Supporting information

for

Synthesis of isoprenoid bisphosphonate ethers through C–P bond formations:

Potential inhibitors of geranylgeranyl diphosphate synthase

Xiang Zhou¹, Jacqueline E. Reilly², Kathleen A. Loerch¹, Raymond J. Hohl²,³, and David F. Wiemer*¹,²,§

Address: ¹Department of Chemistry, University of Iowa, Iowa City, Iowa 52242-1294, USA, ²Department of Pharmacology, University of Iowa, Iowa City, Iowa 52242-1294, USA and ³Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52242-1294, USA

Email: David F. Wiemer - david-wiemer@uiowa.edu

*Corresponding author

Experimental procedures, characterization data, and ¹H and ¹³C NMR spectra are provided for all new compounds
Supplemental Table of Contents

General experimental procedures S4
Representative procedure for monophosphonate ether formation (8) S4
Bisphosphonate ether 9 S5
Representative procedure for bisphosphonate ether alkylation (10) S5
Bisphosphonate salt 11 S6
Bisphosphonate salt 6 S6
Monophosphonate ether 12 S6
Representative procedure for monophosphonate ether phosphonylation (13) S7
Bisphosphonate salt 14 S7
Bisphosphonate ester 15 S8
Bisphosphonate salt 16 S8
Bisphosphonate ester 17 S8
Bisphosphonate salt 18 S9
Bisphosphonate ester 19 S9
Representative procedure for hydrolysis of bisphosphonate esters (20) S9
Synthesis of monophosphonate ether 21 S10
Synthesis of bisphosphonate ether 22 S10
Synthesis of tetraethyl bisphosphonate ether 23 S11
Synthesis of bisphosphonate salt 24 S11
Procedure for the enzyme assays S12
References S12

$^1$H NMR for compound 8 (300 MHz) S13
$^{13}$C NMR for compound 8 (75 MHz) S14
$^1$H NMR for compound 9 (300 MHz) S15
$^{13}$C NMR for compound 9 (75 MHz) S16
$^1$H NMR for compound 10 (300 MHz) S17
$^{13}$C NMR for compound 10 (75 MHz) S18
$^1$H NMR for compound 11 (300 MHz) S19
$^{13}$C NMR for compound 11 (75 MHz) S20
$^1$H NMR for compound 6 (300 MHz) S21
$^{13}$C NMR for compound 6 (125 MHz) S22
$^1$H NMR for compound 12 (300 MHz) S23
$^{13}$C NMR for compound 12 (75 MHz) S24
$^1$H NMR for compound 13 (300 MHz) S25
$^{13}$C NMR for compound 13 (75 MHz) S26
$^1$H NMR for compound 14 (300 MHz) S27
$^{13}$C NMR for compound 14 (100 MHz) S28
$^1$H NMR for compound 15 (400 MHz) S29
$^{13}$C NMR for compound 15 (100 MHz) S30
$^1$H NMR for compound 16 (500 MHz) S31
$^{13}$C NMR for compound 16 (125 MHz) S32
$^1$H NMR for compound 17 (500 MHz) S33
$^{13}$C NMR for compound 17 (125 MHz) S34
$^1$H NMR for compound 18 (500 MHz) S35
<table>
<thead>
<tr>
<th>Compound</th>
<th>NMR Method</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>$^1$H NMR</td>
<td>300 MHz</td>
</tr>
<tr>
<td>19</td>
<td>$^1$H NMR</td>
<td>75 MHz</td>
</tr>
<tr>
<td>20</td>
<td>$^1$H NMR</td>
<td>500 MHz</td>
</tr>
<tr>
<td>21</td>
<td>$^1$H NMR</td>
<td>500 MHz</td>
</tr>
<tr>
<td>22</td>
<td>$^1$H NMR</td>
<td>100 MHz</td>
</tr>
<tr>
<td>23</td>
<td>$^1$H NMR</td>
<td>400 MHz</td>
</tr>
<tr>
<td>24</td>
<td>$^1$H NMR</td>
<td>500 MHz</td>
</tr>
<tr>
<td>13C</td>
<td>$^{13}$C NMR</td>
<td>125 MHz</td>
</tr>
<tr>
<td>13C</td>
<td>$^{13}$C NMR</td>
<td>125 MHz</td>
</tr>
<tr>
<td>13C</td>
<td>$^{13}$C NMR</td>
<td>100 MHz</td>
</tr>
<tr>
<td>13C</td>
<td>$^{13}$C NMR</td>
<td>125 MHz</td>
</tr>
<tr>
<td>13C</td>
<td>$^{13}$C NMR</td>
<td>125 MHz</td>
</tr>
<tr>
<td>13C</td>
<td>$^{13}$C NMR</td>
<td>125 MHz</td>
</tr>
</tbody>
</table>
General experimental procedures. Tetrahydrofuran was freshly distilled from sodium/benzophenone, while methylene chloride was distilled from calcium hydride prior to use. All other reagents and solvents were purchased from commercial sources and used without further purification. All reactions in nonaqueous solvents were conducted in flame-dried glassware under a positive pressure of argon and with magnetic stirring. The NMR spectra were obtained at 300, 400, or 500 MHz for $^1$H, and 75, 100, or 125 MHz for $^{13}$C, with internal standards of (CH$_3$)$_4$Si ($^1$H, 0.00) or CDCl$_3$ ($^1$H, 7.27; $^{13}$C, 77.2 ppm) for non-aqueous samples or D$_2$O ($^1$H, 4.80) and 1,4-dioxane ($^{13}$C, 66.7 ppm) for aqueous samples. The $^{31}$P chemical shifts were reported in ppm relative to 85% H$_3$PO$_4$ (external standard). High resolution mass spectra were obtained at the University of Iowa Mass Spectrometry Facility. Silica gel (60 Å, 0.040–0.063 mm) was used for flash chromatography.

Monophosphonate ether 8. Diethyl hydroxymethylphosphonate (7, 1 mL, 6.8 mmol) was added dropwise to a solution of NaH (60% dispersion in mineral oil, 300 mg, 7.5 mmol) in THF (7 mL) in an ice bath, followed by addition of 15-crown-5 (0.1 mL, 1 M in THF). After 30 minutes, geranyl bromide (1.62 g, 7.5 mmol) was added to the reaction mixture and it was allowed to react at room temperature overnight. Once the reaction was complete based on analysis of the $^{31}$P NMR spectrum, saturated NH$_4$Cl was added. The resulting residue was extracted with Et$_2$O, the organic extracts were combined, dried (Na$_2$SO$_4$), concentrated in vacuo, and purified by column chromatography (5% EtOH in hexane) to afford the desired product 8 as a colorless oil (1.27 g, 62%): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.31 (t, $J = 6.5$ Hz, 1H), 5.08 (t, $J = 4.9$ Hz, 1H), 4.25–4.09 (m, 6H), 3.74 (d, $J_{HP} = 8.6$ Hz, 2H), 2.17–1.98 (m, 4H), 1.68 (s, 6H) 1.60
(s, 3H), 1.35 (t, \( J = 7.3 \) Hz, 6H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 141.7, 131.5, 123.6, 119.6, 69.0 (d, \( J_{CP} = 12.7 \) Hz), 62.9 (d, \( J_{CP} = 166.0 \) Hz), 62.1 (d, \( J_{CP} = 6.1 \) Hz, 2C), 39.4, 26.0, 25.5, 17.4, 16.3, 16.2 (2C); \(^{31}\)P NMR (121 MHz, CDCl\(_3\)) \( \delta \) 22.0.

**Bisphosphonate ether 9.** Prepared according to the general procedure given for compound 13: yield, 44%; colorless oil; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 5.26 (t, \( J = 7.0 \) Hz, 1H), 5.01 (t, \( J = 6.7 \) Hz, 1H), 4.27 (t, \( J = 7.5 \) Hz, 2H), 4.23–4.10 (m, 8H), 3.95 (t, \( J_{HP} = 17.6 \) Hz, 1H), 2.09–1.93 (m, 4H), 1.63 (s, 3H), 1.63 (s, 3H), 1.60 (s, 3H), 1.28 (t, \( J = 7.3 \) Hz, 12H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 142.1, 131.0, 123.2, 119.0, 70.1 (t, \( J_{CP} = 157.9 \) Hz), 69.2 (t, \( J_{CP} = 5.1 \) Hz), 62.7 (t, \( J_{CP} = 4.1 \) Hz, 2C), 62.5 (t, \( J_{CP} = 3.2 \) Hz, 2C), 39.1, 25.7, 25.0, 17.0, 15.9 (t, \( J_{CP} = 2.4 \) Hz, 2C), 15.8 (t, \( J_{CP} = 2.7 \) Hz, 2C), 15.8; \(^{31}\)P NMR (121 MHz, CDCl\(_3\)) \( \delta \) 16.1; HRMS (ES\(^+\), m/z) calcd for (M+Na\(^+\)) \( C_{19}H_{38}O_{7}P_{2}Na \): 463.1991; found: 463.1972.

**Bisphosphonate ether 10.** Compound 9 (325 mg, 0.74 mmol) was added into a solution of NaH (60% dispersion in mineral oil, 50 mg, 1.25 mmol) in THF (3 mL), 15-crown-5 (0.1 mL, 1M in THF) was added, and the reaction mixture was allowed to stir for 30 minutes. Geranyl bromide (300 mg, 1.38 mmol) was then added and the reaction was allowed to stir at room temperature overnight. Reaction progress was monitored by analysis of the \(^{31}\)P NMR spectrum. Once it was complete, water was added to quench the reaction. The resulting solution was then extracted with EtOAc and washed with brine. The organic layer was dried (Na\(_2\)SO\(_4\)) and concentrated in vacuo, and the residue was purified by column chromatography (5% EtOH in hexane) to afford compound 10 as a colorless oil (220 mg, 51%): \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 5.50 (t, \( J = 6.7 \) Hz, 1H), 5.34 (t, \( J = 5.6 \) Hz, 1H), 5.16–5.05 (m, 2H), 4.37 (d, \( J = 6.8 \) Hz, 2H), 4.30–4.17 (m, 8H), 2.98–
2.82 (m, 2H), 2.16–1.98 (m, 8H), 1.68 (s, 12H), 1.61 (s, 6H), 1.35 (t, $J = 6.9$ Hz, 6H), 1.35 (t, $J = 6.9$ Hz, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 139.6, 136.7, 131.4, 131.2, 124.3, 123.9, 120.8, 117.8 (t, $J_{CP} = 7.9$ Hz), 80.7 (t, $J_{CP} = 151.0$ Hz), 63.2 (t, $J_{CP} = 3.6$ Hz), 62.9 (t, $J_{CP} = 3.7$ Hz, 2C), 40.0, 39.5, 30.0, 26.6, 26.3, 25.6 (2C), 17.6 (2C), 16.5 (t, $J_{CP} = 3.0$ Hz, 2C), 16.4 (t, $J_{CP} = 2.5$ Hz, 2C), 16.4, 16.3; $^{31}$P NMR (121 MHz, CDCl$_3$) δ 19.0; HRMS (ES$^+$, $m/z$) calcd for (M+Na)$^+$ C$_{29}$H$_{54}$O$_7$NaP$_2$: 599.3243; found: 599.3244.

Bispophosphonate salt 11. Prepared according to the general procedure given for compound 20: yield, 17%; white solid; $^1$H NMR (300 MHz, D$_2$O) δ 5.49 (t, $J = 6.6$ Hz, 1H), 5.29–5.21 (m, 1H), 4.32 (d, $J = 7.1$ Hz, 2H), 3.67 (t, $J_{HP} = 15.2$ Hz, 1H), 2.25–2.08 (m, 4H), 1.74 (s, 3H), 1.72 (s, 3H), 1.66 (s, 3H); $^{13}$C NMR (75 MHz, D$_2$O) δ 142.1, 133.8, 124.3, 120.8, 75.7 (t, $J_{CP} = 130.3$ Hz), 69.8, 39.0, 25.8, 24.9, 17.0, 15.8; $^{31}$P NMR (121 MHz, D$_2$O) δ 14.1; HRMS (ES$^-$, $m/z$) calcd for (M-H)$^-$ C$_{11}$H$_{21}$O$_7$P$_2$: 327.0763; found: 327.0748.

Bispophosphonate salt 6. Prepared according to the general procedure given for compound 20: yield, 20%; white solid; $^1$H NMR (300 MHz, D$_2$O) δ 5.65 (t, $J = 6.5$ Hz, 1H), 5.39 (t, $J = 6.2$ Hz, 1H), 5.27–5.18 (m, 2H), 4.32 (d, $J = 6.9$ Hz, 2H), 2.88 (td, $J_{HP} = 14.1$ Hz, J = 6.5 Hz, 2H), 2.19–2.12 (m, 4H), 2.11–2.05 (m, 4H), 1.70 (s, 6H), 1.69 (s, 6H), 1.65 (s, 3H), 1.64 (s, 3H); $^{13}$C NMR (125 MHz, D$_2$O) δ 141.2, 137.1, 133.7, 133.5, 125.2, 124.7, 121.3, 119.7 (t, $J_{CP} = 7.8$ Hz), 79.5 (t, $J_{CP} = 131.8$ Hz), 62.7 (t, $J_{CP} = 6.5$ Hz), 39.4, 38.9, 28.7, 26.1, 25.7, 25.0, 18.5, 17.1, 17.1, 15.9, 15.6; $^{31}$P NMR (201 MHz, D$_2$O) 17.5; HRMS (ES$^-$, $m/z$) calcd for (M-H)$^-$ C$_{21}$H$_{37}$O$_7$P$_2$: 463.2015; found: 463.2021.

Monophosphonate ether 12. Yield, 77%; colorless oil; $^1$H NMR (300 MHz, CDCl$_3$) δ 5.36–5.28 (m, 1H), 4.23–4.11 (m, 4H), 4.10 (d, $J = 7.1$ Hz, 2H), 3.74 (d, $J_{HP} = 8.4$ Hz,
Bisphosphonate ether 13. A solution of n-butyllithium in hexanes (8.8 mL, 21.2 mmol) was added to a solution of diisopropylamine (2.75 mL, 19.5 mmol) in THF (16 mL) at −78 °C and the reaction was allowed to stir for 30 minutes. Ether 12 (2 g, 8.5 mmol) was then added to the reaction mixture dropwise (over 90 minutes), allowed to react for one additional hour, and then followed by the careful addition of diethyl chlorophosphate (2.9 mL, 19.5 mmol). After it was allowed to warm to room temperature slowly and to stir overnight, the reaction was quenched by addition of water. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. The resulting residue was purified by column chromatography (5% EtOH in hexane) to afford the desired product 13 as a colorless oil (1.39 g, 44%): ¹H NMR (300 MHz, CDCl₃) δ 5.34 (t, J = 7.2 Hz, 1H), 4.36–4.08 (m, 8H), 4.32 (d, J = 7.1 Hz, 2H), 4.03 (t, Jₜₜ = 17.5 Hz, 1H), 1.77 (s, 3H), 1.72 (s, 3H), 1.37 (t, J = 7.3 Hz, 12H); ¹³C NMR (75 MHz, CDCl₃) δ 139.1, 119.3, 70.1 (t, Jₖₖ = 156.9 Hz), 69.4 (t, Jₖₖ = 5.2 Hz), 62.9 (t, Jₖₖ = 2.6 Hz, 2C), 62.7 (t, Jₖₖ = 3.2 Hz, 2C), 25.4, 17.6, 16.1 (t, Jₖₖ = 2.9 Hz, 2C), 16.0 (t, Jₖₖ = 3.6 Hz, 2C); ³¹P NMR (121 MHz, CDCl₃) δ 16.2; HRMS (ES⁺, m/z) calcd for (M+Na)⁺ C₁₄H₃₀O₇NaP₂: 395.1365; found: 395.1395.

Bisphosphonate salt 14. Yield, 73%; white solid; ¹H NMR (300 MHz, D₂O) δ 5.38 (t, J = 6.7 Hz, 1H), 4.25 (d, J = 7.2 Hz, 2H), 3.67 (t, Jₜₜ = 16.2 Hz, 1H), 1.74 (s, 3H), 1.68 (s, 3H); ¹³C NMR (100 MHz, D₂O) δ 140.2, 119.9, 74.1 (t, Jₖₖ = 140.6 Hz), 69.8, 25.1, 17.5;
31P NMR (121 MHz, D2O) δ 13.9; HRMS (ES−, m/z) calcd for (M-H)− C6H13O7P2: 259.0137; found: 259.0145.

Bisphosphonate ester 15. Yield, 37%; colorless oil; 1H NMR (300 MHz, CDCl3) δ 5.48 (t, J = 6.2 Hz, 1H), 5.31 (t, J = 6.7 Hz, 1H), 5.16–5.04 (m, 1H), 4.33 (d, J = 6.8 Hz, 2H), 4.30–4.10 (m, 8H), 2.89 (td, JHP = 14.5 Hz, J = 6.4 Hz, 2H), 2.15–1.96 (m, 4H), 1.72 (s, 3H), 1.68 (s, 3H), 1.67 (s, 3H), 1.65 (s, 3H), 1.60 (s, 3H), 1.34 (t, J = 7.0 Hz, 6H), 1.34 (t, J = 6.8 Hz, 6H); 13C NMR (100 MHz, CDCl3) δ 136.8, 136.5, 131.4, 124.4, 121.2, 117.9 (t, JCP = 8.1 Hz), 80.8 (t, JCP = 150.6 Hz), 63.4 (t, JCP = 3.2 Hz), 63.4 (t, JCP = 4.5 Hz, 2C), 63.0 (t, JCP = 3.6 Hz, 2C), 40.1, 30.2, 26.7, 25.8, 25.8, 18.2, 17.7, 16.6 (t, JCP = 2.4 Hz, 2C), 16.6 (t, JCP = 3.0 Hz, 2C), 16.5; 31P NMR (121 MHz, CDCl3) δ 19.1; HRMS (ES+ m/z) calcd for (M+H)+ C24H47O7P2: 509.2797; found: 509.2803.

Bisphosphonate salt 16. Yield, 33%; white solid; 1H NMR (500 MHz, D2O) δ 5.65 (t, J = 6.3 Hz, 1H), 5.39 (t, J = 6.6 Hz, 1H), 5.25 (t, J = 6.0 Hz, 1H), 4.31 (d, J = 7.0 Hz, 2H), 2.87 (td, JHP = 13.3 Hz, J = 6.4 Hz, 2H), 2.20–2.12 (m, 2H), 2.11–2.05 (m, 2H), 1.75 (s, 3H), 1.70 (s, 6H), 1.69 (s, 3H), 1.65 (s, 3H); 13C NMR (125 MHz, D2O) δ 138.7, 137.1, 133.6, 124.7, 121.1, 119.7 (t, JCP = 7.8 Hz), 79.5 (t, JCP = 131.7 Hz), 62.7 (t, JCP = 6.0 Hz), 39.3, 28.9, 26.0, 25.0, 24.9, 17.5, 17.1, 15.5; 31P NMR (201 MHz, D2O) δ 17.5; HRMS (ES− m/z) calcd for (M-H)− C16H29O7P2: 395.1389; found: 395.1400.

Bisphosphonate ester 17. Yield, 29%; colorless oil; 1H NMR (500 MHz, CDCl3) δ 5.45 (t, J = 6.5 Hz, 1H), 5.31 (tt, J = 6.9 Hz, JHP = 1.4 Hz, 1H), 4.33 (d, J = 6.7 Hz, 2H), 4.28–4.18 (m, 8H), 2.87 (td, JHP = 14.7 Hz, J = 6.8 Hz, 2H), 1.73 (s, 6H), 1.67 (s, 3H), 1.65 (s, 3H), 1.34 (t, J = 7.6 Hz, 6H), 1.34 (t, J = 7.2 Hz, 6H); 13C NMR (125 MHz, CDCl3) δ 136.6, 133.3, 121.2, 118.1 (t, JCP = 7.8 Hz), 80.8 (t, JCP = 150.5 Hz), 63.5 (t, JCP = 5.2
Hz), 63.3 (t, $J_{CP} = 3.2$ Hz, 2C), 63.1 (t, $J_{CP} = 3.7$ Hz, 2C), 30.4, 26.1, 25.8, 18.2, 18.1, 16.6 (t, $J_{CP} = 2.6$ Hz, 4C); $^{31}$P NMR (201 MHz, CDCl$_3$) δ 19.0; HRMS (ES$^+$, $m/z$) calcd for (M+Na)$^+$ C$_{19}$H$_{38}$O$_7$NaP$_2$: 463.1991; found: 463.1989.

**Bisphosphonate salt 18.** Yield, 87%; white solid; $^1$H NMR (500 MHz, D$_2$O) δ 5.70 (s, 1H), 5.39 (t, $J = 5.9$ Hz, 1H), 4.30 (d, $J = 6.7$ Hz, 2H), 2.82 (td, $J_{HP} = 12.4$ Hz, $J = 5.7$ Hz, 2H), 1.75 (s, 3H) 1.74 (s, 3H), 1.69 (s, 3H), 1.68 (s, 3H); $^{13}$C NMR (125 MHz, D$_2$O) δ 137.9, 132.7, 121.7, 121.5 (t, $J_{CP} = 7.7$ Hz), 80.4 (t, $J_{CP} = 127.1$ Hz), 62.4 (t, $J_{CP} = 5.5$ Hz), 30.3, 25.3, 25.1, 17.5, 17.4; $^{31}$P NMR (201 MHz, D$_2$O) δ 17.7; HRMS (ES$^-$, $m/z$) calcd for (M-H)$^-$ C$_{11}$H$_{21}$O$_7$P$_2$: 327.0763; found: 327.0780.

**Bisphosphonate ester 19.** Yield, 30%; colorless oil; $^1$H NMR (300 MHz, CDCl$_3$) δ 5.45 (t, $J = 6.9$ Hz, 1H), 5.33 (t, $J = 6.5$ Hz, 1H), 5.13–5.04 (m, 1H), 4.37 (d, $J = 6.5$ Hz, 2H), 4.30–4.16 (m, 8H), 2.88 (td, $J_{HP} = 14.2$ Hz, $J = 6.5$ Hz, 2H), 2.15–1.97 (m, 4H), 1.73 (s, 3H), 1.68 (s, 3H), 1.66 (s, 6H), 1.61 (s, 3H), 1.35 (t, $J = 6.9$ Hz, 6H), 1.34 (t, $J = 7.3$ Hz, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 139.7, 133.2, 131.6, 124.0, 120.8, 118.0 (t, $J_{CP} = 7.5$ Hz), 80.7 (t, $J_{CP} = 150.9$ Hz), 63.3 (t, $J_{CP} = 5.9$ Hz), 63.3 (t, $J_{CP} = 3.2$ Hz, 2C), 63.0 (t, $J_{CP} = 3.0$ Hz, 2C), 39.5, 30.2, 26.4, 26.0, 25.7, 18.1, 17.7, 16.5 (t, $J_{CP} = 3.2$ Hz, 4C), 16.5; $^{31}$P NMR (121 MHz, CDCl$_3$) δ 19.0; HRMS (ES$^+$, $m/z$) calcd for (M+Na)$^+$ C$_{24}$H$_{46}$O$_7$P$_2$Na: 531.2617; found: 531.2619.

**Bisphosphonate salt 20.** 2,4,6-Collidine (0.22 mL, 1.67 mmol) was added to an ice cold solution of bisphosphonate 19 (85 mg, 0.17 mmol) in CH$_2$Cl$_2$ (5 mL) followed by the addition of excess TMSBr (0.27 mL, 2.00 mmol). The reaction was allowed to warm slowly to rt and allowed to stir overnight. Once the reaction was complete based on analysis of the $^{31}$P NMR spectrum, the volatile materials were removed in vacuo. The
resulting residue was washed with toluene and concentrated repeatedly to remove any remaining TMSBr. It was treated with NaOH (0.27 mL 5M NaOH, 2 mL H2O) for 10 minutes and then the water was removed on a lyophilizer to obtain the crude salt. This material was precipitated from water by addition of acetone to obtain the desired product, the salt 20 as a white solid (77 mg, 94%): 1H NMR (500 MHz, D2O) δ 5.84 (s, 1H), 5.39 (t, J = 6.8 Hz, 1H), 5.22–5.17 (m, 1H), 4.17 (d, J = 7.0 Hz, 2H), 2.89–2.79 (m, 2H), 2.16–2.03 (m, 4H), 1.72 (s, 3H), 1.69 (s, 3H), 1.68 (s, 3H), 1.65 (s, 3H), 1.62 (s, 3H); 13C NMR (125 MHz, D2O) δ 140.6, 133.7, 131.4, 124.2, 123.1 (t, JCP = 6.4 Hz), 122.4, 82.4 (t, JCP = 134.7 Hz), 61.1 (t, JCP = 6.2 Hz), 39.1, 29.7, 25.9, 25.4, 25.0, 17.4, 17.1, 15.6; 31P NMR (201 MHz, D2O) δ 17.8; HRMS (ES−, m/z) calcd for (M−H)− C16H29O7P2: 395.1389; found: 395.1388.

Monophosphonate ether 21. Yield, 34%; colorless oil; 1H NMR (500 MHz, CDCl3) δ 5.10–5.05 (m, 1H), 4.20–4.12 (m, 4H), 3.75 (dd, JHP = 8.7 Hz, J = 1.9 Hz, 2H), 3.63–3.57 (m, 2H), 2.05–1.89 (m, 2H), 1.70–1.53 (m, 2H), 1.66 (s, 3H), 1.59 (s, 3H), 1.43–1.28 (m, 2H), 1.34 (t, J = 7.4 Hz, 6H), 1.23–1.12 (m, 1H), 0.90 (d, J = 6.7 Hz, 3H); 13C NMR (125 MHz, CDCl3) δ 130.1, 124.0, 71.2 (d, JCP = 11.5 Hz), 64.3 (d, JCP = 165.6 Hz), 61.4 (d, JCP = 5.7 Hz, 2C), 36.4, 35.7, 28.6, 24.9, 24.7, 18.7, 16.8, 15.7 (d, JCP = 5.4 Hz, 2C); 31P NMR (201 MHz, CDCl3) δ 21.0; HRMS (ES+, m/z) calcd for (M+H)+ C15H32O4P: 307.2038; found: 307.2044.

Bisphosphonate ether 22. Yield, 53%; colorless oil; 1H NMR (400 MHz, CDCl3) δ 5.12–5.05 (m, 1H), 4.30–4.20 (m, 8H), 3.91 (t, JHP = 17.6 Hz, 1H), 3.85–3.75 (m, 2H), 2.06–1.88 (m, 2H), 1.73–1.49 (m, 2H), 1.67 (s, 3H), 1.60 (s, 3H), 1.47–1.25 (m, 2H), 1.36 (t, J = 7.0 Hz, 12H), 1.23–1.12 (m, 1H), 0.91 (d, J = 6.7 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 130.1, 124.0, 71.2 (d, JCP = 11.5 Hz), 64.3 (d, JCP = 165.6 Hz), 61.4 (d, JCP = 5.7 Hz, 2C), 36.4, 35.7, 28.6, 24.9, 24.7, 18.7, 16.8, 15.7 (d, JCP = 5.4 Hz, 2C); 31P NMR (201 MHz, CDCl3) δ 21.0; HRMS (ES+, m/z) calcd for (M+H)+ C15H32O4P: 307.2038; found: 307.2044.
MHz, CDCl$_3$) δ 130.5, 124.3, 73.2 (t, $J_{CP} = 157.0$ Hz), 73.0 (t, $J_{CP} = 4.6$ Hz), 62.9 (t, $J_{CP} = 3.1$ Hz), 62.8 (t, $J_{CP} = 3.3$ Hz), 62.7 (t, $J_{CP} = 3.5$ Hz), 62.7 (t, $J_{CP} = 3.2$ Hz), 36.7, 36.4, 28.8, 25.2, 25.0, 19.0, 17.2, 16.1 (t, $J_{CP} = 3.6$ Hz, 2C), 16.0 (t, $J_{CP} = 3.1$ Hz, 2C); $^{31}$P NMR (121 MHz, CDCl$_3$) δ 15.8; HRMS (ES$^+$, $m/z$) calcd for (M+H)$^+$ C$_{19}$H$_{41}$O$_7$P$_2$: 443.2328; found: 443.2325.

**Tetraethyl bisphosphonate ether 23.** Yield, 45%; colorless oil; $^1$H NMR (400 MHz, CDCl$_3$) δ 5.46 (t, $J = 6.3$ Hz, 1H), 5.15–5.05 (m, 2H), 4.29–4.16 (m, 8H), 3.87–3.78 (m, 2H), 2.79 (td, $J_{HP} = 14.8$ Hz, $J = 6.6$ Hz, 2H), 2.14–1.90 (m, 6H), 1.76–1.52 (m, 4H), 1.68 (s, 3H), 1.65 (s, 3H), 1.60 (s, 3H), 1.60 (s, 3H), 1.40–1.30 (m, 15H), 1.22–1.12 (m, 1H), 0.89 (d, $J = 6.5$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 136.8, 131.3, 131.1, 124.9, 124.4, 117.8 (t, $J_{CP} = 7.9$ Hz), 81.0 (t, $J_{CP} = 150.4$ Hz), 84.7 (t, $J_{CP} = 5.5$ Hz), 63.3 (t, $J_{CP} = 2.9$ Hz), 63.3 (t, $J_{CP} = 3.6$ Hz), 62.9 (t, $J_{CP} = 3.2$ Hz), 62.9 (t, $J_{CP} = 4.3$ Hz), 40.0, 37.4 (2C), 29.9, 29.4, 26.7, 25.7 (2C), 25.5, 19.6, 17.7, 17.6, 16.6 (t, $J_{CP} = 3.3$ Hz, 2C), 16.5 (t, $J_{CP} = 3.3$ Hz, 2C), 16.4; $^{31}$P NMR (121 MHz, CDCl$_3$) δ 19.2; HRMS (ES$^+$, $m/z$) calcd for (M+H)$^+$ C$_{29}$H$_{57}$O$_7$P$_2$: 579.3580; found: 579.3573.

**Bisphosphonate salt 24.** Yield, 17%; white solid; $^1$H NMR (500 MHz, D$_2$O) δ 5.73 (t, $J = 5.5$ Hz, 1H), 5.28–5.22 (m, 2H), 3.85–3.73 (m, 2H), 2.87–2.75 (m, 2H), 2.20–1.95 (m, 6H), 1.71 (s, 3H), 1.70 (s, 3H), 1.67 (s, 3H), 1.65 (s, 3H), 1.64 (s, 3H), 1.61–1.45 (m, 2H), 1.44–1.31 (m, 2H), 1.22–1.12 (m, 1H), 0.89 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (125 MHz, D$_2$O) δ 136.0, 133.5, 133.1, 125.5, 124.9, 121.6, 80.0 (t, $J_{CP} = 126.5$ Hz), 64.9, 39.5, 37.3, 36.9, 29.4, 26.2, 25.0 (3C), 24.9, 19.2, 17.1, 17.0, 15.5; $^{31}$P NMR (201 MHz, D$_2$O) δ 17.7; HRMS (ES$^-$, $m/z$) calcd for (M-H)$^-$ C$_{21}$H$_{39}$O$_7$P$_2$: 465.2171; found: 465.2168.
FDPS and GGDPS enzyme assays. The enzymes FDPS and GGDPS were kindly provided by Dr. James E. Dunford. Both the FDPS and GGPDS assays were implemented with a method modified from Dunford et al. [2]. Enzymes were diluted to 2 µg/mL (10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM TCEP, and 5 µg/mL BSA) and pre incubated with inhibitors in the reaction buffer (50 mM Tris, pH 7.7, 2 mM MgCl₂, 0.5 mM TCEP, and 50 µg/mL BSA) for 10 min at room temperature. Both FDPS and GGDPS enzyme assay reactions were initiated by the simultaneous addition of either 10 µM GPP or 10 µM FPP and ¹⁴C-IPP and were allowed to proceed at 37 °C for 3 min and 15 min respectively, at which point no more than 20% of the substrate was used. Reactions were terminated by the addition of 200 µL saturated NaCl and isoprenoids were extracted with 1 mL saturated butanol. Incorporated ¹⁴C was detected by liquid scintillation counting.

References.


300 MHz $^1$H NMR Spectrum of Compound 8.
$\text{150} \quad \text{100} \quad \text{50} \quad \text{PPM}$

$\left(\text{EtO}\right)_2\text{PO}$

$\text{8}$

75 MHz $^{13}\text{C}$ NMR Spectrum of Compound 8.
300 MHz $^1$H NMR Spectrum of Compound 9.
75 MHz $^{13}$C NMR Spectrum of Compound 9.
300 MHz $^1$H NMR Spectrum of Compound 10.
75 MHz $^1$H NMR Spectrum of Compound 10.
300 MHz $^1$H NMR Spectrum of Compound 11.
75 MHz $^1$H NMR Spectrum of Compound 11.
$300 \text{ MHz } ^1\text{H NMR Spectrum of Compound 6.}$
125 MHz $^{13}$C NMR Spectrum of Compound 6.
300 MHz $^1$H NMR Spectrum of Compound 12.
75 MHz $^{13}\text{C}$ NMR Spectrum of Compound 12.
300 MHz $^1$H NMR Spectrum of Compound 13.
75 MHz $^{13}$C NMR Spectrum of Compound 13.
300 MHz $^1$H NMR Spectrum of Compound 14.
100 MHz $^{13}$C NMR Spectrum of Compound 14.
$400 \text{ MHz } ^1\text{H NMR Spectrum of Compound 15.}$
100 MHz $^{13}$C NMR Spectrum of Compound 15.
500 MHz $^1$H NMR Spectrum of Compound 16.
125 MHz $^{13}$C NMR Spectrum of Compound 16.
500 MHz $^1$H NMR Spectrum of Compound 17.
125 MHz $^{13}$C NMR Spectrum of Compound 17.
500 MHz $^1$H NMR Spectrum of Compound 18.
125 MHz $^{13}$C NMR Spectrum of Compound 18.
300 MHz $^1$H NMR Spectrum of Compound 19.
$^{13}$C NMR Spectrum of Compound 19.

$\text{(EtO)}_2P\text{P(OEt)}_2$
500 MHz $^1$H NMR Spectrum of Compound 20.
125 MHz $^{13}$C NMR Spectrum of Compound 20.
500 MHz $^1$H NMR Spectrum of Compound 21.
125 MHz $^{13}$C NMR Spectrum of Compound 21.
400 MHz $^1$H NMR Spectrum of Compound 22.
100 MHz $^1$H NMR Spectrum of Compound 22.
400 MHz $^1$H NMR Spectrum of Compound 23.
100 MHz $^{13}$C NMR Spectrum of Compound 23.
500 MHz $^1$H NMR Spectrum of Compound 24.
125 MHz $^{13}$C NMR Spectrum of Compound 24.