGtase-I. Experiments were performed in triplicate and data points represent the mean.
5.4 Conclusions

The goal of this chapter was to focus on the synthesis of a small library of alkynyl-GGPP analogs to utilize as potential chemical tools to evaluate GGTase-I via proteomics. Previously, our lab synthesized alkynyl-FPP analogs that displayed interesting results in vivo and were later used to identify over 190 farnesylated proteins. Therefore, our laboratory aimed to expand this concept to include analogs that could aid in identifying geranylgeranylated proteins.

The synthesis of the two terminal alkynes (5.6 and 5.7) was accomplished in accordance with the procedures of Charron and colleagues. The triazole containing alkynes were quickly generated using the previously synthesized intermediate 4.26 and commercially available dialkynes. The triazole-containing analogs had to be pyrophosphorylated via the mesylate to avoid unwanted side reactions with NCS and the triazole ring. The 7-propargyl GGPP analog (5.25) was more difficult to accomplish. The synthesis was carried out in the same manner as previously developed by Dr. Andrew Placzek in our laboratory. The problematic step in this synthesis was the coupling of the vinyl-iodide 3.14, which resulted in very poor yields.

We have successfully synthesized a small library of alkynyl-GGPP analogs. Of the analogs synthesized, only the terminal alkyne, 5.7, showed moderate substrate activity. Terminal alkyne 5.6 also demonstrated substrate activity, albeit it was a poorer substrate than 5.7. Again, chain length seems to be an important factor. Although our alkynyl-GGPP analogs did not live up to our expectations, it is important to keep in mind that our preliminary biochemical testing utilized only one CaaX sequence, dansyl-GCVLL. It is possible that these analogs are more selective chemical tools and although most are weak substrates with dansyl-CVLL, they could potentially be strong co-substrates with other CaaX sequences. In the future, these compounds will be tested as inhibitors of GGTase-I. If no inhibitor activity is detected, an extensive in vitro
screening with a large library of dansyl-GCaaX sequences will determine if these analogs are selective substrates.
5.5 Experimental Procedures Utilized for the Synthesis & Biochemical Evaluation of Alkynyl-Tagged Geranylgeranyl Pyrophosphate Analogs

**General Experimental Procedures:** All reactions were performed with oven-dried or flame-dried glassware and under dry argon gas. All commercial reagents and solvents were used directly without subsequent purification. For the organometallic coupling reactions, anhydrous THF was freshly distilled from sodium and benzophenone. All other anhydrous solvents were purchased from Acros Organics as extra dry solvents and were bottled over molecular sieves. Reactions were monitored by thin layer chromatography and visualized with one or more of the following: UV light, iodine, vanillin solution, potassium permanganate solution, dinitrophenylhydrazine solution, and/or phosphomolybdic acid solution. All products were purified using flash chromatography silica gel 60 M purchased from Macherey-Nagel. All reactions involving either triphenyl phosphine or triphenyl phosphine oxide were first dry-loaded with sodium sulfated before column purification. NOTE: *Dry glassware is critical for the organometallic reactions in this publication. This was accomplished by taking oven-dried glassware (dried for at least 24 hr and then cooled under argon) and flame drying the round bottom flask under vacuum. The flask was then purged with argon and cooled to room temperature. This process was repeated 3 times to produce a completely water free environment.*  **Deoxygenated solvents are extremely crucial for successful organometallic reactions in this publication. In order to achieve completely deoxygenated solutions, the solvent was placed in a flame-dried round bottom and under vacuum was sonicated for 30 seconds and then purged with argon for 30 seconds. This process was repeated 3 times to yield completely deoxygenated solvents.**  All NMR spectra were taken either on a 300 MHz Bruker ARX300 or a 500 MHz Bruker DRX500 spectrometer. Low-resolution MS (EI/CI) were recorded with a
Hewlett Packard Engine and low-resolution MS (ESI) were taken on a Thermoquest LCQ. All high-resolution mass spectra were recorded on a FinniganMAT XL95.

\[
\text{Br} \begin{array}{c}
\text{Br} \\
\text{Br} \\
\end{array} \\
\text{OTHP}
\]

2-(((2E,6E,10E)-12-bromo-3,7,11-trimethyldec-2,6,10-trien-1-yl)oxy)tetrahydro-2H-pyran (5.1):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added NBS (2.0 eq, 2.0 mmol) in 10 mL of CH\textsubscript{2}Cl\textsubscript{2} and cooled to -30°C where dimethyl sulfide (2.0 eq, 2.0 mmol) was added dropwise to the reaction. Following the addition, the mixture is then placed in a 0°C ice bath and stirred for 5 minutes before being recooled back to -30°C. Next, alcohol 2.2 (1 eq, 1 mmol) is dissolved in 5 mL of CH\textsubscript{2}Cl\textsubscript{2} and added dropwise to the reaction mixture. The mixture is then placed in a 0°C ice bath and stirred for 2.5 hours coming to room temperature. After the allotted time, brine is added to the reaction mixture and the organic layer was extracted. The aqueous layer was further extracted 3 × 10 mL CH\textsubscript{2}Cl\textsubscript{2} and the organic layers were combined, dried with magnesium sulfate, and concentrated. The crude reaction product was passed through a short plug of silica gel using 5%-10% EtOAc/hexanes as the eluent. Bromide 5.1 was used immediately in the next step.

\[
\begin{array}{c}
\text{Br} \\
\text{Br} \\
\text{Br} \\
\end{array} \\
\text{OH}
\]

(2E,6E,10E)-3,7,11-trimethyltetradeca-2,6,10-trien-13-yn-1-ol (5.3):

To an oven-dried round bottom flask is added powdered molecular sieves and is cooled under argon. The round bottom was then flamed-dried under vacuum and cooled under argon; this process was repeated 3 times. Next, ethynyltrimethylsilane (3.0 eq, 4 mmol) and 12 mL of anhydrous DMF were added to the round bottom flask. To the round bottom flask was added K\textsubscript{2}CO\textsubscript{3} (1.5 eq, 2.7 mmol), Na\textsubscript{2}SO\textsubscript{3} (1.0 eq, 1.33 mmol), bromide 5.1, and Cu(I)I (0.05 eq, 0.07mmol) sequentially and the reaction was stirred at room temperature for 12 hours. The
reaction was quenched by the addition of 10% NH₄Cl, the organic layer was isolated, and the aqueous layer was extracted 3 × CH₂Cl₂ (15 mL). The organic layers were combined, washed with brine, dried with MgSO₄, filtered, and concentrated. The crude reaction product was purified by column chromatography using 3% Ethyl Acetate/Hexanes as the mobile phase to afford 5.2 in 27% yield (2-steps from 2.2).

In a scintillation vial equipped with a magnetic stir bar, 5.2 (1.0 eq, 0.47 mmol) was dissolved in 5 mL of absolute EtOH and PPTS (0.1 eq, 0.05 mmol) was added to the vial. The reaction mixture was heated to 75°C and stirred for 12 hours. Next, the reaction was cooled to room temperature where it was poured into a separator funnel containing water and Et₂O and the aqueous layer was extracted 3 × 10 mL Et₂O. The organic layers were combined, washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was then dissolved in 5 mL of THF and cooled to 0°C where TBAF (2.0 eq, 1.0 mmol, 1.0 M in THF) was added to the reaction flask. The reaction was allowed to stir for 3 hours and then quenched with 10% NH₄Cl(aq). Next, the organic layer was removed, and the aqueous layer was extracted 3 × Et₂O (10 mL). The organic layers are combined, washed with brine, dried with MgSO₄, filtered, and concentrated. The crude reaction product was purified by column chromatography using 20% Ethyl Acetate/Hexanes as the mobile phase to afford alcohol 5.3 in 21% yield (7.7:2.3; S₅N₂:S₅N₂'). ¹H NMR (300 MHz, Chloroform-d) δ 5.39 (t, J = 7.0 Hz, 2H), 5.09 (t, J = 8.4 Hz, 1H), 4.13 (d, J = 6.5 Hz, 2H), 2.93 – 2.80 (m, 2H), 2.14 – 1.91 (m, 10H), 1.77 (t, J = 1.1 Hz, 1H), 1.66 (d, J = 1.4 Hz, 6H), 1.58 (d, J = 1.4 Hz, 3H). MS (EI) m/z 246; MS (CI) m/z 229 [M⁺-H-H₂O].
An oven-dried round bottom flask equipped with a magnetic stir bar was charged with 1-(trimethylsilyl)propyne (3.0 eq, 4.2 mmol) dissolved in 5.5 mL of anhydrous THF and cooled to -78°C. Next, n-BuLi (2.5 M in hexanes, 3.0 eq, 4.2 mmol) was slowly added to the mixture and the reaction was allowed to stir 1.5 hours. After the allotted time, 5.1 (1.0 eq, 1.4 mmol) in 7 mL of THF was added dropwise to the reaction vessel at -78°C. The reaction was allowed to stir for an additional 12 hours after which time the reaction was quenched by the addition of 10% NH₄Cl.

The organic layer was removed, and the aqueous layer was extracted 3 × Et₂O (15 mL). The organic layers were combined, washed with brine, dried with MgSO₄, filtered, and concentrated. The crude reaction product was purified by column chromatography using 5% Ethyl Acetate/Hexanes as the mobile phase to afford 5.4 in 49% yield (2-steps from 2.2). ¹H NMR (300 MHz, CDCl₃) δ 5.33 (t, J = 6.4 Hz, 1H), 5.10 (dt, J = 12.8, 6.4 Hz, 2H), 4.60 (t, J = 3.3 Hz, 1H), 4.29 – 3.41 (m, 4H), 2.33 – 1.90 (m, 11H), 1.79 (ddd, J = 20.6, 12.5, 8.6 Hz, 1H), 1.66 (d, J = 5.7 Hz, 4H), 1.62 – 1.42 (m, 11H), 0.14 – 0.00 (m, 9H). ¹³C NMR (75 MHz, Chloroform-d) δ 140.44, 135.30, 133.54, 125.67, 124.15, 120.74, 107.57, 97.94, 84.68, 63.81, 62.44, 39.82, 39.79, 38.84, 30.90, 26.79, 26.48, 25.69, 19.81, 19.43, 16.63, 16.22, 16.01, 0.35.

The same procedure was used as in 5.3. Yield (53%). ¹H NMR (300 MHz, CDCl₃) δ 5.40 (td, J = 6.9, 1.1 Hz, 1H), 5.11 (dt, J = 12.5, 6.7 Hz, 2H), 4.13 (d, J = 6.9 Hz, 2H), 2.31 – 1.89
(m, 13H), 1.66 (s, 3H), 1.58 (s, 6H). $^{13}$C NMR (75 MHz, Chloroform-$d$) δ 140.04, 135.40, 133.38, 125.71, 124.09, 123.49, 84.66, 68.53, 59.63, 39.75, 39.73, 38.61, 26.75, 26.48, 17.82, 16.5, 16.23, 16.01. MS (EI) $m/z$ 260; MS (CI) $m/z$ 261 [M$^+$+H].

**Representative procedure for the synthesis of triazole containing alkynyl-GGPP analogs:**

![Triazole Containing Alkynyl-GGPP Analog](image)

1-((4E,8E)-4,8-dimethyl-10-((tetrahydro-2H-pyran-2-yl)oxy)deca-4,8-dien-1-yl)-4-(4-(triisopropylsilyl)but-3-yn-1-yl)-1H-1,2,3-triazole (5.9):

To a scintillation vial equipped with a magnetic stir bar was added iodide 4.26 (1.0 eq, 0.5 mmol) and 2.5 mL of DMF. Next, NaN$_3$ (3 eq, 1.5 mmol), sodium ascorbate (0.5 eq, 0.25 mmol), and CuSO$_4$·H$_2$O (0.25 eq, 0.125 mmol) are added sequentially to the vial. Alkyne 5.8 (3.6 eq, 1.8 mmol) was then dissolved in 1.3 mL of DMF and added to the vial. The reaction was heated to 70°C and allowed to stir for 36 hours. The reaction was then quenched with 10% NH$_4$Cl and the aqueous layer was extracted 3 × EtOAC (10 mL). The organic layers were combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was purified by column chromatography using 25% Ethyl Acetate/Hexanes as the mobile phase to afford 5.9 in 70% yield. $^1$H NMR (300 MHz, Chloroform-$d$) δ 7.42 (s, 1H), 5.35 (ddd, $J$ = 7.6, 6.1, 1.4 Hz, 1H), 5.21 – 5.04 (m, 1H), 4.61 (dd, $J$ = 4.4, 2.7 Hz, 1H), 4.24 (q, $J$ = 6.4 Hz, 3H), 4.01 (dd, $J$ = 11.9, 7.3 Hz, 1H), 3.88 (dd, $J$ = 11.2, 7.3, 3.2 Hz, 1H), 3.60 – 3.42 (m, 1H), 2.95 (t, $J$ = 7.1 Hz, 3H), 2.62 (td, $J$ = 7.1, 4.1 Hz, 3H), 2.18 – 1.90 (m, 8H), 1.91 – 1.69 (m, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 1.52 (dq, $J$ = 8.6, 5.2 Hz, 4H), 1.02 (s, 18H).
Deprotection was accomplished using the same procedure as 5.3. The crude reaction product was purified by column chromatography using 50 to 100% Ethyl Acetate/Hexanes as the mobile phase to afford 5.10 in 62% yield. $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.40 (s, 1H), 5.46 – 5.35 (m, 1H), 5.10 (t, $J = 6.4$ Hz, 1H), 4.26 (t, $J = 7.0$ Hz, 2H), 4.14 (d, $J = 6.8$ Hz, 2H), 2.93 (t, $J = 7.1$ Hz, 2H), 2.54 (td, $J = 7.1$, 2.6 Hz, 2H), 2.11 (t, $J = 6.6$ Hz, 1H), 2.03 (dd, $J = 9.4$, 6.8 Hz, 2H), 1.97 (dt, $J = 5.2$, 3.0 Hz, 4H), 1.65 (s, 2H), 1.61 (s, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 146.23 (s), 138.90 (s), 133.22 (s), 125.79 (s), 124.11 (s), 121.32 (s), 83.69 (s), 69.28 (s), 59.40 (s), 49.61 (s), 39.41 (s), 36.22 (s), 28.19 (s), 26.12 (s), 25.09 (s), 18.90 (s), 16.34 (s), 15.95 (s). MS (ESI) m/z 302 [M$^+$+H].

Synthesis was accomplished following the same procedures as 5.9 and 5.10 to afford 5.13 in 15% yield (2-steps). $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.44 (s, 1H), 5.49 – 5.23 (m, 1H), 5.06 (t, $J = 6.5$ Hz, 1H), 4.26 – 4.16 (m, 2H), 4.08 (d, $J = 6.9$ Hz, 2H), 3.84 – 3.55 (m, 2H), 2.11 – 2.01 (m, 3H), 2.01 – 1.87 (m, 6H), 1.59 (d, $J = 1.4$ Hz, 3H), 1.52 (d, $J = 1.5$ Hz, 3H). $^{13}$C NMR (75 MHz, Chloroform-$d$) $\delta$ 143.44, 138.56, 133.07, 125.74, 124.09, 121.67, 80.27, 70.03, 59.18, 49.68, 39.33, 36.12, 28.07, 26.07, 16.32, 16.25, 15.84. MS (EI) m/z 287; MS (Cl) m/z 288 [M$^+$+H].
5-iodo-2-methylpent-2-ene (5.16):

An oven-dried round bottom flask equipped with a magnetic stir bar was charged with MeMgI (3.0 \( M \) in \( \text{Et}_2\text{O} \), 1.0 eq, 50.5 mmol) and an additional 150 mL of \( \text{Et}_2\text{O} \). The mixture was then cooled to 0\(^\circ\)C and cyclopropyl methyl ketone, 5.15, (1.0 eq, 50.5 mmol) in 25 mL of \( \text{Et}_2\text{O} \) was added dropwise to the reaction mixture and stirred for 1 hour at room temperature. After the allotted time, 200 mL of 6 \( M \) \( \text{H}_2\text{SO}_4(aq) \) was cooled to 0\(^\circ\)C in a beaker. Next, the reaction mixture was very slowly poured into the acidic solution and allowed to stir for 45 minutes. Next, the reaction was poured into a separator funnel and the organic layer was isolated. The aqueous layer was further extracted 2 × 100 mL \( \text{Et}_2\text{O} \). The organic layers were combined, washed 2 × 100 mL of saturated \( \text{Na}_2\text{S}_2\text{O}_3 \), washed 1 × brine, dried with magnesium sulfate, and concentrated. The crude reaction product was purified by column chromatography 100% hexanes as the mobile phase to afford iodide 5.16 in 68% yield. \(^1\)H NMR (300 MHz, Chloroform-d) \( \delta \) 5.07 (tq, \( J = 7.1, 5.7, 2.8, 1.4 \) Hz, 1H), 3.08 (t, \( J = 7.4 \) Hz, 2H), 2.69 – 2.36 (m, 2H), 1.68 (s, 3H), 1.59 (s, 3H). \(^13\)C NMR (75 MHz, Chloroform-d) \( \delta \) 134.74, 123.26, 32.72, 25.93, 18.18, 6.36.

(E)-4,8-dimethylnona-3,7-dien-1-ol (5.17):

To an oven-dried round bottom flask is added powdered molecular sieves and is cooled under argon. The round bottom was then flamed-dried under vacuum and cooled under argon; this process was repeated 3 times. Next, 10 mL of anhydrous THF was added to the round bottom flask which was sonicated under vacuum for 30 seconds and then the atmosphere was replaced with argon for 30 seconds; this process was also repeated 3 times. Next, 2,3-dihydrofuran (stored over molecular sieves, 3.0 eq, 51.4 mmol) was added to the reaction vessel and the solution was cooled to -78\(^\circ\)C where \( t\)-BuLi (3.0 eq, 51.4 mmol, 1.7 \( M \) in heptanes) was
slowly added dropwise over 5 – 10 minutes. The reaction was stirred at -78°C for 10 minutes then placed in a 0°C ice bath and continued to stir for 1 hour. Next, the reaction mixture was cooled back -78°C and **5.16** (1.0 eq, 17.14 mmol) in 7 mL of anhydrous THF (also sonicated as described above) was added to the reaction vessel. The reaction was stirred for 20 hours slowly coming to room temperature and then poured into a beaker containing ice-cold 50 mL of saturated NH₄Cl(aq) and 5 mL of NH₄OH. The mixture was allowed to stir for 20 minutes and then extracted with 3 × 50 mL Et₂O, the organic layers were combined, washed with brine, dried with magnesium sulfate, concentrated (at room temperature), and used immediately in the next step.

To an oven-dried round bottom flask is added NiCl₂(PPh₃)₂ (0.05 eq, 1.72 mmol) dissolved in anhydrous benzene (100 mL). The round bottom flask was sonicated under vacuum for 30 seconds and then the atmosphere was replaced with argon for 30 seconds; this process was repeated 3 times. Next, MeMgBr (3.0 M in Et₂O, 2.9 eq, 99.4 mmol) was added slowly to the Ni-catalyst solution and stirred for 30 minutes at room temperature. Next, a solution of the newly alkylated-furan (1.0 eq, 17.14 mmol) was dissolved in benzene (50 mL) and deoxygenated by sonication as previously described. After deoxygenation, the alkylated-furan solution was added slowly to the Ni-catalyst solution and the reaction was refluxed at 80°C for 12 hours. The reaction is then cooled to room temperature and poured into a beaker containing 500 mL of saturated NH₄Cl(aq) and 150 mL of Et₂O. The organic layer was isolated and the aqueous layer was extracted 3 × Et₂O (50 mL). The organic layers were pooled together, washed with brine, dried with MgSO₄, filtered, and concentrated. Purification by flash chromatography using 20% EtOAc/hexanes as the eluent afford **5.17** in 61% yield. ¹H NMR (300 MHz, Chloroform-d) δ 5.30 – 4.95 (m, 2H), 3.57 (p, J = 5.9, 4.8 Hz, 2H), 2.41 – 2.19 (m, 2H), 2.02 (tq, J = 10.4, 6.7, 5.3 Hz, 4H), 1.73 (s, 1H), 1.64 (d, J = 1.5 Hz, 3H), 1.60 (d, J = 1.3 Hz, 3H), 1.56 (d, J = 1.3 Hz, 3H).
(E)-9-iodo-2,6-dimethylnona-2,6-diene (5.18):

To a round bottom flask was added 5.17 (1 eq, 18.34 mmol), PPh₃ (1.3 eq, 23.84 mmol) and imidazole (1.7 eq, 31.17 mmol) dissolved in dichloromethane (70 mL). Next, the solution was cooled to 0°C with an ice-bath and I₂ (1.3 eq, 23.84 mmol) was added to the reaction flask. The reaction was allowed to stir 0.5 hour at 0°C then allowed to warm to room temperature and stirred for an additional 1.5 hour. Upon completion of the reaction, 10% Na₂S₂O₃ was added to the flask and stirred for 20 minutes. The organic layer was isolated and the aqueous layer was extracted 3 × DCM (50 mL). The organic layers were pooled together, washed with brine, dried with MgSO₄, filtered and concentrated. Purification by flash chromatography using hexanes as the eluent afforded 5.18 in 81% yield. ¹H NMR (300 MHz, Chloroform-d) δ 5.08 (ddq, J = 8.5, 5.4, 1.4 Hz, 2H), 3.09 (t, J = 7.4 Hz, 2H), 2.56 (q, J = 7.3 Hz, 2H), 2.16 – 1.82 (m, 4H), 1.66 (s, 3H), 1.58 (s, 6H). ¹³C NMR (75 MHz, Chloroform-d) δ 138.20, 131.66, 124.21, 123.14, 39.79, 32.57, 26.62, 25.92, 17.92, 16.47, 6.25.

(3Z,7E)-8,12-dimethyl-4-(3-(trimethylsilyl)prop-2-yn-1-yl)trideca-3,7,11-trien-1-ol (5.22):

To an oven-dried round bottom flask was added powdered molecular sieves and is cooled under argon. The round bottom was then flamed-dried under vacuum and cooled under argon; this process was repeated 3 times. Next, iodide 5.19 (1.0 eq, 7.29 mmol) and 11 mL of anhydrous Et₂O was added to the round bottom flask which was sonicated under vacuum for 30 seconds and then the atmosphere was replaced with argon for 30 seconds; this process was also repeated 3
times. The solution was cooled to -78°C and t-BuLi (1.7 M in heptanes, 2.2 eq, 16 mmol) was added dropwise to the reaction mixture over a period of 5-10 minutes. The solution was allowed to stir for 30 minutes at -78°C and for 1 hour at 0°C. Next, THF (1.1 mL) was added to the reaction and it continued to stir for 1 hour at room temperature.

In a second oven-dried round bottom flask was added powdered molecular sieves and is cooled under argon. The round bottom was charged with CuCN (0.5 eq, 3.65 mmol) then flame-dried under vacuum and cooled under argon; this process was repeated 3 times. Next, 7.3 mL of Et₂O followed by dimethyl sulfide (9.1 mL) were added to the round bottom flask and sonicated as previously described. The mixture was cooled to -78°C and the organo-lithium solution of 5.19 was added dropwise. The mixture was stirred for 15 minutes at -78°C and then 30 minutes at 0°C to furnish the corresponding organocuprate.

To a third oven-dried round bottom flask was added powdered molecular sieves and is cooled under argon. This flask was also flame-dried as described previously and then was charged with 2,3-dihydrofuran (1.0 eq, 7.3 mmol) and 1.5 mL of THF and sonicated as described above. The solution was then cooled to -78°C and t-BuLi (1.7 M in heptanes, 1.0 eq, 7.3 mmol) was added dropwise over a 5-10 minute period. The solution was then warmed to 0°C and stirred for 45 minutes. After the allotted time, Et₂O (6.7 mL) was added to dilute the organo-lithium solution which was then slowly added to the organocuprate solution at -78°C. The reaction was stirred at this temperature for 15 minutes and then placed in a 0°C ice bath and allowed to slowly warm to room temperature over 16 hours. Next, the mixture was cooled back down to 0°C and PBu₃ (0.55 eq, 4.0 mmol) was added to the round bottom flask and stirred for 15 minutes. Following this addition, 5.19 (1.5 eq, 10.9 mmol) dissolved in 19 mL of Et₂O and added to the reaction mixture which was allowed to warm to room temperature and stirred for 3 hours. The reaction was quenched by the addition of 10% NH₄Cl and the organic layer was isolated. The aqueous layer was extracted 3 × Et₂O (40 mL), the organic layers were combined, washed with
brine, dried with MgSO₄, filtered, and concentrated. The crude reaction product was purified by column chromatography using 20% Ethyl Acetate/Hexanes as the mobile phase to afford 5.20 in 19% yield. ¹H NMR (300 MHz, Chloroform-d) δ 5.21 (t, J = 7.5 Hz, 1H), 5.16 – 4.96 (m, 2H), 3.62 (t, J = 6.3 Hz, 2H), 2.98 (s, 2H), 2.30 (q, J = 6.6 Hz, 2H), 2.22 – 1.84 (m, 8H), 1.65 (s, 3H), 1.58 (s, 6H), 0.11 (s, 9H). ¹³C NMR (75 MHz, Chloroform-d) δ 137.78, 135.68, 131.54, 124.49, 123.95, 122.42, 104.98, 85.04, 62.44, 39.90, 37.34, 31.69, 26.92, 26.72, 25.92, 21.54, 17.90, 16.28, 0.25.

((4Z,7E)-4-(3-iodopropylidene)-8,12-dimethyltrideca-7,11-dien-1-yn-1-yl)trimethylsilane (5.23): The synthesis of 5.21 was achieved using the same procedure utilized for 5.18. The crude reaction product was purified by column chromatography using 0.5 to 1.0% Ethyl Acetate/Hexanes as the mobile phase to afford alcohol 5.21 in 77% yield. ¹H NMR (300 MHz, Chloroform-d) δ 5.17 (t, J = 7.2 Hz, 1H), 5.14 – 5.03 (m, 2H), 3.12 (t, J = 7.4 Hz, 2H), 2.95 (s, 2H), 2.61 (q, J = 7.3 Hz, 2H), 2.19 – 2.09 (m, 4H), 2.10 – 1.86 (m, 4H), 1.66 (s, 3H), 1.58 (d, J = 2.1 Hz, 6H), 0.12 (s, 9H). ¹³C NMR (75 MHz, Chloroform-d) δ 137.09, 135.64, 131.52, 125.18, 124.52, 123.86, 104.51, 85.16, 39.91, 37.34, 32.46, 26.94, 26.58, 25.94, 21.72, 17.93, 16.32, 5.42, 0.28.

(2E,6Z,10E)-3,11,15-trimethyl-7-(prop-2-yn-1-yl)hexadeca-2,6,10,14-tetraen-1-ol (5.24): An oven-dried round-bottom flask containing powdered molecular sieves was charged with 5.16 (stored over sieves; 1.5 eq, 1.1 mmol) which was dissolved in 3.6 mL of anhydrous Et₂O and cooled to -78°C. Next, t-BuLi (1.7M in pentane; 3.0 eq, 1.64 mmol) was slowly added to the reaction flask and stirred for 1 hour at -78°C. Afterward, β-MeO-9-BBN (1.0M in
Hexanes; 3.8 eq, 2.07 mmol) was slowly added to the reaction vessel and the mixture was stirred for 16 hours slowly warming to room temperature.

In a scintillation vial, **3.14** (1.0 eq, 0.72 mmol) and Pd(dppf)Cl$_2$ (0.3 eq, 0.22 mmol) are dissolved in 2.8 mL of DMF. Next, K$_3$PO$_4$ (3.0 eq, 2.16 mmol) is added to the vial and after deoxygenating the solvent, the vinyl-iodide solution is added to the round-bottom flask containing the newly formed organoborane. The reaction mixture is allowed to stir for an additional 16 hours at 85°C after which it is poured into a separatory funnel containing water and Et$_2$O and the aqueous layer was extracted 3 × 20 mL Et$_2$O. The organic layers were combined and washed with water then brine, dried with magnesium sulfate, and concentrated. The crude reaction product was run through a silica column (20% EtOAc/Hexanes) to remove most of the impurities and any oxidizing species.

In a scintillation vial, the semi-crude product was dissolved in 5.0 mL of THF, cooled to 0°C, and TBAF (4.9 eq, 3.5 mmol, 1.0 M in THF) was added to the vial. The reaction was allowed to stir for 4 hours and then quenched with 10% NH$_4$Cl$_{aq}$. Next, the organic layer was removed, and the aqueous layer was extracted 3 × Et$_2$O (10 mL). The organic layers are combined, washed with brine, dried with MgSO$_4$, filtered, and concentrated. The crude reaction product was purified by column chromatography using 20% Ethyl Acetate/Hexanes as the mobile phase to afford alcohol **5.22** in 6% yield. $^1$H NMR (300 MHz, Chloroform-$d$) δ 5.49 – 5.40 (m, 1H), 5.22 (t, $J$ = 6.7 Hz, 1H), 5.18 – 4.98 (m, 2H), 4.15 (d, $J$ = 7.0 Hz, 2H), 2.93 (d, $J$ = 2.8 Hz, 2H), 2.28 – 1.87 (m, 13H), 1.68 (s, 6H), 1.60 (s, 6H). $^{13}$C NMR (75 MHz, Chloroform-$d$) δ 139.44, 135.47, 134.23, 131.48, 126.12, 124.46, 123.94, 123.85, 82.56, 68.34, 59.51, 39.85, 39.39, 36.83, 26.87, 26.65, 26.36, 25.86, 19.84, 17.84, 16.46, 16.20. MS (EI) m/z 296 [M$^+$-H$_2$O]; MS (CI) m/z 297 [(M$^+$+H)-H$_2$O].
Representative procedure for the synthesis of non-triazole containing pyrophosphates:

(2E,6E,10E)-3,7,11-trimethylpentadeca-2,6,10-trien-14-yn-1-yl diphosphate (5.7):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added NCS (2.5 eq, 0.24 mmol) in 0.5 mL of CH₂Cl₂ and cooled to -30°C where dimethyl sulfide (2.5 eq, 0.24 mmol) was added dropwise to the reaction. Following the addition, the mixture is then placed in a 0°C ice bath and stirred for 5 minutes before being recooled back to -30°C. Next, alcohol 5.5 (1 eq, 0.096 mmol) is dissolved in 0.2 mL CH₂Cl₂ and added dropwise to the reaction mixture. The mixture is then placed in a 0°C ice bath and stirred for 2.5 hours coming to room temperature. After the allotted time, brine is added to the reaction mixture and the organic layer was extracted. The aqueous layer was further extracted 3 × 5 mL CH₂Cl₂ and the organic layers were combined, dried with magnesium sulfate, and concentrated. The crude reaction product was used immediately in the following step.

To another oven-dried round bottom flask equipped with a magnetic stir bar was added tris (tetrabutylammonium) hydrogen pyrophosphate (3.0eq, 0.29 mmol) dissolved in 1.3 mL of acetonitrile. Next, a solution of crude allylic chloride dissolve in 0.9 mL acetonitrile was added dropwise to the reaction vessel. The reaction was stirred for 3 hours at room temperature and then the solvent was removed by rotary evaporation at 34°C. The residue was the dissolve in a minimal amount of ion exchange NH₃HCO₃ buffer (700mg NH₃HCO₃, 1 L of deionized H₂O, 20 mL of isopropanol) and the resulting solution was passed through a Dowex AG 50 × 8 ion exchange column (2 × 8 cm) using the NH₃HCO₃ buffer as an eluent and 25 mL was collected in a flask. The resulting solution was lyophilized for 3-5 hours. The resulting residue was then redissolved in deionized watered and purified by cellulose flash column chromatography (3 × 15 cm) using isopropanol:deionized H₂O:acetonitrile: NH₃HCO₃ buffer (500 mL : 250 mL : 250 mL
4 g) as the eluent. In a beaker was collected 40 mL of eluent, then twenty-four 2.5 mL fractions were collected. Typically, fractions 12-18 were collected and the organic solvents were removed by rotary evaporation at 34°C. The resulting solution was then lyophilized to afford pyrophosphate (5.6) as a white fluffy solid in 84% yield. 

$^1$H NMR (500 MHz, Deuterium Oxide) δ 5.39 (s, 1H), 5.13 (d, $J = 6.3$ Hz, 2H), 4.41 (s, 2H), 2.33 – 1.98 (m, 12H), 1.95 (d, $J = 6.1$ Hz, 1H), 1.66 (s, 3H), 1.56 (s, 6H). $^{31}$P NMR (202 MHz, D$_2$O) δ -9.89, -13.73. HRMS 419.1391 [M+2H]$^+$, calculated 419.1389 (C$_{18}$H$_{29}$O$_7$P$_2$).

(2E,6E,10E)-3,7,11-trimethyltetradeca-2,6,10-trien-13-yn-1-yl diphosphate (5.6):

Yield (91%). $^1$H NMR (500 MHz, Deuterium Oxide) δ 5.41 (s, 2H), 5.15 (s, 1H), 4.43 (s, 2H), 2.26 – 1.84 (m, 10H), 1.73 (s, 1H), 1.66 (s, 3H), 1.62 (s, 3H), 1.57 (s, 3H). $^{31}$P NMR (202 MHz, Deuterium Oxide) δ -10.08 (d, $J = 37.0$ Hz), -13.92 (d, $J = 40.4$ Hz). HRMS 405.1237 [M+2H]$^+$, calculated 405.1232 (C$_{17}$H$_{27}$O$_7$P$_2$).

(2E,6Z,10E)-3,11,15-trimethyl-7-(prop-2-yn-1-yl)hexadeca-2,6,10,14-tetraen-1-yl diphosphate (5.25):

Yield (71%). $^{31}$P NMR (202 MHz, Deuterium Oxide) δ -10.12, -14.20.
Representative procedure for the synthesis of triazole containing pyrophosphates:

(2E,6E)-10-(4-(but-3-yn-1-yl)-1H-1,2,3-triazol-1-yl)-3,7-dimethyldeca-2,6-dien-1-yl diphosphate (5.11):

To an oven-dried round bottom flask equipped with a magnetic stir bar was added methanesulfonyl chloride (1.3 eq, 0.15 mmol), DMAP (1.5 eq, 0.17 mmol) and 0.6 mL of dichloromethane. The mixture was cooled to 0°C and a solution of alcohol 5.10 in 0.2 mL of dichloromethane was added dropwise to the reaction vessel and allowed to stir for 2.5 hours. Hexanes were then added to the reaction vessel and the solution was filtered and concentrated. Next, Et2O was added to the crude product and was again filtered and concentrated. The crude allylic chloride was then converted to the pyrophosphate following the same method described for 5.6. Yield (80%). 1H NMR (500 MHz, Deuterium Oxide) δ 7.79 (s, 1H), 5.39 (d, J = 6.9 Hz, 1H), 5.06 (s, 1H), 4.41 (t, J = 6.3 Hz, 2H), 4.29 (t, J = 6.3 Hz, 2H), 2.85 (t, J = 6.8 Hz, 2H), 2.58 – 2.46 (m, 2H), 2.28 (s, 1H), 2.04 (dd, J = 12.9, 5.8 Hz, 4H), 1.99 – 1.93 (m, 2H), 1.90 (d, J = 6.5 Hz, 2H), 1.66 (s, 3H), 1.51 (s, 3H). 31P NMR (202 MHz, Deuterium Oxide) δ -10.07 (d, J = 21.3 Hz), -13.96 (d, J = 22.0 Hz). HRMS 460.1403 [M+2H]+, calculated 460.1403 (C18H28N3O7P2).

(2E,6E)-3,7-dimethyl-10-(4-(prop-2-yn-1-yl)-1H-1,2,3-triazol-1-yl)deca-2,6-dien-1-yl diphosphate (5.14):

Yield (73%). 1H NMR (500 MHz, Deuterium Oxide) δ 7.84 (s, 1H), 5.41 (t, J = 6.7 Hz, 1H), 5.07 (t, J = 6.8 Hz, 1H), 4.43 (t, J = 6.1 Hz, 2H), 4.32 (td, J = 6.7, 2.4 Hz, 2H), 3.65 (t, 1H), 2.11 – 2.02 (m, 4H), 2.01 – 1.87 (m, 4H), 1.67 (s, 3H), 1.52 (s, 3H). 31P NMR (202 MHz,
Deuterium Oxide) δ -13.07 (d, J = 16.7 Hz), -14.29 (d, J = 17.3 Hz). HRMS 446.1250 [M+2H]^+, calculated 446.1246 (C_{17}H_{27}N_{3}O_{7}P_{2}).

**General procedure for in vitro biochemical substrate screening:**

All biochemical evaluations were performed in our collaborator Dr. Carol Fierke’s laboratory at the University of Michigan by Elia Wright. Preliminary evaluation of all pyrophosphate analogs were performed using GGPP analog (1, 5, or 10 µM), the peptide dansyl-GCVLL (5 µM), recombinant mammalian GGTase-I (50 nM), 50 nM HEPPSO pH 7.8, 5 mM tris(2carboxyethyl)phosphine (TCEP), and 5 mM MgCl$_2$ at 25°C in 96 well plates (Corning). Protein prenylation was determined by monitoring the dansylated peptide using a continuous spectrofluorometric assay and all assays were performed in triplicate. Upon prenylation of the peptide, the activity was measured by an increase in fluorescence intensity of the dansyl group ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 520$ nm) in a POLARstar Galaxy plate reader. Peptide in assay buffer was utilized as a negative control and the baseline fluorescence of the peptide was subtracted from the reaction signal.
CHAPTER 6. CONCLUSIONS & FUTURE DIRECTIONS

This work served as a preliminary study of the isoprene requirements and their effect on GGTase-I activity. Several small libraries of aryl-modified, saturated, frame-shifted, and alkynyl-GGPP compounds were synthesized and evaluated as co-substrates with dansyl-GCVLL in a fluorescence-based assay. Interestingly, the majority of our compounds displayed in vitro biochemical activity and provided us with interesting insights into GGTase-I binding (Figure 6.1).

6.1 Aryl-Modified GGPP Analogs

Replacing the ω-isoprene unit with an aryl motif was well tolerated by the enzyme; however, the number of methylene spacers between the aryl group and the γ-isoprene unit was important. When the number of methylene spacers was two, this yielded compounds that had substrate activity comparable to GGPP (2.6a, d, f; Figure 2.6). The longer carbon chain allows the aryl-moiety to be positioned in a more spacious area of the exit groove (Figure 6.3, yellow). When the number of methylene spacers were decreased by one, there was diminished substrate activity, although these compounds still retained some ability to be turned over by the GGTase-I. One possible reason for the observations noticed with the shorter chain analogs (2.6b-d) could be that the aryl-analogs bind in such a way that the pyrophosphate head group is further away from the Zn\(^{2+}\) ion (i.e. bound deeper within the pocket). Therefore, coordination to the zinc ion would be attenuated and in return the catalytic ability of the enzyme to transfer the isoprenoid chain would be diminished (Figure 6.2 & 6.4A).
Additionally, the aryl-group of the shorter chained analog (2.6b) is less flexible and modeling in the exit groove indicates a potential unfavorable interaction (Figure 6.3). Due to the sp$^{2}$ carbons being more electronegative than the hydrogen atoms, the benzene ring exhibits a negative potential on the pi face. This could result in an unfavorable interaction with the electrons of the carbonyl backbone group of the neighboring P317β. Alternatively, aligning the aryl-moiety of 2.6b (Figure 6.3, purple) with the ω-isoprene of GGPP (Figure 6.3, green) indicates that in this position the steric bulk of the aryl-motif would potentially be too great to fit into the narrow exit groove. In order to alleviate these issues, the aryl-motif of 2.6b could rotate into an alternative pocket of the exit groove which would allow the analog to adopt a more extended conformation into a different pocket of the exit groove which may or may not affect product release (Figure 6.3, dashed circle).

Surprisingly, the cyclohexyl analog (2.6c) also displayed substrate activity; in fact, it appeared to be a more efficient substrate than its aryl-counterpart (2.6b). This led us to question whether or not the aryl-motif is beneficial or if it is simply a matter of hydrophobicity. An alternative explanation could lie in the differences in flexibility between the two motifs and the product release of the prenylated protein. In order for the product to be released from the enzyme, the isoprenoid group must rotate into an exit groove of the enzyme followed by the binding of a new molecule of the pyrophosphate analog. The cyclohexyl motif is a much more flexible group; thus, it could be speculated that as the isoprenoid chain is shifting into the exit groove, the more flexible cyclohexyl group is able to undergo conformational changes in order to allow the isoprenoid shift to occur more efficiently (Figure 6.3, red). The aryl-motif, which is a more rigid structure, could potentially hinder the ability of the isoprenoid chain to undergo this conformational shift into the exit groove.

Another potential benefit of the cyclohexyl group of 2.6c is lack of aromaticity when the carbon chain is shorter than GGPP. This would remove any potential unfavorable electronic
interaction between the carbonyl backbone of P317β and the pi face. It would be intriguing to synthesize a cyclohexyl-analog that contains two methylene spacers between the cyclic group and the γ-isoprene unit to determine if any additional activity could be gained by increasing the length of the isoprene chain (Figure 6.1). In general, the length of the carbon chain seems to greatly influence whether or not the aromatic moiety is a beneficial modification.

6.2 Saturated GGPP Analogs

The saturated analogs revealed that GGTase-I can recognize and utilize compounds that contain only the α-isoprene unit; however, length of the analog has a significant effect upon substrate activity. Generally, compounds that are the same length as GGPP display the greatest amount of substrate activity. The attenuated activity of the shorter analogs could be due to the position of the isoprene in the binding pocket (Figure 6.4). One possibility is that the carbon chain binds in the pocket in such a way that the carbon adjacent to the pyrophosphate head group is further away from the Zn$^{2+}$ ion (Figure 6.4A, yellow) which could diminish catalysis.

Analogs containing only the α-isoprene unit (3.3a-c) revealed that only α-isoprene is required for enzyme recognition and catalysis; however, only moderate substrate activity was observed. Possible explanations for this observation include: 1) loss of potential pi interactions between the β, γ, and ω-isoprene units with aromatic groups aligning the binding pocket and/or 2) effects of changing hydrophobicity on the dansyl-fluorophore. By taking a closer look at the binding pocket of GGTase-I, it is possible that the β and γ-isoprene units could interact with W275β via pi-pi interactions (Figure 6.4C). Additionally, the γ and ω-isoprene units have the potential to have pi-pi interactions with Y176β and the ω-isoprene unit could have additional pi-pi interactions with F52β, F53β, Y126β, W275β, and F324β. Therefore, the lack of these interactions may result in the observed decrease in enzymatic catalysis by potentially lowering
the affinity of the enzyme for the saturated analogs. On the other hand, the observation could be due to the nature of the pyrophosphate analogs. The substrate assays were developed for the use of an isoprenoid chain with a dansyl-fluorophore. Increasing the hydrophobic environment around the dansyl-fluorophore with an isoprenoid chain leads to an increase in fluorescence which can be easily measured. This begs the question of whether or not the fluorescence change/intensity will be the same between a saturated analog and GGPP. Thus, further studies need to done to address this issue and any definitive conclusions cannot be made until such experiments have been conducted. One possible method is to use HPLC analyses which would allow us to quantify the amount of unprenylated peptide versus prenylated product which could then be related back to the change in fluorescence observed.

The saturated analogs also revealed that incorporation of the ω-isoprene unit is very beneficial for substrate activity, but only when the carbon chain is the length of GGPP. This modification yields a compound (3.16b) that was comparable to GGPP and indicated that the β- and γ-isoprene units are not essential for GGTase-I recognition and turn over. However, by decreasing the length of the carbon chain by one (3.16a) resulted in a drastic decrease in enzyme activity. Once again, this could potentially be due to pi-pi interactions. As mentioned previously, the ω-isoprene unit has the potential to have pi-pi interactions with F52β, F53β, Y126β, Y176β, W275β, and F324β (Figure 6.4C). By decreasing the carbon length by one methylene unit, this increases the distance between the ω-isoprene unit and four of the aromatic residues lining the binding pocket (F52β, F53β, Y126β, and F324β) (Figure 6.4B). Therefore, the decrease in or lose of pi-pi interactions could account for the observed decrease in enzymatic activity.

Interestingly, replacing the β-isoprene has little effect on substrate activity when the carbon chain is the length of GGPP. Instead, the replacement of the β-isoprene has the most pronounced effect on compounds that are lacking in length. As mentioned previously, the
attenuated activity of the shorter analogs could be due to the position of the isoprene in the binding pocket (Figure 6.2 & 6.4A). Without the β-isoprene unit, perhaps it is more likely that the carbon chain binds in the pocket in such a way that the carbon adjacent to the pyrophosphate head group is further away from the Zn$^{2+}$ ion (Figure 6.4A, yellow) which could diminish catalytic ability. However, by reintroducing the β-isoprene unit back into the molecule, this could provide an additional pi-pi interaction between the β-isoprene unit and W275β (Figure 6.4C). Therefore, instead of binding deep in the pocket (Figure 6.3A, yellow) where the pyrophosphate group is further away from the Zn$^{2+}$ ion, perhaps the added pi-pi interaction aids in the analogs binding in the correct orientation (i.e. the pyrophosphate head group is near the Zn$^{2+}$). Thus, for analogs which shorter carbons chains, this modification would be beneficial.

### 6.3 Frame-Shifted GGPP Analogs

The majority of the compounds in our frame-shifted GGPP library were determined to display substrate activity. The information gained from these results can be helpful when designing new analogs. Once again, the length of the carbon chain appears to be a major factor in determining substrate ability. As mentioned previously, this is most likely due to the ω-isoprene unit having pi-pi interactions with F52β, F53β, Y126β, Y176β, W275β, and F324β (Figure 6.4C). Interestingly, compounds 1,2,2,1-OPP and 5,2,1-OPP have similar substrate abilities indicated that chain length is more important than flexibility. It is possible that both molecules bind to the enzyme as shown in Figure 6.4B. Both analogs increase the distance between the ω-isoprene unit and four of the aromatic residues lining the binding pocket (F52β, F53β, Y126β, and F324β) (Figure 6.4B). Therefore, the decrease in or lose of pi-pi interactions could account for the observed decrease in enzymatic activity and similar activities.
Additionally, increasing the number of methylene spacers between the α- and β- isoprene units from two to three seems to be very detrimental to substrate ability. Preliminary docking of analogs 2,3,1-OOP (4.17, Figure 6.5B) and 3,3,1-OOP (4.11, Figure 6.5C) indicates that this modification could lead to potential unwanted steric interactions with C177β and C225β (Figure 6.5B & 6.5C, dashed circles). This could potentially explain their poor substrate ability. By reverting back to the two methylene units between the α and β-subunits, analog 3,2,1-OOP (4.28) does not appear to have these steric interactions and regains minimal substrate activity (Figure 6.5A). It seems for analog 3,2,1-OOP the major factor is carbon chain length. An analog synthesized by Dr. Andrew Placzek (2,2,1,1-OOP) where the number of methylene units between the α- and β- isoprene units was decreased to one was also determined to be a poor substrate of GGTase-I. Thus, the position and orientation of the β-isoprene unit is crucial.

This class of compounds also revealed the γ-isoprene unit is unnecessary for activity (4.40c; 6,2,1x-OOP). This is perhaps not surprising when taken into consideration the majority of possible pi-pi interactions occur between the enzyme and the β and ω-isoprene units (Figure 6.4C). Moreover, converting the allylic pyrophosphate into a homo-allylic pyrophosphate produced an inhibitor. This is most likely due to the decreased nucleophilicity of the non-allylic pyrophosphate. It would be interesting to synthesized frame-shifted analogs with a carbon chain length of 16 such as 1,3,2,1-OOP, 2,3,1,1-OOP and 3,2,1,1-OOP (Figure 6.1). Additionally, it would also be interesting to synthesize an analog in which all the isoprene units except the β-isoprene are present (2,6,1-OOP) (Figure 6.1). These new analogs could provide further insight into GGTase-I activity.
6.4 Alkynyl-GGPP Analogs

Unfortunately, the alkynyl-GGPP analogs did not show as much promise in our \textit{in vitro} biochemical screening assays. Looking at the transformations that occurs throughout the reaction pathway of GGTase-I could provide a possible understanding of why the triazole compounds did not behave as substrates. Preliminary modeling of analog 5.11 in the catalytic site to GGTase-I indicates the possibility of an unfavorable steric interaction with C177β (Figure 6.6A, dashed circle). This observation taken together with the fact that introduction of the triazole into the compound decreases the flexibility of the analog could hinder binding to GGTase-I. Additionally, due to increased rigidity, it is possible that the prenylated product cannot shift readily into the exit groove of GGTase-I which would hinder product releases (Figure 6.1). It is also possible that the prenylated product may not fit into the exit groove (Figure 6.6B, orange). Moreover, analog 5.11 could adopt an alternate conformation with in the exit groove (Figure 6.6B, violet). This alternative position allows for favorable, stabilizing interactions between the triazole nitrogens and the hydroxyl group of Y40β and the backbone carbonyl of P317β. Such stabilizing interactions could result in attenuated product release.

Only the terminal alkyne (5.7) showed moderate substrate activity; however, as mentioned previously, it’s important to note that these compounds were only screened with one dansyl-GCaαX sequence. Using combinatorial screening in the past, our laboratory has demonstrated that some modified FPP and GGPP analogs act as selective substrates. These substrates only modify a very limited amount of CaαX sequences.\textsuperscript{73,144} Thus, it is possible these analogs are substrates of GGTase-I but they are not a co-substrate with dansyl-CVLL. Therefore, all these compounds will be evaluated more extensively in the future with combinatorial assays in which several libraries of dansyl-CaαX peptides will be screened. These screens will provide us with a good indication of whether or not our substrates are global or selective prenyl donors.
Additionally, compounds that do not display substrate activity will be tested in a similar fluorescence-based assay to determine if these analogs are inhibitors of GGTase-I. Finally, we also anticipate screening these analogs in a combinatorial screen and/or inhibitor screen with FTase. These screens will provide insight into the differences and/or similarities of isoprene requirements of FTase and GGTase-I leading to a deeper understanding of the dynamic roles farnesylation and geranylgeranylation play in cellular processes.
Figure 6.1. Summary of project findings and proposed future analogs for synthesis.
**Figure 6.2.** Structure of GGPP and CVLL or CVIL bound in the β-subunit of GGTase-I. A) Overlay of GGPP (magenta) and CVLL (light purple) and prenylated CVIL (blue). B) Overlay of prenylated CVIL (blue) in the catalytic site and prenylated CVIL in the exit groove (green) of GGTase-I (PDB: 1TNZ, 1N4R, 1N4S).

**Figure 6.3.** Aryl-modified analogs bound in the exit groove of GGTase-I. Overlay of analog 2.6a (yellow, homobenzyl), 2.6b (purple, benzyl), 2.6c (red, cyclohexyl), & GGPP (green). Alternative binding mode of 2.6b is highlighted in dashed circle (PDB: 1N4S).
Figure 6.4. Analyzing the distances between the $\text{Zn}^{2+}$ ion or aromatic residues in the binding pocket of GGTase-I with isoprenoid chains. A) Shorter carbon chain analogs (yellow) that could bind deep in the GGTase-I pocket. B) Bound isoprenoid that is one carbon shorter than GGPP (1,2,2,10-OPP; red). C) Bound GGPP (blue) (PDB: 1TNZ).
Figure 6.5. Analyzing the affects of increasing the number of carbons between the α and β-isoprene units to three carbons. Overlay of GGPP (blue) and A) 3,2,1-OPP (green), B) 2,3,1-OPP (purple) and C) 3,3,1-OPP (pink). Potential unfavorable steric interactions are shown in dashed circles (PDB: 1N4R).
Figure 6.6. Triazole-containing alkynyl-GGPP analogs bound in GGTase-I. A) Overlay of analog 5.11 (orange) with GGPP (magenta) bound in the catalytic site. B) Overlay of analog 5.11 (orange) and GGPP (green) bound in the exit groove of GGTase-I. Alternative binding mode of analog 5.11 is also shown (violet). Unfavorable steric interactions shown dashed circles (PDB: 1TNZ & 1N4S).
LIST OF REFERENCES
LIST OF REFERENCES


77. Aditya, A. Investigation of Peptide Substrate Specificity and Design of Novel Bisubstrate Inhibitors of Protein Geranylgeranyl Transferase-1. Purdue University, West Lafayette, 2010.


APPENDIX
APPENDIX: NMR SPECTRA OF FINAL ALCOHOLS & PYROPHOSPHATES
Figure A.1. $^1$H NMR of 2.5a.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Bruker Analytik GmbH</td>
</tr>
<tr>
<td>Solvent</td>
<td>CDCl3</td>
</tr>
<tr>
<td>Spectrometer Frequency</td>
<td>75.48</td>
</tr>
<tr>
<td>Nucleus</td>
<td>13C</td>
</tr>
</tbody>
</table>

**2.5a**

Figure A. 2. $^{13}$C NMR of 2.5a.
Figure A. 3. $^1$H NMR of 2.6a.
Figure A.4. $^{31}$P NMR of 2.6a.
Figure A. 5. $^1$H NMR of 2.5b.

![Figure A. 5. $^1$H NMR of 2.5b.](image-url)
Figure A. 6. $^{13}$C NMR of 2.5b.
Figure A. 7. $^1$H NMR of 2.6b.
Figure A. 8. \(^{31}\)P NMR of 2.6b.
Figure A. 9. $^1$H NMR of 2.5c.
Figure A.10. $^{13}$C NMR of 2.5c.
Figure A.11. $^1$H NMR of 2.6c.
Figure A. 12. $^{31}$P NMR of 2.6c.
Figure A.13. $^1$H NMR of 2.5d.
Figure A. 14. $^{13}$C NMR of 2.5d.
Figure A. 15. \(^1\)H NMR of 2.6d.
Figure A. 16. $^{31}$P NMR of 2.6d.
Figure A. 17. $^1$H NMR of 2.5e.
Figure A. 18. $^{13}$C NMR of 2.5e.
Figure A. 19. $^1$H NMR of 2.6e.
Figure A. 20. $^{31}$P NMR of 2.6e.
Figure A. 21. $^1$H NMR of 2.5f.
Figure A.22. $^{13}$C NMR of 2.5f.
Figure A. 23. $^{19}\text{F}$ NMR of 2.5f.
Figure A. 24. $^1$H NMR of 2.6f.
Figure A. 25. $^{31}$P NMR of 2.6f.
Figure A.26. $^1$H NMR of 2.14.
Figure A. 27. $^{13}$C NMR of 2.14.
Figure A.28. $^1$H NMR of 2.15.
Figure A. 29. $^{31}$P NMR of 2.15.
Figure A. 30. $^1$H NMR of 3.2a.
Figure A. 31. $^{13}\text{C}$ NMR of 3.2a.
Figure A. 32. $^1$H NMR of 3.3a.
Figure A. 33. $^{31}$P NMR of 3.3a.
Figure A. 34. $^1$H NMR of 3.2b.
Figure A. 35. $^{13}$C NMR of 3.2b.
Figure A. 36. $^1$H NMR of 3.3b.
Figure A. 37. $^{31}$P NMR of 3.3b.
Figure A. 38. $^1$H NMR of 3.2c.
Figure A. 39. $^{13}$C NMR of 3.2c.
Figure A. 40. $^1$H NMR of 3.3c.
Figure A. 41. $^{31}$P NMR of 3.3c.
Figure A. 42. $^1$H NMR of 3.6a.
Figure A. 43. $^{13}$C NMR of 3.6a.
Figure A. 44. $^1$H NMR of 3.7a.
Figure A. 45. $^{31}$P NMR of 3.7a.
Figure A.46. $^1$H NMR of 3.6b.
Figure A. 47. $^{13}$C NMR of 3.6b.
Figure A. 48. $^1$H NMR of 3.7b.
Figure A. 49. $^{31}$P NMR of 3.7b.
Figure A. 50. $^1$H NMR of 3.6c.
Figure A. 51. $^{13}$C NMR of 3.6c.
Figure A. 52. $^1$H NMR of 3.7c.
Figure A. 53. $^{31}\text{P}$ NMR of 3.7c.
Figure A. 54. $^1$H NMR of 3.6d.
Figure A. 55. $^{13}$C NMR of 3.6d.
Figure A. 56. $^1$H NMR of 3.7d.
Figure A. 57. $^{31}P$ NMR of 3.7d.
Figure A. 58. $^1$H NMR of 3.15a.
Figure A. 59. $^{13}$C NMR of 3.15a.
Figure A. 60. $^1$H NMR of 3.16a.
Figure A. 61. $^{31}$P NMR of 3.16a.
Figure A. 62. $^1$H NMR of 3.15b.
Figure A. 63. $^{13}$C NMR of 3.15b.
Figure A. 64. $^1$H NMR of 3.16b.
Figure A. 65. $^{31}$P NMR of 3.16b.
Figure A.66. $^1$H NMR of 3.20.
Figure A. 67. $^{13}$C NMR of 3.20.
Figure A. 68. $^1$H NMR of 3.21.
Figure A. 69. $^{31}$P NMR of 3.21.
Figure A. 70. $^1$H NMR of 3.25.
Figure A. 71. $^{13}$C NMR of 3.25.
Figure A. 72. $^1$H NMR of 3.26.
Figure A. 73. $^{31}$P NMR of 3.26.
Figure A. 74. $^1$H NMR of 4.10.
Figure A. 75. $^{13}$C NMR of 4.10.
Figure A. 76. $^1$H NMR of 4.11.
Figure A. 77. $^{31}$P NMR of 4.11.
Figure A. 78. $^1$H NMR of 4.16.
Figure A. 79. $^{13}$C NMR of 4.16.
Figure A. 80. $^1$H NMR of 4.17.
Figure A. 81. $^{31}$P NMR of 4.17.
Figure A. 82. $^1$H NMR of 4.20.
Figure A. 83. $^{13}$C NMR of 4.20.
Figure A. 84. $^1$H NMR of 4.21.
Figure A. 85. $^{31}$P NMR of 4.21.
Figure A. 86. $^1$H NMR of 4.27.
Figure A. 87. $^{13}$C NMR of 4.27.
Figure A. 88. $^1$H NMR of 4.28.
Figure A. 89. $^{31}$P NMR of 4.28.
Figure A. 90. $^1$H NMR of 4.31.
Figure A. 91. $^{13}$C NMR of 4.31.
Figure A. 92. $^1$H NMR of 4.32.
Figure A. 93. $^{31}$P NMR of 4.32.
Figure A. 94. $^1$H NMR of 4.39a.
Figure A. 96. $^1$H NMR of 4.40a.
Figure A. 97. $^{31}$P NMR of 4.40a.
Figure A. 98. $^1$H NMR of 4.39b.
Figure A. 99. $^{13}$C NMR of 4.39b.
Figure A. 100. $^1$H NMR of 4.40b.
Figure A.101. $^{31}$P NMR of 4.40b.
Figure A. 102. $^1$H NMR of 4.39c.
Figure A.103. $^{13}$C NMR of 4.39c.
Figure A. 104. ¹H NMR of 4.40c.
Figure A. 105. $^{31}$P NMR of 4.40c.
Figure A. 106. $^1$H NMR of 5.3.
Figure A. 107. $^1$H NMR of 5.5.
Figure A. 108. $^{13}$C NMR of 5.5.
Figure A. 109. $^{31}$P NMR of 5.6.
Figure A.110. $^1$H NMR of 5.6.
Figure A.111. $^1$H NMR of 5.7.
Figure A.112. $^{31}$P NMR of 5.7.
Figure A.113. $^1$H NMR of 5.10.
Figure A. 114. $^{13}$C NMR of 5.10.
Figure A. 115. $^1$H NMR of 5.11.
Figure A.116. $^{31}$P NMR of 5.11.
Figure A. 117. $^1$H NMR of 5.13.
Figure A.118. $^{13}$C NMR of 5.13.
Figure A.119. $^1$H NMR of 5.14.
Figure A. 120. $^{31}$P NMR of 5.14.
Figure A. 121. $^1$H NMR of 5.24.
Figure A. 122. $^{13}$C NMR of 5.24.
Figure A. 123. $^{31}$P NMR of 5.25.
VITA
Kayla Temple, daughter of Terry & Beverly Temple, was born on August 14, 1985 in Wheeling, WV. She attended John Marshall High School in Glen Dale, WV, where she graduated in June 2003. She went on to further her education by pursuing a chemistry degree from Wheeling Jesuit University. During her undergraduate career, she joined ΑΕ∆ (the Health Pre-professional Honor Society) as well as ΓΣΕ (the Chemistry Honor Society) and volunteered at Wheeling Hospital in the Pharmacy. In May 2007, she graduated summa cum laude from Wheeling Jesuit University with a Bachelor of Science degree in Chemistry. In August of 2007, she joined the Department of Medicinal Chemistry & Molecular Pharmacology at Purdue University in pursuit of a Doctor of Philosophy degree. She joined the laboratory of Dr. Richard Gibbs where she researched protein geranylgeranylation. Kayla received her Ph.D. in December 2013.