

# Supporting Information

## Chemistry and Biology of Macrolide Antiparasitic Agents

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**Table S1**

	<i>D. radiodurans</i>	<i>E. coli</i>	<i>H. marismortui</i>	<i>T. gondii</i>	<i>B. subtilis</i>
<b>Domain II of 23S rRNA</b>	<b>A764Dr</b>	<b>A751Ec</b>	<b>A844Hm</b>	<b>A673Tg</b>	<b>A798Bs</b>
	C765Dr	A752Ec	T845Hm	A674Tg	A799Bs
	A802Dr	A789Ec	A882Hm	G711Tg	A836Bs
	C803Dr	U790Ec	U883Hm	U712Tg	U837Bs
<b>Domain V of 23S rRNA</b>	A2041Dr	A	G	A	A
	<b>A2042Dr</b>	<b>A</b>	<b>A</b>	<b>A</b>	<b>A</b>
	<b>A2045Dr</b>	<b>A</b>	<b>A</b>	<b>A</b>	<b>A</b>
	<b>A2482Dr</b>	<b>A</b>	<b>A</b>	<b>A</b>	<b>A</b>
	<b>G2484Dr</b>	<b>G</b>	<b>G</b>	<b>G</b>	<b>G</b>
	C2565Dr	U	C	C	C
	U2588Dr	U	C	U	U
	C2589Dr	C	U	C	C
U2590Dr	C	G	C	C	
<b>L22</b>	Ile107	Met86		Phe97	Arg86
	Pro108	Pro87		Phe98	Pro87
	<b>Arg109</b>	<b>Arg88</b>		<b>Arg99</b>	<b>Arg88</b>
	<b>Ala110</b>	<b>Ala89</b>		<b>Ala100</b>	<b>Ala89</b>
	Arg111	Lys90		Arg101	Met90
	<b>Gly112</b>	<b>Gly91</b>		<b>Gly102</b>	<b>Gly91</b>
	Ser113	Arg92		Arg103	Arg92
	Ala114	Ala93		Met104	Ala93
	Asn115	Asp94		Asp105	Ser94
	Ile116	Arg95		Ile106	Gln95
Ile117	Ile96		Arg107	Ile96	
<b>L4</b>	Lys57	Lys57		Lys74	Arg62
	Met58	Lys58		Lys75	Lys63
	Tyr59	Pro59		Leu76	Pro64
		Trp60		Arg77	Trp65
	Gly60	Arg61		Gln78	Arg66
	<b>Gln61</b>	<b>Gln62</b>		<b>Gln79</b>	<b>Gln67</b>
	<b>Lys62</b>	<b>Lys63</b>		<b>Lys80</b>	<b>Lys68</b>
	<b>Gly63</b>	<b>Gly64</b>		<b>Gly81</b>	<b>Gly69</b>

**Table S1.** Multiple sequence alignment of selected residues from domains II and V of 23S rRNA and the ribosomal proteins L4 and L22 from *D. radiodurans*, *E. coli*, *H. harismortui*, *T. gondii* and *B. subtilis*. Residues that are completely conserved are in bold.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

**Erythromycin A 9-oxime propyl ether, 5.** Erythromycin A 9-oxime, **1a** (0.27  $\mu\text{mol}$ , 200 mg), 1-iodopropane (0.32  $\mu\text{mol}$ , 55 mg) and 1 M potassium *tert*-butoxide in THF (0.30  $\mu\text{mol}$ , 300  $\mu\text{L}$ ) in 1:1 mixture of THF and DMF (2 mL) yielded a white solid (48 mg, 22%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  5.08 (dd,  $J = 11.1, 2.1$  Hz, 1H), 4.90 (d,  $J = 4.8$  Hz, 1H), 4.47 (s, 1H), 4.40 (d,  $J = 7.2$  Hz, 1H), 4.02 (d,  $J = 8.4$  Hz, 1H), 4.01 – 3.98 (m, 1H), 3.97 – 3.92 (m, 2H), 3.72 – 3.63 (m, 2H), 3.66 (s, 1H), 3.56 (d,  $J = 7.4$  Hz, 1H), 3.47 (tdd,  $J = 11.8, 5.9, 1.7$  Hz, 1H), 3.40 (s, 1H), 3.30 (s, 3H), 3.20 (dd,  $J = 10.2, 7.2$  Hz, 1H), 3.11 (s, 1H), 3.00 (t,  $J = 9.5$  Hz, 1H), 2.89 (dq,  $J = 14.4, 7.1$  Hz, 1H), 2.63 (q,  $J = 7.2$  Hz, 1H), 2.41 (ddd,  $J = 12.3, 10.4, 3.9$  Hz, 1H), 2.34 (d,  $J = 15.1$  Hz, 1H), 2.26 (s, 6H), 2.24 (d,  $J = 10.2$  Hz, 1H), 2.04 (s, 1H), 2.01 – 1.94 (m, 1H), 1.89 (dq,  $J = 15.2, 7.5, 2.1$  Hz, 1H), 1.78 (s, 1H), 1.63 (ddd,  $J = 13.9, 7.1, 2.4$  Hz, 3H), 1.56 (td,  $J = 12.5, 7.7$  Hz, 3H), 1.45 (s, 3H), 1.50 – 1.39 (m, 1H), 1.28 (d,  $J = 6.2$  Hz, 3H), 1.22 (s, 3H), 1.20 (d,  $J = 6.1$  Hz, 3H), 1.16 (t,  $J = 7.2$  Hz, 6H), 1.10 (s, 2H), 1.08 (d,  $J = 7.5$  Hz, 3H), 1.00 (d,  $J = 7.0$  Hz, 3H), 0.91 (t,  $J = 7.4$  Hz, 3H), 0.82 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  175.29, 171.40, 103.04, 96.35, 83.17, 80.00, 78.10, 77.00, 75.64, 75.46, 74.32, 72.79, 71.08, 70.65, 68.89, 65.60, 49.58, 44.79, 40.38, 39.04, 37.82, 35.15, 32.89, 28.74, 27.13, 26.46, 22.40, 21.60, 21.54, 21.17, 18.80, 18.72, 16.30, 14.57, 10.78, 10.52, 9.28. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 791.5; found 761.6.

**Erythromycin A 9-oxime allyl ether, 6.** Erythromycin A 9-oxime, **1a** (0.27  $\mu\text{mol}$ , 200 mg), allyl iodide (0.4  $\mu\text{mol}$ , 67 mg) and 1 M potassium *tert*-butoxide in THF (0.30  $\mu\text{mol}$ , 300  $\mu\text{L}$ ) in 1:1 mixture of THF and DMF (2 mL) yielded a white solid (54 mg, 25%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  5.95 (ddd,  $J = 16.2, 11.0, 5.7$  Hz, 1H), 5.29 (dd,  $J = 17.2, 1.5$  Hz, 1H), 5.24 (dd,  $J = 10.5, 1.1$  Hz, 1H), 5.11 (dd,  $J = 11.0, 2.1$  Hz, 1H), 4.93 (d,  $J = 4.8$  Hz, 1H), 4.52 (d,  $J = 5.7$  Hz, 2H), 4.43 (d,  $J = 7.3$  Hz, 1H), 4.41 (s, 1H), 4.09 – 3.97 (m, 2H), 3.75 – 3.70 (m, 1H), 3.69 (s, 1H), 3.58 (d,  $J = 7.5$  Hz, 1H), 3.49 (tdd,  $J = 11.7, 6.8, 4.9$  Hz, 1H), 3.42 (s, 1H), 3.32 (s, 3H), 3.23 (dd,  $J = 10.2, 7.2$  Hz, 1H), 3.13 (s, 1H), 3.03 (t,  $J = 9.6$  Hz,

1H), 2.91 (dq,  $J = 14.3, 7.0$  Hz, 1H), 2.67 (q,  $J = 6.8$  Hz, 1H), 2.43 (ddd,  $J = 12.3, 10.4, 3.8$  Hz, 1H), 2.37 (d,  $J = 15.1$  Hz, 1H), 2.29 (s, 6H), 2.27 (d,  $J = 15.4$  Hz, 1H), 2.10 (s, 1H), 2.00 (p,  $J = 7.6$  Hz, 1H), 1.96 – 1.87 (m, 1H), 1.78 (s, 1H), 1.69 – 1.65 (m, 1H), 1.59 (dt,  $J = 11.9, 9.0$  Hz, 3H), 1.48 (s, 3H), 1.52 – 1.44 (m, 1H), 1.31 (d,  $J = 6.2$  Hz, 3H), 1.25 (s, 3H), 1.23 (d,  $J = 6.0$  Hz, 3H), 1.18 (t,  $J = 7.7$  Hz, 6H), 1.13 (s, 3H), 1.11 (d,  $J = 7.5$  Hz, 3H), 1.04 (d,  $J = 7.0$  Hz, 3H), 0.84 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  175.34, 171.84, 133.90, 118.15, 103.06, 96.35, 83.25, 79.99, 78.09, 77.01, 75.45, 74.81, 74.32, 72.78, 71.06, 70.59, 68.91, 65.60, 49.58, 44.79, 40.39, 39.13, 37.81, 35.13, 33.01, 28.73, 27.13, 26.60, 21.59, 21.53, 21.18, 18.74, 18.72, 16.33, 16.23, 14.63, 10.77, 9.27. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 789.5; found 789.5.

**Erythromycin A 9-oxime benzyl ether**, 7. Erythromycin A 9-oxime, **1a** (0.27  $\mu\text{mol}$ , 200 mg), benzyl bromide (0.32  $\mu\text{mol}$ , 55 mg) and 1 M potassium *tert*-butoxide in THF (0.30  $\mu\text{mol}$ , 300  $\mu\text{L}$ ) in 1:1 mixture of THF and DMF (2 mL) yielded a white solid (63 mg, 28%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.34 – 7.30 (m, 2H), 7.29 – 7.27 (m, 2H), 7.26 – 7.24 (m, 1H), 5.07 (dd,  $J = 11.0, 2.1$  Hz, 1H), 5.04 – 4.97 (m, 2H), 4.87 (d,  $J = 4.7$  Hz, 1H), 4.35 (d,  $J = 7.0$  Hz, 2H), 3.99 – 3.91 (m, 2H), 3.72 – 3.63 (m, 2H), 3.65 (s, 1H), 3.49 (d,  $J = 7.6$  Hz, 1H), 3.46 – 3.39 (m, 1H), 3.37 (s, 1H), 3.27 (s, 3H), 3.16 (dd,  $J = 10.3, 7.2$  Hz, 1H), 3.08 (s, 1H), 2.98 (t,  $J = 9.5$  Hz, 1H), 2.85 (dq,  $J = 14.2, 7.1$  Hz, 1H), 2.60 (q,  $J = 7.0$  Hz, 1H), 2.37 (ddd,  $J = 12.8, 10.7, 4.0$  Hz, 1H), 2.32 (d,  $J = 15.2$  Hz, 1H), 2.23 (s, 6H), 2.19 (d,  $J = 10.7$  Hz, 1H), 1.34 (s, 3H), 1.25 (d,  $J = 6.2$  Hz, 3H), 1.20 (s, 3H), 1.21 – 1.18 (m, 1H), 1.17 (d,  $J = 6.1$  Hz, 3H), 1.15 (d,  $J = 7.2$  Hz, 3H), 1.09 (d,  $J = 7.0$  Hz, 3H), 1.07 (s, 3H), 1.05 (d,  $J = 7.5$  Hz, 3H), 0.94 (d,  $J = 7.0$  Hz, 3H), 0.80 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  175.32, 172.36, 137.45, 128.61, 128.32, 128.07, 103.08, 96.39, 83.25, 80.03, 78.14, 76.96, 76.14, 75.29, 74.34, 72.79, 71.04, 70.57, 68.93, 65.63, 65.58, 49.59, 44.77, 40.40, 39.13, 37.82, 35.16, 33.08, 28.72, 27.08, 26.76, 21.62, 21.54, 21.20, 18.76, 18.72, 16.35, 16.26, 14.69, 10.79, 9.26. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 839.5; found 839.5.

***N9-benzyl azithromycin, 11.*** 9-Deoxo-9a-aza-homoerythromycin A, **8** (204  $\mu\text{mol}$ , 150 mg), benzylaldehyde (1020  $\mu\text{mol}$ , 108 mg), acetic acid (2041  $\mu\text{mol}$ , 123 mg) and sodium cyanoborohydride (306  $\mu\text{mol}$ , 19 mg) in DMF (1.5 mL) yielded a white solid (36 mg, 21%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  4.96 (d,  $J = 3.2$  Hz, 1H), 4.77 (d,  $J = 8.0$  Hz, 1H), 4.42 (d,  $J = 7.3$  Hz, 1H), 4.31 (s, 1H), 4.23 (dd,  $J = 5.7, 3.7$  Hz, 1H), 4.08 (dt,  $J = 15.4, 6.3$  Hz, 2H), 3.85 (s, 1H), 3.58 (d,  $J = 13.6$  Hz, 2H), 3.54 (d,  $J = 5.7$  Hz, 2H), 3.53 – 3.47 (m, 1H), 3.36 (d,  $J = 15.1$  Hz, 1H), 3.31 (s, 3H), 3.28 – 3.22 (m, 2H), 3.03 (s, 1H), 2.90 (d,  $J = 13.8$  Hz, 1H), 2.82 (dd,  $J = 13.2, 6.5$  Hz, 1H), 2.79 – 2.72 (m, 1H), 2.64 (s, 2H), 2.52 (dd,  $J = 21.0, 8.9$  Hz, 2H), 2.35 (d,  $J = 15.3$  Hz, 1H), 2.28 (s, 6H), 2.26 – 2.18 (m, 1H), 2.02 (s, 1H), 1.76 (s, 1H), 1.65 (d,  $J = 12.7$  Hz, 2H), 1.57 (dd,  $J = 15.2, 4.9$  Hz, 1H), 1.45 – 1.37 (m, 2H), 1.32 (d,  $J = 6.2$  Hz, 3H), 1.23 (s, 6H), 1.22 – 1.19 (m, 6H), 1.12 (d,  $J = 6.8$  Hz, 3H), 1.08 (d,  $J = 7.4$  Hz, 3H), 1.03 (s, 2H), 0.93 (s, 3H), 0.86 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  177.81, 139.76, 129.92, 129.63, 128.51, 128.25, 127.35, 127.06, 103.71, 96.22, 85.86, 78.95, 78.09, 78.01, 75.48, 74.93, 72.90, 70.93, 69.16, 66.02, 65.29, 57.96, 54.00, 53.56, 49.57, 45.77, 41.75, 41.27, 40.59, 40.52, 39.77, 37.97, 35.00, 29.41, 29.14, 22.18, 21.87, 21.67, 21.42, 21.32, 18.15, 16.77, 15.24, 11.50, 10.00. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 825.5; found 825.6.

***N9-benzyl amide azithromycin, 13.*** 9-Deoxo-9a-aza-homoerythromycin A, **8** (79  $\mu\text{mol}$ , 58 mg) and benzyl isocyanate (79  $\mu\text{mol}$ , 11 mg) in toluene (1.8 mL) yielded a white solid (24 mg, 35%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.35 – 7.28 (m, 4H), 7.27 – 7.22 (m, 1H), 5.03 (s, 1H), 5.00 (dd,  $J = 9.3, 2.7$  Hz, 1H), 4.84 (d,  $J = 4.3$  Hz, 1H), 4.45 (d,  $J = 7.3$  Hz, 1H), 4.45 – 4.43 (m, 1H), 4.34 (d,  $J = 10.9$  Hz, 1H), 4.08 (td,  $J = 12.7, 6.4$  Hz, 1H), 4.04 – 3.99 (m, 1H), 3.83 (s, 1H), 3.58 (dd,  $J = 9.5, 6.0$  Hz, 1H), 3.51 (d,  $J = 4.3$  Hz, 1H), 3.52 – 3.43 (m, 1H), 3.38 – 3.30 (m, 1H), 3.28 (s, 3H), 3.03 (d,  $J = 9.1$  Hz, 1H), 2.72 – 2.65 (m, 1H), 2.62 – 2.51 (m, 2H), 2.31 (s, 6H), 2.36 – 2.24 (m, 1H), 2.04 – 1.79 (m, 5H), 1.68 (dd,  $J = 12.9, 1.7$  Hz, 2H), 1.56 (dd,  $J = 15.1, 5.0$  Hz, 1H), 1.52 – 1.45 (m, 1H), 1.34 (d,  $J = 6.9$  Hz, 3H), 1.29 (d,

$J = 6.2$  Hz, 5H), 1.25 – 1.19 (m, 9H), 1.15 (s, 3H), 1.07 (d,  $J = 7.3$  Hz, 3H), 1.00 (d,  $J = 6.6$  Hz, 3H), 0.91 (t,  $J = 7.5$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  159.73, 139.35, 128.71, 127.68, 127.30, 104.94, 97.03, 79.33, 78.33, 77.67, 74.72, 72.92, 70.80, 69.56, 66.30, 64.78, 49.51, 46.47, 45.14, 41.09, 40.60, 35.19, 29.42, 28.23, 22.49, 21.65, 21.30, 21.23, 17.99, 17.50, 15.36, 12.75, 11.59, 10.53. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 868.6; found 868.6.

***C6-methoxyl N9-methyl azithromycin, 15.*** C6-Methoxyl 9-deoxo-9a-aza-homoerythromycin A, **14** (107  $\mu\text{mol}$ , 80 mg), 37% formaldehyde (534  $\mu\text{mol}$ , 48 mg), acetic acid (1068  $\mu\text{mol}$ , 61 mg) and sodium cyanoborohydride (160  $\mu\text{mol}$ , 10 mg) in DMF (1 mL) yielded a white solid (8 mg, 10%). ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 763.5; found 763.7.

***C6-methoxyl N9-propyl azithromycin, 16.*** C6-Methoxyl 9-deoxo-9a-aza-homoerythromycin A, **14** (107  $\mu\text{mol}$ , 80 mg), propionaldehyde (534  $\mu\text{mol}$ , 31 mg), acetic acid (1068  $\mu\text{mol}$ , 61 mg) and sodium cyanoborohydride (160  $\mu\text{mol}$ , 10 mg) in DMF (1 mL) yielded a white solid (17 mg, 20%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  4.98 (dd,  $J = 10.0, 2.3$  Hz, 1H), 4.95 (d,  $J = 4.4$  Hz, 1H), 4.47 (d,  $J = 7.1$  Hz, 1H), 4.05 (dt,  $J = 15.0, 6.1$  Hz, 1H), 3.97 (d,  $J = 8.2$  Hz, 1H), 3.87 (s, 1H), 3.71 (d,  $J = 6.3$  Hz, 1H), 3.52 (s, 1H), 3.54 – 3.48 (m, 1H), 3.37 – 3.32 (m, 3H), 3.30 (d,  $J = 17.5$  Hz, 2H), 3.25 (s, 3H), 3.21 (dd,  $J = 10.0, 7.4$  Hz, 1H), 3.03 (d,  $J = 8.7$  Hz, 2H), 2.94 (dd,  $J = 13.6, 6.7$  Hz, 1H), 2.84 (dt,  $J = 14.1, 7.1$  Hz, 1H), 2.70 (dd,  $J = 13.8, 4.5$  Hz, 1H), 2.63 (s, 1H), 2.46 (ddd,  $J = 22.5, 12.7, 4.9$  Hz, 3H), 2.34 (d,  $J = 15.0$  Hz, 1H), 2.31 – 2.24 (m, 9H), 2.16 (dd,  $J = 9.2, 8.3$  Hz, 3H), 2.03 – 1.99 (m, 1H), 1.97 – 1.83 (m, 5H), 1.71 (s, 1H), 1.64 (d,  $J = 11.5$  Hz, 1H), 1.59 (dd,  $J = 15.0, 4.9$  Hz, 1H), 1.51 – 1.45 (m, 1H), 1.42 – 1.36 (m, 6H), 1.30 (d,  $J = 6.3$  Hz, 3H), 1.25 (s, 6H), 1.22 (dd,  $J = 6.6, 2.9$  Hz, 9H), 1.12 (d,  $J = 8.8$  Hz, 6H), 1.05 (dd,  $J = 11.6, 7.2$  Hz, 6H), 0.91 – 0.87 (m, 6H), 0.82 (t,  $J = 7.2$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  176.84, 102.83, 102.72, 95.96, 80.69, 80.20, 78.95, 78.44, 78.04, 75.99, 72.93, 71.27, 70.30, 69.65, 68.82, 68.71, 65.97, 65.72, 65.55, 59.62, 59.10, 57.17, 53.89, 53.58, 50.76, 49.54, 45.58, 41.36, 40.53,

40.27, 36.14, 35.17, 31.94, 31.89, 30.67, 29.84, 29.40, 29.03, 28.37, 22.68, 22.29, 22.05, 21.95, 21.70, 21.62, 21.44, 21.01, 18.81, 18.66, 16.93, 16.07, 12.14, 11.91, 11.43, 11.31, 10.27, 9.46. ESI mass spectrum; Calcd (MH<sup>+</sup>) 763.5; found 763.7.

**C6-methoxyl N9-allyl azithromycin, 17.** C6-Methoxyl 9-deoxo-9a-aza-homoerythromycin A, **14** (107  $\mu$ mol, 80 mg), allyl acetate (5340  $\mu$ mol, 535 mg), TEA (282  $\mu$ mol, 29 mg) and Pd(PPh<sub>3</sub>)<sub>4</sub> (11  $\mu$ mol, 12 mg) yielded a white solid (29 mg, 23%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  5.82 (dddd,  $J$  = 15.5, 10.2, 6.7, 5.5 Hz, 1H), 5.10 (dd,  $J$  = 17.2, 1.7 Hz, 1H), 5.03 (d,  $J$  = 10.2 Hz, 1H), 4.96 (dd,  $J$  = 11.4, 3.6 Hz, 2H), 4.48 (d,  $J$  = 7.2 Hz, 1H), 4.11 (s, 1H), 4.05 (dq,  $J$  = 12.6, 6.3 Hz, 1H), 3.96 (dd,  $J$  = 8.4, 1.3 Hz, 1H), 3.73 (d,  $J$  = 6.3 Hz, 1H), 3.55 – 3.46 (m, 2H), 3.33 (s, 3H), 3.32 – 3.28 (m, 1H), 3.26 (s, 3H), 3.21 (dd,  $J$  = 10.3, 7.2 Hz, 2H), 3.15 (dd,  $J$  = 15.0, 6.7 Hz, 1H), 3.03 (t,  $J$  = 9.2 Hz, 1H), 2.98 (q,  $J$  = 6.9 Hz, 1H), 2.86 (p,  $J$  = 7.3 Hz, 1H), 2.74 (dd,  $J$  = 13.8, 4.8 Hz, 1H), 2.44 (ddd,  $J$  = 12.4, 10.5, 3.8 Hz, 1H), 2.34 (dd,  $J$  = 15.0, 1.1 Hz, 1H), 2.28 (s, 6H), 2.24 (d,  $J$  = 10.4 Hz, 1H), 2.18 – 2.12 (m, 2H), 2.03 (dd,  $J$  = 14.9, 6.8 Hz, 1H), 1.97 (dd,  $J$  = 13.8, 9.1 Hz, 1H), 1.86 (tdd,  $J$  = 15.3, 7.5, 2.5 Hz, 2H), 1.78 – 1.70 (m, 1H), 1.64 (ddd,  $J$  = 12.5, 3.6, 1.9 Hz, 1H), 1.60 (dd,  $J$  = 15.0, 4.9 Hz, 1H), 1.52 – 1.42 (m, 1H), 1.37 (s, 3H), 1.30 (d,  $J$  = 6.3 Hz, 3H), 1.23 (dd,  $J$  = 13.3, 6.4 Hz, 12H), 1.11 – 1.06 (m, 6H), 1.03 (d,  $J$  = 6.9 Hz, 3H), 0.88 (t,  $J$  = 7.5 Hz, 6H). <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  176.85, 137.88, 115.67, 102.76, 96.07, 80.49, 80.19, 79.08, 78.04, 77.57, 76.76, 76.04, 72.93, 71.24, 68.70, 66.00, 65.59, 59.80, 59.13, 56.70, 50.75, 49.55, 45.55, 40.51, 40.08, 36.18, 35.21, 29.84, 28.95, 28.91, 22.46, 22.17, 21.96, 21.69, 21.63, 18.68, 16.91, 16.17, 11.38, 9.89, 9.53. ESI mass spectrum; Calcd (MH<sup>+</sup>) 789.5; found 789.6.

**C6-methoxyl N9-benzyl azithromycin, 18.** C6-Methoxyl 9-deoxo-9a-aza-homoerythromycin A, **14** (134  $\mu$ mol, 100 mg), benzyl aldehyde (670  $\mu$ mol, 70 mg), acetic acid (1340  $\mu$ mol, 80 mg) and sodium cyanoborohydride (201  $\mu$ mol, 13 mg) in DMF (1.5 mL) yielded a white solid (26 mg, 23%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.34 (d,  $J$  = 7.2 Hz, 2H), 7.27 (t,  $J$  = 7.4 Hz, 2H), 7.18 (t,  $J$  = 7.3 Hz, 1H), 4.96 (d,

$J = 3.9$  Hz, 2H), 4.92 (dd,  $J = 10.0, 2.5$  Hz, 2H), 4.52 (d,  $J = 7.2$  Hz, 2H), 4.15 (s, 1H), 4.07 (dq,  $J = 9.0, 6.4$  Hz, 2H), 3.98 (d,  $J = 7.7$  Hz, 2H), 3.78 – 3.71 (m, 4H), 3.64 (d,  $J = 14.0$  Hz, 2H), 3.52 (ddd,  $J = 11.5, 7.2, 5.2$  Hz, 4H), 3.50 (s, 1H), 3.36 (s, 6H), 3.31 (d,  $J = 13.7$  Hz, 2H), 3.28 (s, 6H), 3.22 (dd,  $J = 10.3, 7.2$  Hz, 2H), 3.05 (d,  $J = 8.8$  Hz, 2H), 2.96 (q,  $J = 6.9$  Hz, 2H), 2.90 – 2.84 (m, 4H), 2.51 – 2.42 (m, 4H), 2.36 (dd,  $J = 15.1, 1.1$  Hz, 2H), 2.29 (s, 12H), 2.27 (s, 1H), 2.14 (d,  $J = 4.9$  Hz, 1H), 2.12 – 2.03 (m, 4H), 1.86 (s, 3H), 1.75 (dtd,  $J = 21.5, 7.4, 2.3$  Hz, 4H), 1.67 – 1.63 (m, 2H), 1.61 (dd,  $J = 15.1, 5.0$  Hz, 2H), 1.42 (s, 6H), 1.38 – 1.34 (m, 2H), 1.31 (d,  $J = 6.3$  Hz, 6H), 1.26 (s, 6H), 1.25 (d,  $J = 2.0$  Hz, 6H), 1.23 (d,  $J = 3.8$  Hz, 6H), 1.21 (d,  $J = 3.5$  Hz, 6H), 1.19 (s, 3H), 1.18 (s, 3H), 1.13 (dd,  $J = 14.7, 8.6$  Hz, 1H), 1.04 (d,  $J = 6.9$  Hz, 6H), 0.97 (dd,  $J = 16.7, 6.7$  Hz, 2H), 0.91 (d,  $J = 6.7$  Hz, 6H), 0.84 (t,  $J = 7.4$  Hz, 6H), 0.57 (d,  $J = 12.8$  Hz, 6H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  176.80, 141.51, 128.97, 128.19, 126.76, 102.83, 95.93, 80.97, 80.39, 78.79, 78.01, 76.99, 76.31, 72.95, 71.27, 68.69, 66.02, 65.57, 62.23, 59.28, 55.86, 55.69, 50.96, 49.56, 45.55, 40.54, 40.16, 36.06, 35.16, 30.97, 29.84, 29.03, 28.48, 22.66, 22.25, 22.22, 21.70, 21.64, 18.69, 16.14, 11.40, 9.48, 8.60. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 839.6; found 839.5.

***C6-methoxyl N9-ethyl amino azithromycin, 19.*** C6-Methoxyl 9-deoxo-9a-aza-homoerythromycin A, **14** (80  $\mu\text{mol}$ , 60 mg) and ethyl isocyanate (96  $\mu\text{mol}$ , 7 mg) in toluene (1.5 mL) and DMF (0.1 mL) yielded a white solid (42 mg, 64%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  5.03 (s, 1H), 4.91 (d,  $J = 4.5$  Hz, 1H), 4.43 (d,  $J = 7.2$  Hz, 1H), 4.01 (dq,  $J = 12.5, 6.2$  Hz, 1H), 3.89 (d,  $J = 9.0$  Hz, 1H), 3.67 (d,  $J = 6.4$  Hz, 1H), 3.63 (s, 1H), 3.48 (dq,  $J = 12.0, 6.0, 1.8$  Hz, 1H), 3.30 (s, 3H), 3.23 (s, 3H), 3.15 (dd,  $J = 10.2, 7.2$  Hz, 2H), 3.01 (t,  $J = 7.8$  Hz, 1H), 2.84 (s, 1H), 2.58 (s, 1H), 2.44 – 2.37 (m, 1H), 2.32 (d,  $J = 15.1$  Hz, 1H), 2.28 – 2.22 (m, 6H), 2.01 (s, 1H), 1.85 (ddd,  $J = 14.0, 8.2, 6.5$  Hz, 1H), 1.66 – 1.61 (m, 1H), 1.57 (dd,  $J = 15.1, 5.0$  Hz, 1H), 1.52 – 1.43 (m, 1H), 1.37 (s, 3H), 1.27 (d,  $J = 6.2$  Hz, 3H), 1.23 (s, 6H), 1.21 – 1.16 (m, 6H), 1.12 (t,  $J = 7.2$  Hz, 3H), 1.02 (s, 3H), 0.93 (d,  $J = 6.4$  Hz, 4H), 0.85 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  159.08, 102.65, 96.07, 80.56, 79.58, 78.00, 74.54, 72.85, 71.30, 68.65,



65.85, 65.43, 53.56, 50.99, 49.53, 45.33, 40.49, 35.95, 35.14, 29.03, 28.25, 22.17, 21.62, 20.63, 18.74, 17.08, 16.20, 15.64, 12.56, 11.18, 9.32. ESI mass spectrum; Calcd (MH<sup>+</sup>) 820.6; found 820.6.

**C6-methoxyl N9-benzyl amino azithromycin, 20.** C6-Methoxyl 9-deoxo-9a-aza-homoerythromycin A, **14** (80 μmol, 60 mg) and benzyl isocyanate (96 μmol, 13 mg) in toluene (1.5 mL) and DMF (0.1 mL) yielded a white solid (23 mg, 33%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 5.03 (s, 1H), 4.91 (d, *J* = 4.5 Hz, 1H), 4.43 (d, *J* = 7.2 Hz, 1H), 4.01 (dq, *J* = 12.5, 6.2 Hz, 1H), 3.89 (d, *J* = 9.0 Hz, 1H), 3.67 (d, *J* = 6.4 Hz, 1H), 3.63 (s, 1H), 3.52 – 3.42 (m, 1H), 3.30 (s, 3H), 3.23 (s, 3H), 3.15 (dd, *J* = 10.2, 7.2 Hz, 2H), 3.01 (t, *J* = 7.8 Hz, 1H), 2.84 (s, 1H), 2.58 (s, 1H), 2.44 – 2.37 (m, 1H), 2.32 (d, *J* = 15.1 Hz, 1H), 2.29 – 2.21 (m, 6H), 2.17 – 2.10 (m, 1H), 2.05 – 1.95 (m, 1H), 1.85 (dd, *J* = 12.4, 7.4 Hz, 1H), 1.66 – 1.61 (m, 1H), 1.57 (dd, *J* = 15.1, 5.0 Hz, 1H), 1.51 – 1.44 (m, 1H), 1.37 (s, 3H), 1.27 (d, *J* = 6.2 Hz, 3H), 1.23 (s, 6H), 1.19 (dd, *J* = 6.6, 3.6 Hz, 6H), 1.12 (t, *J* = 7.2 Hz, 3H), 1.02 (d, *J* = 3.5 Hz, 3H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.85 (t, *J* = 7.4 Hz, 3H). <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>) δ 139.41, 128.72, 128.07, 127.36, 102.70, 79.60, 78.02, 74.50, 72.88, 71.21, 68.67, 65.92, 65.59, 51.10, 49.56, 45.33, 40.53, 35.18, 29.06, 28.15, 22.17, 21.65, 20.60, 18.75, 17.06, 16.18, 12.63, 11.22, 9.44. ESI mass spectrum; Calcd (MH<sup>+</sup>) 882.6; found 882.7.

**C6-alloxyl N9-methyl azithromycin, 22.** C6-alloxyl 9-deoxo-9a-aza-homoerythromycin A, **21** (52 μmol, 40 mg) and 37 % formaldehyde (258 μmol, 8 mg), acetic acid (516 μmol, 31 mg) and sodium cyanoborohydride (52 μmol, 3 mg) in DMF (1 mL) yielded a white solid (7 mg, 18%). ESI mass spectrum; Calcd (MH<sup>+</sup>) 789.5; found 789.6.

**C6-alloxyl N9-propyl azithromycin, 23.** C6-alloxyl 9-deoxo-9a-aza-homoerythromycin A, **21** (52 μmol, 40 mg), propionaldehyde (258 μmol, 15 mg), acetic acid (516 μmol, 31 mg) and sodium cyanoborohydride (52 μmol, 3 mg) in DMF (1 mL) yielded a white solid (11 mg, 26%). <sup>1</sup>H NMR (500

MHz, CDCl<sub>3</sub>) δ 5.92 (dtd,  $J = 15.5, 10.3, 5.0$  Hz, 1H), 5.24 (dd,  $J = 17.3, 2.0$  Hz, 1H), 5.01 (dd,  $J = 10.5, 1.9$  Hz, 1H), 4.92 (d,  $J = 4.5$  Hz, 1H), 4.67 (dd,  $J = 10.7, 1.6$  Hz, 1H), 4.46 (d,  $J = 7.2$  Hz, 1H), 4.31 (d,  $J = 4.0$  Hz, 1H), 4.18 – 4.15 (m, 2H), 4.08 (td,  $J = 12.4, 6.2$  Hz, 1H), 3.79 (d,  $J = 7.6$  Hz, 1H), 3.51 (dd,  $J = 8.9, 6.2$  Hz, 1H), 3.30 (d,  $J = 6.3$  Hz, 5H), 3.24 (dd,  $J = 10.3, 7.3$  Hz, 1H), 3.08 (d,  $J = 5.8$  Hz, 1H), 3.03 (d,  $J = 9.4$  Hz, 1H), 2.84 – 2.78 (m, 1H), 2.53 (s, 3H), 2.50 – 2.41 (m, 3H), 2.32 (d,  $J = 14.9$  Hz, 2H), 2.27 (s, 6H), 2.14 (s, 3H), 1.98 (dd,  $J = 13.1, 3.4$  Hz, 2H), 1.87 (dd,  $J = 13.9, 6.3$  Hz, 3H), 1.79 (d,  $J = 12.5$  Hz, 3H), 1.63 (dd,  $J = 9.7, 2.8$  Hz, 2H), 1.59 – 1.54 (m, 2H), 1.32 (d,  $J = 6.3$  Hz, 3H), 1.23 (s, 3H), 1.21 (dd,  $J = 6.7, 3.3$  Hz, 6H), 1.15 – 1.12 (m, 6H), 1.09 (s, 3H), 0.93 – 0.87 (m, 6H), 0.84 (t,  $J = 7.3$  Hz, 3H). <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>) δ 208.98, 179.15, 137.21, 114.26, 102.80, 96.00, 81.38, 80.46, 78.84, 78.65, 78.37, 76.02, 73.03, 71.29, 68.85, 65.67, 63.21, 58.09, 55.85, 54.09, 52.79, 49.49, 49.24, 45.35, 42.53, 40.50, 40.06, 35.28, 32.08, 30.97, 29.06, 27.66, 21.91, 21.76, 21.66, 21.14, 20.49, 18.82, 18.40, 16.34, 15.53, 12.19, 11.29, 10.05, 6.99. ESI mass spectrum; Calcd (MH<sup>+</sup>) 817.6; found 817.5.

***C6-alloxy N9-allyl azithromycin, 24.*** C6-alloxy 9-deoxo-9a-aza-homoerythromycin A, **21** (52 μmol, 40 mg), allyl acetate (2581 μmol, 26 mg), TEA (136 μmol, 14 mg) and Pd(PPh<sub>3</sub>)<sub>4</sub> (5 μmol, 6 mg) yielded a white solid (14 mg, 34%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 5.93 (ddt,  $J = 22.4, 15.6, 5.2$  Hz, 1H), 5.75 (ddd,  $J = 18.0, 11.2, 4.2$  Hz, 1H), 5.33 – 5.18 (m, 1H), 5.11 (dd,  $J = 9.7, 8.0$  Hz, 1H), 5.02 (dd,  $J = 16.1, 5.7$  Hz, 1H), 4.97 – 4.89 (m, 1H), 4.65 (dt,  $J = 22.3, 11.1$  Hz, 1H), 4.56 – 4.41 (m, 1H), 4.31 (d,  $J = 3.6$  Hz, 1H), 4.08 – 4.21 (m, 4H), 4.05 – 3.95 (m, 1H), 3.79 (dd,  $J = 9.3, 6.9$  Hz, 1H), 3.50 (dt,  $J = 26.7, 11.2$  Hz, 1H), 3.38 (s, 3H), 3.30 – 3.18 (m, 3H), 3.11 (dd,  $J = 18.3, 11.5$  Hz, 1H), 3.04 (dd,  $J = 9.1, 5.9$  Hz, 1H), 2.99 – 2.88 (m, 1H), 2.86 – 2.76 (m, 1H), 2.65 – 2.55 (m, 1H), 2.54 (s, 3H), 2.50 – 2.39 (m, 1H), 2.37 – 2.30 (m, 1H), 2.30 – 2.21 (d, 6H), 2.16 (s, 3H), 2.05 – 1.97 (m, 3H), 1.88 (ddd,  $J = 14.4, 10.3, 1.8$  Hz, 4H), 1.71 (t,  $J = 14.7$  Hz, 3H), 1.66 – 1.58 (m, 2H), 1.58 – 1.44 (m, 2H), 1.34 (dd,  $J = 16.3, 10.0$  Hz, 3H), 1.24 (s, 3H), 1.22 (t,  $J = 7.0$  Hz, 3H), 1.11 – 1.01 (m, 6H), 0.92 (d,  $J = 6.7$  Hz, 3H), 0.88

(dd,  $J = 14.0, 6.6$  Hz, 6H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  209.98, 179.38, 138.11, 137.29, 136.60, 116.23, 115.24, 114.31, 102.70, 102.62, 96.80, 95.74, 81.45, 80.94, 80.15, 78.54, 78.39, 78.26, 78.02, 75.91, 75.60, 73.07, 72.89, 71.29, 68.82, 68.64, 66.07, 65.81, 65.69, 65.61, 64.04, 63.51, 57.52, 55.81, 55.16, 52.60, 49.52, 49.26, 45.52, 45.35, 42.95, 41.37, 40.51, 39.96, 35.52, 35.17, 32.08, 31.94, 30.94, 30.67, 29.84, 28.95, 27.36, 21.77, 21.71, 21.04, 20.86, 18.80, 18.48, 18.37, 16.50, 16.40, 15.53, 11.29, 9.83, 6.63. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 815.6; found 815.6.

***C6-alloxyl N9-benzyl azithromycin, 25.*** C6-alloxyl 9-deoxo-9a-aza-homoerythromycin A, **21** (52  $\mu\text{mol}$ , 40 mg), benzaldehyde (258  $\mu\text{mol}$ , 27 mg), acetic acid (516  $\mu\text{mol}$ , 31 mg) and sodium cyanoborohydride (52  $\mu\text{mol}$ , 3 mg) in DMF (1 mL) yielded a white solid (7 mg, 16%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.36 – 7.27 (m, 4H), 7.17 (t,  $J = 6.3$  Hz, 1H), 5.92 (ddd,  $J = 15.4, 10.0, 4.7$  Hz, 1H), 5.25 (d,  $J = 16.0$  Hz, 1H), 5.02 (d,  $J = 10.6$  Hz, 1H), 4.91 (d,  $J = 4.6$  Hz, 1H), 4.59 (d,  $J = 10.0$  Hz, 1H), 4.49 (d,  $J = 7.3$  Hz, 1H), 4.42 (d,  $J = 5.6$  Hz, 1H), 4.18 (dd,  $J = 16.1, 4.4$  Hz, 1H), 4.11 (dt,  $J = 15.6, 6.3$  Hz, 1H), 3.84 (d,  $J = 8.0$  Hz, 1H), 3.71 (d,  $J = 13.7$  Hz, 1H), 3.52 (dd,  $J = 9.7, 6.0$  Hz, 1H), 3.44 (d,  $J = 13.8$  Hz, 1H), 3.31 (s, 3H), 3.36 – 3.25 (m, 2H), 3.04 (d,  $J = 9.1$  Hz, 1H), 2.89 (dd,  $J = 13.4, 6.7$  Hz, 1H), 2.86 – 2.83 (m, 1H), 2.69 – 2.62 (m, 1H), 2.54 – 2.47 (m, 1H), 2.32 (d,  $J = 15.3$  Hz, 1H), 2.29 (s, 1H), 2.27 (s, 6H), 2.21 – 2.17 (m, 2H), 2.11 (d,  $J = 14.1$  Hz, 1H), 1.99 – 1.95 (m, 1H), 1.79 (dd,  $J = 13.9, 6.9$  Hz, 2H), 1.64 (d,  $J = 10.6$  Hz, 2H), 1.56 (dd,  $J = 15.1, 4.7$  Hz, 1H), 1.47 – 1.41 (m, 1H), 1.40 (s, 1H), 1.34 (d,  $J = 6.2$  Hz, 3H), 1.27 (s, 3H), 1.24 (s, 3H), 1.21 (d,  $J = 4.9$  Hz, 6H), 1.19 – 1.16 (m, 3H), 1.14 – 1.08 (m, 2H), 0.95 (t,  $J = 6.6$  Hz, 6H), 0.85 (t,  $J = 7.3$  Hz, 3H), 0.60 (s, 2H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  179.07, 140.60, 137.26, 129.16, 128.23, 126.85, 114.05, 103.12, 96.42, 81.31, 80.58, 79.24, 78.39, 78.26, 76.76, 76.03, 73.05, 71.21, 68.95, 65.86, 65.71, 63.18, 57.43, 55.79, 51.88, 49.48, 45.47, 42.48, 40.88, 40.47, 35.45, 29.84, 28.90, 27.23, 21.78, 21.63, 21.11, 20.06, 18.95, 18.27, 15.74, 15.55, 11.29, 10.20, 6.65. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 865.6; found 865.5.

**C6-alloxy N9-ethyl amide azithromycin, 26.** C6-alloxy 9-deoxo-9a-aza-homoerythromycin A, **21** (65  $\mu\text{mol}$ , 50 mg), and ethyl isocyanate (71  $\mu\text{mol}$ , 5 mg) in toluene (1 mL) yielded a white solid (7 mg, 13%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  5.84 (s, 1H), 5.17 (d,  $J = 17.2$  Hz, 1H), 5.09 (dd,  $J = 10.6, 1.6$  Hz, 2H), 4.90 (d,  $J = 4.4$  Hz, 1H), 4.68 (s, 1H), 4.48 (d,  $J = 7.2$  Hz, 1H), 4.13 (d,  $J = 12.8$  Hz, 1H), 4.04 (dq,  $J = 12.2, 6.0$  Hz, 1H), 3.89 (d,  $J = 8.7$  Hz, 1H), 3.79 (d,  $J = 6.0$  Hz, 1H), 3.72 (d,  $J = 6.4$  Hz, 1H), 3.67 – 3.63 (m, 1H), 3.50 (dtd,  $J = 12.5, 6.2, 4.3$  Hz, 1H), 3.31 (s, 3H), 3.33 – 3.23 (m, 2H), 3.19 (dd,  $J = 10.2, 7.2$  Hz, 3H), 3.05 – 2.99 (m, 1H), 2.94 (td,  $J = 13.8, 6.9$  Hz, 1H), 2.50 – 2.38 (m, 2H), 2.34 (d,  $J = 14.3$  Hz, 1H), 2.28 (s, 6H), 2.20 – 2.15 (m, 1H), 2.02 (s, 2H), 1.90 (ddd,  $J = 9.8, 6.1, 2.5$  Hz, 1H), 1.75 – 1.63 (m, 6H), 1.60 (dd,  $J = 15.0, 5.1$  Hz, 2H), 1.42 (d,  $J = 4.7$  Hz, 1H), 1.37 (s, 3H), 1.31 (d,  $J = 6.2$  Hz, 3H), 1.28 (d,  $J = 6.8$  Hz, 3H), 1.25 (s, 3H), 1.22 (d,  $J = 6.1$  Hz, 3H), 1.19 – 1.17 (m, 3H), 1.14 – 1.07 (m, 9H), 0.94 (d,  $J = 6.7$  Hz, 2H), 0.84 (t,  $J = 7.3$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  159.69, 135.40, 115.11, 102.53, 96.92, 80.36, 79.46, 78.05, 74.86, 72.88, 71.27, 68.65, 65.98, 65.65, 63.90, 49.56, 45.15, 40.43, 37.31, 35.84, 35.52, 30.98, 29.85, 28.78, 28.60, 21.82, 21.72, 21.64, 20.71, 18.94, 17.23, 16.79, 15.51, 12.65, 10.88. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 846.6; found 846.7.

**C6-alloxy N9-benzyl amide azithromycin, 27.** C6-alloxy 9-deoxo-9a-aza-homoerythromycin A, **21** (77  $\mu\text{mol}$ , 60 mg), and benzyl isocyanate (85  $\mu\text{mol}$ , 11 mg) in toluene (1 mL) yielded a white solid (6.5 mg, 9%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.34 – 7.28 (m, 4H), 7.25 – 7.23 (m, 1H), 5.86 (s, 1H), 5.20 (d,  $J = 16.6$  Hz, 1H), 5.11 (dd,  $J = 10.6, 1.6$  Hz, 1H), 5.04 (s, 1H), 4.90 (d,  $J = 4.4$  Hz, 1H), 4.49 (d,  $J = 7.2$  Hz, 1H), 4.47 – 4.44 (m, 1H), 4.42 – 4.31 (m, 1H), 4.28 (dd,  $J = 14.6, 4.5$  Hz, 1H), 4.17 (dd,  $J = 12.8, 4.4$  Hz, 1H), 4.05 (dt,  $J = 15.3, 6.2$  Hz, 1H), 3.92 (d,  $J = 11.0$  Hz, 1H), 3.80 (d,  $J = 6.0$  Hz, 1H), 3.71 (s, 1H), 3.50 (dd,  $J = 8.8, 6.0$  Hz, 1H), 3.32 (s, 3H), 3.29 – 3.22 (m, 1H), 3.19 (dd,  $J = 10.3, 7.2$  Hz, 1H), 3.03 (d,  $J = 9.2$  Hz, 1H), 2.96 (dd,  $J = 15.3, 8.4$  Hz, 1H), 2.45 – 2.38 (m, 1H), 2.35 (d,  $J = 14.4$  Hz, 1H), 2.27 (s, 6H), 2.31 – 2.23 (m, 6H), 2.17 (d,  $J = 3.0$  Hz, 1H), 1.96 (s, 1H), 1.92 (d,  $J = 2.0$  Hz, 1H), 1.91 – 1.87 (m, 1H), 1.86 (s, 1H), 1.65 (dd,  $J = 10.0, 2.9$  Hz, 1H), 1.60 (dd,  $J = 15.0, 5.1$  Hz, 1H), 1.50 (dd,  $J = 15.7, 9.6$

Hz, 2H), 1.35 (dt,  $J = 15.9, 5.5$  Hz, 9H), 1.25 (s, 3H), 1.21 (d,  $J = 6.0$  Hz, 3H), 1.19 (d,  $J = 7.6$  Hz, 3H), 1.13 (dt,  $J = 16.7, 6.1$  Hz, 9H), 1.03 (s, 1H), 0.88 (d,  $J = 6.7$  Hz, 3H), 0.84 (t,  $J = 7.3$  Hz, 3H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  139.41, 128.70, 127.77, 127.50, 127.31, 115.13, 102.51, 92.11, 79.50, 78.02, 74.85, 72.88, 71.23, 69.64, 68.63, 66.02, 65.68, 65.22, 63.74, 53.88, 49.55, 45.14, 42.01, 41.37, 40.43, 37.38, 35.52, 32.07, 31.93, 31.54, 30.66, 29.84, 29.39, 28.51, 28.36, 25.32, 21.71, 21.63, 21.30, 20.66, 18.96, 17.20, 16.78, 12.82, 10.86, 9.98. ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 908.6; found 908.6.

***N9-(p-methyl benzyl) azithromycin, 28.*** 9-Deoxo-9a-aza-homoerythromycin A, **8** (109  $\mu\text{mol}$ , 80 mg), *p*-tolualdehyde (871  $\mu\text{mol}$ , 105 mg), acetic acid (1633  $\mu\text{mol}$ , 98 mg) and sodium cyanoborohydride (163  $\mu\text{mol}$ , 10 mg) in DMF (2 mL) yielded a white solid (8 mg, 9%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.24 (d,  $J = 7.9$  Hz, 2H), 7.16 (dd,  $J = 12.4, 4.6$  Hz, 1H), 7.10 (t,  $J = 6.8$  Hz, 2H), 4.94 (d,  $J = 3.4$  Hz, 1H), 4.78 (d,  $J = 8.0$  Hz, 1H), 4.44 (d,  $J = 7.2$  Hz, 1H), 4.26 (dd,  $J = 5.5, 4.0$  Hz, 1H), 4.09 (dq,  $J = 12.4, 6.1$  Hz, 1H), 3.99 (s, 2H), 3.85 (s, 1H), 3.60 (d,  $J = 13.7$  Hz, 1H), 3.55 (dd,  $J = 15.5, 5.2$  Hz, 2H), 3.35 – 3.29 (m, 1H), 3.32 (s, 3H), 3.29 – 3.24 (m, 1H), 3.06 – 3.01 (m, 1H), 2.92 (d,  $J = 36.7$  Hz, 1H), 2.84 (d,  $J = 6.2$  Hz, 1H), 2.77 – 2.72 (m, 1H), 2.53 (t,  $J = 8.7$  Hz, 1H), 2.43 (d,  $J = 8.7$  Hz, 1H), 2.35 (d,  $J = 15.1$  Hz, 1H), 2.31 (s, 3H), 2.29 (s, 6H), 2.23 – 2.17 (m, 3H), 2.03 (d,  $J = 3.2$  Hz, 2H), 1.66 (d,  $J = 10.6$  Hz, 2H), 1.57 (dd,  $J = 15.2, 4.9$  Hz, 1H), 1.32 (d,  $J = 6.2$  Hz, 3H), 1.25 – 1.20 (m, 12H), 1.12 – 1.07 (m, 9H), 0.93 (m, 6H), 0.85 (t,  $J = 7.4$  Hz, 3H). ESI mass spectrum; Calcd ( $\text{MH}^+$ ) 839.6; found 839.6.

***N9-(p-fluorobenzyl) azithromycin, 29.*** 9-Deoxo-9a-aza-homoerythromycin A, **8** (109  $\mu\text{mol}$ , 80 mg), *p*-fluorobenzaldehyde (871  $\mu\text{mol}$ , 108 mg), acetic acid (1633  $\mu\text{mol}$ , 98 mg) and sodium cyanoborohydride (163  $\mu\text{mol}$ , 10 mg) in DMF (2 mL) yielded a white solid (9 mg, 10%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.32 (dd,  $J = 8.6, 5.5$  Hz, 2H), 7.25 – 7.21 (m, 1H), 7.02 – 6.94 (m, 2H), 4.94 (d,  $J = 4.1$  Hz, 1H), 4.75 (d,  $J = 8.0$  Hz, 1H), 4.43 (d,  $J = 7.2$  Hz, 1H), 4.22 (dd,  $J = 6.6, 3.7$  Hz, 1H), 4.14 (d,  $J = 3.0$  Hz, 1H), 4.08 (dt,  $J = 15.7, 6.3$  Hz, 1H), 3.83 (s, 1H), 3.54 (d,  $J = 5.6$  Hz, 1H), 3.52 (s, 1H), 3.31 (s, 2H), 3.36 –

3.23 (m, 3H), 3.03 (d,  $J = 9.4$  Hz, 1H), 2.89 – 2.76 (m, 2H), 2.56 – 2.41 (m, 2H), 2.35 (d,  $J = 15.2$  Hz, 1H), 2.29 (s, 3H), 2.27 (dd,  $J = 7.1, 4.1$  Hz, 2H), 2.23 – 2.16 (m, 2H), 2.00 (d,  $J = 10.0$  Hz, 1H), 1.76 (s, 1H), 1.69 – 1.63 (m, 2H), 1.57 (dd,  $J = 15.2, 5.0$  Hz, 1H), 1.39 (dd,  $J = 16.5, 11.4$  Hz, 2H), 1.31 (t,  $J = 6.0$  Hz, 3H), 1.26 – 1.22 (m, 6H), 1.21 (d,  $J = 6.1$  Hz, 3H), 1.10 (dd,  $J = 10.8, 5.4$  Hz, 6H), 0.91 (s, 3H), 0.87 (t,  $J = 7.4$  Hz, 3H). ESI mass spectrum; Calcd ( $MH^+$ ) 843.5; found 843.6.

***N9-(phenyl propyl) azithromycin, 30.*** 9-Deoxo-9a-aza-homoerythromycin A, **8** (109  $\mu\text{mol}$ , 80 mg), hydrocinnamaldehyde (544  $\mu\text{mol}$ , 73 mg), acetic acid (1088  $\mu\text{mol}$ , 65 mg) and sodium cyanoborohydride (163  $\mu\text{mol}$ , 10 mg) in DMF (2 mL) yielded a white solid (10 mg, 11%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.30 – 7.27 (m, 2H), 7.24 – 7.20 (m, 1H), 7.20 – 7.16 (m, 2H), 5.06 (t,  $J = 4.4$  Hz, 1H), 4.72 (d,  $J = 8.2$  Hz, 1H), 4.43 (d,  $J = 7.3$  Hz, 1H), 4.21 (dd,  $J = 5.2, 2.5$  Hz, 1H), 4.08 (tt,  $J = 12.5, 6.3$  Hz, 1H), 3.74 (s, 1H), 3.60 (d,  $J = 6.4$  Hz, 1H), 3.56 – 3.47 (m, 2H), 3.33 (s, 3H), 3.31 – 3.28 (m, 1H), 3.24 (dt,  $J = 13.9, 5.5$  Hz, 2H), 3.03 (t,  $J = 9.0$  Hz, 1H), 2.99 – 2.91 (m, 1H), 2.76 (dd,  $J = 13.5, 5.8$  Hz, 3H), 2.72 – 2.66 (m, 1H), 2.56 (t,  $J = 7.6$  Hz, 2H), 2.50 – 2.44 (m, 2H), 2.35 (d,  $J = 15.1$  Hz, 2H), 2.29 (s, 6H), 2.27 (dd,  $J = 8.6, 6.3$  Hz, 3H), 2.20 (d,  $J = 8.7$  Hz, 1H), 2.17 – 2.16 (m, 1H), 2.08 – 1.99 (m, 3H), 1.95 – 1.85 (m, 3H), 1.76 – 1.70 (m, 1H), 1.67 (d,  $J = 14.4$  Hz, 1H), 1.58 (dd,  $J = 15.2, 5.0$  Hz, 1H), 1.49 (ddd,  $J = 16.8, 6.3, 3.2$  Hz, 1H), 1.32 (dd,  $J = 6.3, 2.5$  Hz, 3H), 1.27 (s, 3H), 1.25 – 1.24 (m, 6H), 1.23 – 1.18 (m, 9H), 1.13 (d,  $J = 6.8$  Hz, 3H), 1.09 – 1.05 (m, 6H), 0.89 (t,  $J = 7.4$  Hz, 6H). ESI mass spectrum; Calcd ( $MH^+$ ) 853.6; found 853.7.

***N9-cyclohexyl azithromycin, 31.*** 9-Deoxo-9a-aza-homoerythromycin A, **8** (109  $\mu\text{mol}$ , 80 mg), cyclohexane carbaldehyde (544  $\mu\text{mol}$ , 61 mg), acetic acid (1088  $\mu\text{mol}$ , 65 mg) and sodium cyanoborohydride (163  $\mu\text{mol}$ , 10 mg) in DMF (2 mL) yielded a white solid (19 mg, 21%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  4.93 (d,  $J = 4.1$  Hz, 1H), 4.78 (d,  $J = 9.3$  Hz, 1H), 4.45 (d,  $J = 7.3$  Hz, 1H), 4.21 (dd,  $J = 6.4, 3.5$  Hz, 1H), 4.08 (dq,  $J = 12.5, 6.3$  Hz, 1H), 3.75 (s, 1H), 3.55 (d,  $J = 3.8$  Hz, 2H), 3.30 (s,

3H), 3.28 (dd,  $J = 5.4, 2.4$  Hz, 1H), 3.02 (s, 1H), 2.91 (s, 1H), 2.81 (d,  $J = 10.4$  Hz, 1H), 2.77 – 2.73 (m, 1H), 2.56 – 2.49 (m, 2H), 2.34 (d,  $J = 15.0$  Hz, 2H), 2.30 (s, 3H), 2.19 (d,  $J = 22.1$  Hz, 1H), 1.98 (s, 2H), 1.92 – 1.84 (m, 2H), 1.70 – 1.67 (m, 3H), 1.66 – 1.61 (m, 3H), 1.56 (dd,  $J = 15.2, 5.0$  Hz, 2H), 1.49 (ddd,  $J = 15.9, 10.3, 4.4$  Hz, 3H), 1.32 – 1.30 (m, 6H), 1.24 – 1.21 (m, 9H), 1.11 (s, 3H), 1.08 (d,  $J = 7.3$  Hz, 6H), 0.98 (d,  $J = 6.8$  Hz, 3H), 0.90 (t,  $J = 7.4$  Hz, 3H), 0.87 – 0.83 (m, 3H). ESI mass spectrum; Calcd (MH<sup>+</sup>) 831.6; found 831.7.

### Molecular modeling

23S RNA sequences of the *T. gondii* apicoplast ribosome and the *B. subtilis* ribosome, obtained from NCBI (GeneID: 1466597 and 1799971, respectively), were aligned with those of *D. radiodurans*, *E. coli*, *H. marismortui*, *B. subtilis* and *T. thermophilus* using ClustalW2<sup>1</sup>. Structures of twelve ribosomal RNA fragments (ca. 25 Å from azithromycin) of *T. gondii* were generated based on the structure of *D. radiodurans*<sup>2</sup> by base mutation, using the MOE software package and the results of multiple sequence alignment. Sequences of the apicoplast-encoded L4 and the nuclear-encoded homologue of bacterial and chloroplast L22 of *T. gondii* were also obtained from NCBI (GeneID; L4: 1466596, and L22: 7895696). Each sequence was aligned with homologues from *B. subtilis*, *E. coli*, *T. thermophilus*, and *D. radiodurans* using the MOE software package. Blosum62 substitution matrix and tree-based build-up methods were applied with other default parameters. A homology model of the *T. gondii* L4 was built based on the corresponding structure of *D. radiodurans*<sup>3</sup>. Ten intermediates were generated. The model with the highest score was selected, and then minimized using the Amber99 force-field and 0.5 RMS gradient. A homology model of L22 of *T. gondii* was also generated by the same procedure. These L4 and L22 models were then combined with the rRNA model. Lastly, the structures of **11-1** and **11-2**, based on that of azithromycin, were combined to generate the complete model. Since two azithromycin molecules (**AZ-1** and **AZ-2**) are present in the active site of the *T. gondii* rRNA co-crystal structure, two molecules of **11** (**11-1** and **11-2**) were used in model building. **11-1** (**AZ-1**) and **11-2** (**AZ-2**) are in

domain V and domain II of the 23S RNA subunit, respectively <sup>2</sup>. The combined model of *T. gondii* rRNA (twelve rRNA fragments, L4, L22, and **11-1/11-2**) was initially minimized to remove steric clashes using the Amber99 force-field and 0.1 RMS gradient. Constraints were applied in the initial minimization step by fixing potentials at the terminal nucleotide of each rRNA fragment as well as at **11-1/11-2** (except for the phenyl ring, C-8, N-9, and C-10). The final homology model of *T. gondii* rRNA with **11-1/11-2** was obtained from full minimization with 0.05 RMS gradient. The final homology model of *B. subtilis* rRNA with **11-1/11-2** was also obtained following the same procedure; GeneID of L4 and L22 of *B. subtilis* is 936690 and 938123, respectively. The final model of *D. radiodurans* rRNA with **11-1/11-2** was also obtained following the same procedure with solved rRNA structures (PDBID: 2ZJR and 1NWY).

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