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Structure−Activity Relationships of 9-Substituted-9-Dihydroerythromycin-Based Motilin Agonists: Optimizing for Potency and Safety

Simon J. Shaw,*[,†] Yue Chen,[†] Hao Zheng,[†] Hong Fu,[†] Mark A. Burlingame,[†] Saul Marquez,[†] Yong Li,[†] Mark Claypool,[‡] Christopher W. Carreras,[⊥,‡] William Crumb,[§] Dwight J. Hardy,[∥] David C. Myles,[†] and Yaoquan Liu[†]

[†]*Departments of Chemistry and* [‡] *Pharmacology, Kosan Biosciences, Inc., 3832 Bay Center Place, Hayward, California 94545,* [§] *Zenas Technologies LLC, 4609 Fairfield Street, Metairie, Louisiana 70006, and* [∥]*Clinical Microbiology Laboratories, University of Rochester Medical Center, Rochester, New York 14642-8710.* [⊥] *Current address: Ardelyx Inc., 34175 Ardenwood Boulevard, Fremont, CA 94555.*

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A series of 9-dihydro-9-acetamido-*N*-desmethyl-*N*-isopropyl erythromycin A analogues and related derivatives was generated as motilin agonists. The compounds were optimized for potency while showing both minimal antibacterial activity and hERG inhibition. As the substituent on the amide was increased in lipophilicity the potency and hERG inhibition increased, while polar groups lowered potency, without significantly impacting hERG inhibition. The *N*-methyl acetamide **7a** showed the optimal in vitro profile and was probed further by varying the chain length to the macrocycle as well as changing the macrocycle scaffold. **7a** remained the compound with the best in vitro properties.

Introduction

The 22-amino acid hormone motilin plays a critical role in the normal regulation of gastrointestinal (GI[a]) motility.[1,2] The activity of motilin is mediated through a G-protein coupled receptor found on smooth muscle and enteric neurons in the GI tract.[3−6] The macrolide erythromycin A (EryA) **1** is an agonist of the motilin receptor (EC_{50} ∼1 uM) (Figure 1);[1,7] it competes with labeled motilin for receptor sites in membrane preparations,[8] causes Ca^{2+} currents in whole cell systems expressing the receptor,[9,10] induces contractions in isolated GI smooth muscle,[8,11] and is an effective prokinetic in animal models which measure gastric emptying[12] and motility.[7,13] Indeed, both motilin and **1** stimulate gastric motility in patients with gastroparesis,[14,15] and clinical studies indicate that **1** has a stronger effect on gastric emptying than metoclopramide, domperidone, or cisapride and is capable of providing symptomatic relief to gastroparesis patients.[16,17] Nevertheless, the antibiotic activity of **1** is undesirable in a prokinetic agent that could require chronic use. Nonantibiotic erythromycin derived motilin agonists (often referred to as "motilides") have therefore been proposed for the treatment of GI motility disorders such as gastresophageal reflux disease (GERD) and gastroparesis.[18]

A number of efforts have been made to generate nonantibiotic, bioavailable motilides.[19−21] Of these, the motilide ABT-229 **2** displayed high potency in vitro but failed in clinical studies due to loss of efficacy upon repeated administration−a phenomenon known as tachyphylaxis that is mediated through a receptor desensitization mechanism.[22,23] While **2** was potent in in vitro muscle contractility assays[19] and promoted gastric emptying following a single administration,[24] it was ineffective when administered as repeated doses.[25−28] In vitro assays for tachyphylaxis were developed and indicated that agonist potency and tachyphylaxis were separable,[22,29,30] validating the potential for finding motilides that retain activity through multiple administrations.

In addition to agonist potency, tachyphylaxis, and antibiotic activity, we were also conscious of the ability of macrolides to block the hERG potassium channel found in the heart, potentially causing a fatal cardiac arrhythmia.[31−34] We therefore included hERG assessment as a regular part of the in vitro characterization of newly synthesized motilides.

During our initial investigation of the properties of simple derivatives of **1**, we learned that 9-dihydroerythromycin derivatives can be potent motilin agonists with dramatically reduced antibiotic activity.[35] In this class, the (9*S*)-9-dihydro-*N*-desmethyl-*N*-isopropylerythromycin **3** showed good potency without causing tachyphylaxis. However, **3** retains a weak antibacterial activity and blocks the hERG channel with an IC_{50} of less than 30 μM. Here we describe the effects of alkylating the 9-hydroxyl group as a route to lowering both the hERG inhibition and antibacterial activity without causing tachyphylaxis or having a deleterious effect on the motilin agonist activity.

Results and Discussion

In our initial attempts to develop an erythromycin-based motilin agonist, we had attempted to obtain rapid access to compounds without antibiotic activity and improved acid stability.[35] The (9*S*)-9-dihydroerythromycins were an ideal starting point because reduction of the 9-ketone improved the acid stability, increased motilin agonist potency, and greatly reduced antibiotic activity. It was found that the residual

*To whom correspondence should be addressed. Phone: (650)624-1423. Fax: (650)624-1423. E-mail: sshaw@rigel.com; simonjshaw@gmail.com. Current address: Rigel, Inc., 1180 Veterans Boulevard, South San Francisco, CA 94080.

[a] Abbreviations: GI, gastrointestinal; EryA, erythromycin A; hERG, human ether-a-go-go related gene; GERD, gastresophageal reflux disease; TMSE, trimethylsilylethyl; TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ELSD, evaporative light scattering detector; MIC, minimal growth inhibitory concentration.

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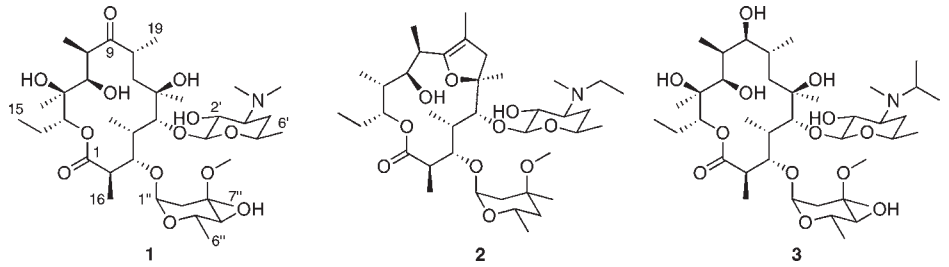
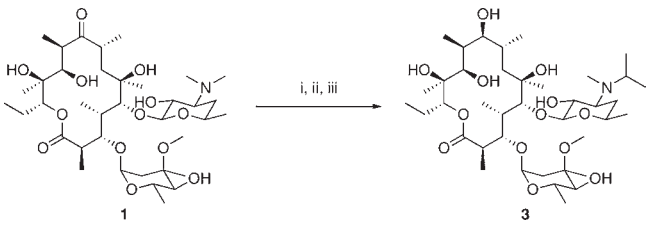


Figure 1. **1** Erythromycin A, **2** ABT-229, **3** 9-dihydro-*N*-desmethyl-*N*-isopropylerythromycin A.

Scheme 1. Synthesis of **3**[a]



[a] (i) $NaBH_4$, $^{i}PrOH \cdot Et_2O$, 0 °C to rt, 3 h; (ii) I_2, NaOAc, MeOH-H_2O, 50 °C, 4 h; (iii) $ICH(CH_3)_2$, $^{i}Pr_2NEt$, CH_3CN, 70 °C, 24 h.

antibiotic activity could be eliminated by replacing a methyl group on the desosamine with sterically larger alkyl group of which the isopropyl gave the optimal properties in terms of potency and ease of synthesis. Thus, (9*S*)-9-dihydro-*N*-desmethyl-*N*-isopropylerythromycin **3** was available in three steps (Scheme 1).

The 9-hydroxyl can be regioselectively alkylated under basic conditions.[36] It was considered that this would be an ideal position to introduce functional groups to tune both the hERG and antibacterial properties and potentially to modulate potency. We began our studies by investigating the feasibility of an acetamide addition. Both 2-chloroacetamide and 2-bromo-*N*, *N*-dimethylacetamide are commercially available. Thus, treatment of **3** with potassium *tert*-butoxide in the presence of either alkylating agent resulted in clean formation of the corresponding 9-*O*-acetamides, **4a** and **4b** (Scheme 2).

None of the mono-*N*-substituted substituted acetamides were commercially available, but they were readily synthesized from bromoacetyl bromide **5** and the corresponding amine in the presence of sodium bicarbonate (Scheme 3).[37,38]

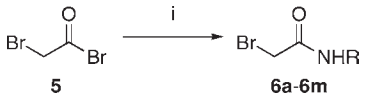
Treatment of **3** under similar alkylating conditions as in Scheme 2 furnished each of the 9-*O*-acetamide derivatives **7a**−**7m** with two exceptions. In the case of the methoxyamide **7g**, the trimethylsilylethyl (TMSE) ester was coupled,[39] which could be deprotected using tetrabutylammonium fluoride (TBAF) and methoxylamine coupled to the free acid. For the 2-hydroxyethylamide **7h**, the coupling was carried out with the hydroxyl protected as the *tert*-butyldimenthysilyl (TBDMS) ether,[40] which was later removed with TBAF.

Investigation of the ^{1}H NMR spectrum of each compound showed a 0.2−0.3 ppm downfield shift of the H-9 proton relative to **3**, suggesting that alkylation had occurred at this position. To confirm this observation, extensive NMR characterization was carried out with **7a**. The HMBC spectrum clearly shows a correlation between H-9 and the methylene carbon of the acetamide, as well as a correlation between the methylene protons of the acetamide and C-9 (see Supporting Information, Figure 7). In this way a series of 9-substituted acetamides was synthesized to probe the impact of the substituent on the nitrogen of the acetamide on the potency and other properties of the molecules (Scheme 4).

The compounds were initially tested for motilin agonist potency and antibacterial activity. Compounds that showed strong potency (EC_{50} < 150 nM) and low antibacterial activity were further tested for tachyphylaxis and inhibition of the hERG channel (Table 1).

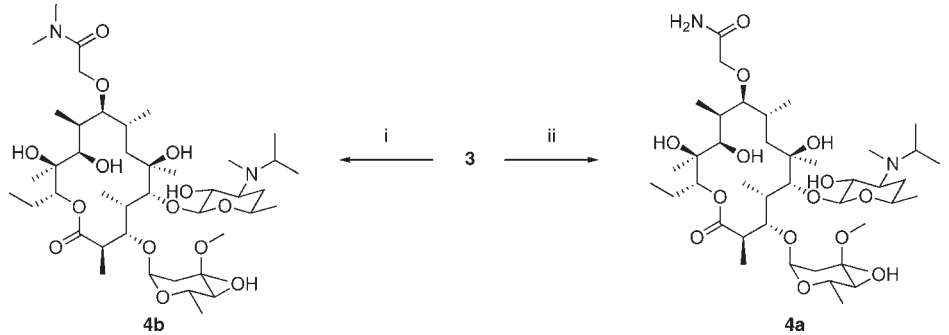
These experiments show that the 9-*O*-acetamides are, in general, a potent class of motilin agonists, with lower antibiotic activity than **3**. The dimethyl acetamide **4b** is a significantly weaker agonist than all the other compounds, suggesting a possible role for a free N−H bond or a steric requirement around the amide nitrogen. There is a trend toward lower potency as the lipophilicity of the side chain is increased (e.g., **7a** vs **7e**, **7f**, and **7i** vs **7h**), however, increasing lipophilicity also increases the hERG inhibition.

Scheme 3. Synthesis of Bromoacetamides **6a**−**6m**[a]



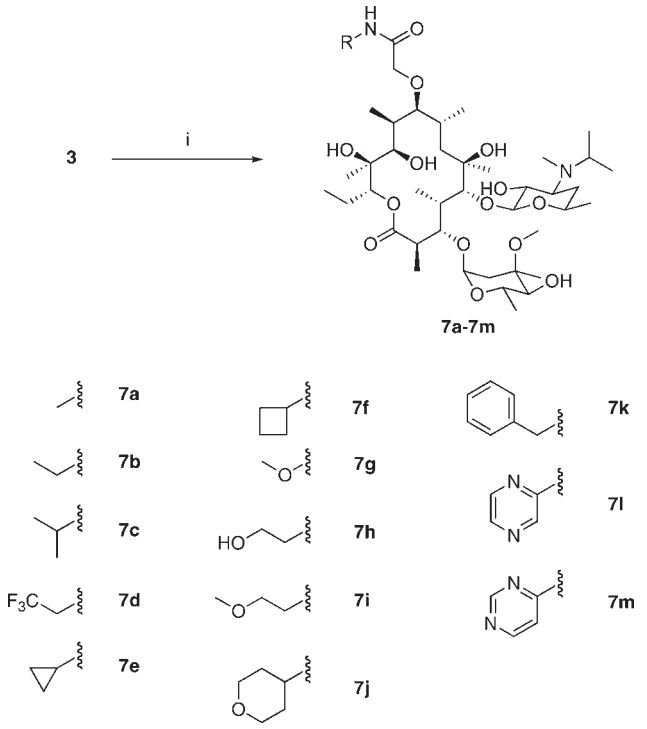
[a] (i) H_2NR, $NaHCO_3$, THF, 0 °C to rt, 16 h.

Scheme 2. Synthesis of 9-*O*-Acetamides **4a** and **4b**[a]



[a] (i) ^{t}BuOK, $ClCH_2CONH_2$, DME, 0 °C, 4 h; (ii) ^{t}BuOK, $BrCH_2CON(CH_3)_2$, DME, 0 °C, 2 h.

Scheme 4. Synthesis of 9-*O*-Acetamides **7a**−**7m**[a]



[a] (i) ^{t}BuOK, **6a**−**6m**, DME, rt, 2 h.

It is interesting to note the series of aromatic side chains **7k**, **7l**, and **7m** in which the introduction of heteroatoms into the ring attenuates the hERG inhibition while also improving potency. In general, as the compounds become more potent, there is more tachyphylaxis observed.

Of these compounds, the *N*-methyl acetamide **7a** stands out for its combination of potency and lack of hERG inhibition. To probe the methyl amide further, a series of *N*-methyl amides was synthesized to study the effect of increasing the distance between the amide and the macrolide as well as the effect of introducing rigidity in this region. An alkylation procedure similar to that described above was used. As before, the required alkylating agents could be readily synthesized from the commercially available ω-halo carboxylic acids and methylamine under standard 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) conditions.[41] A series of five compounds was synthesized in this way (**8a**−**8c**, **9**, and **10**, Figure 2, Table 2).

Table 1. In Vitro Assay Results for Compounds **4a**, **4b**, and **7a**−**7m**

compd	potency EC_{50} (nM)	tachyphylaxis %@ 4th dose	*Streptococcus pneumoniae* ATCC 6301 MIC (μg/mL)	hERG % inhibition @30 μM	hERG % inhibition @300 μM
1	1200	97 ± 1	0.0025	27	90
2	7	22 ± 10	64	98	100
3	260	85 ± 3	32	80	100
4a	52	92 ± 3	> 128	10	51
4b	660	95 ± 8	64	8	29
7a	58	89 ± 8	> 128	7	37
7b	67	89 ± 11	> 128	24	75
7c	140	nd[a]	128	26	89
7d	160	nd	128	30	82
7e	31	82 ± 4	128	23	60
7f	28	75 ± 1	128	64	100
7g	92	nd	nd	33	82
7h	120	87 ± 8	128	29	68
7i	50	nd	128	33	93
7j	120	88 ± 9	> 128	23	67
7k	79	nd	128	67	100
7l	69	nd	128	40	88
7m	25	63 ± 3	> 128	16	60

[a] nd = not determined.

Table 2. In Vitro Assay Results for Compounds **8a**−**8c**, **9**, and **10**

compd	potency EC_{50} (nM)	tachyphylaxis %@ 4th dose	*Streptococcus pneumoniae* ATCC 6301 MIC (μg/mL)	hERG % inhibition @30 μM	hERG % inhibition @300 μM
7a	58	89 ± 8	> 128	7	37
8a	84	79 ± 14	128	13	56
8b	270	nd[a]	128	3	45
8c	150	nd	128	13	61
9	2100	nd	128	63	100
10	1500	nd	nd	nd	nd

[a] nd = not determined.

The location of the amide is important for potency, suggesting that the amide is making a specific interaction with the receptor. As the chain length is increased, the potency decreases (e.g., **7a** vs **8b**). However, the five-carbon linker in **8c** is approximately 2-fold more potent than the four carbon linked compound **8b**. This effect may result from the increased flexibility of the five carbon side-chain in that it may allow

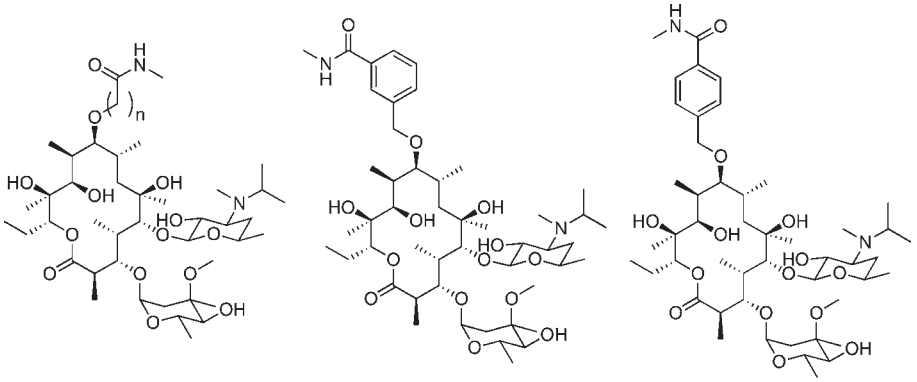
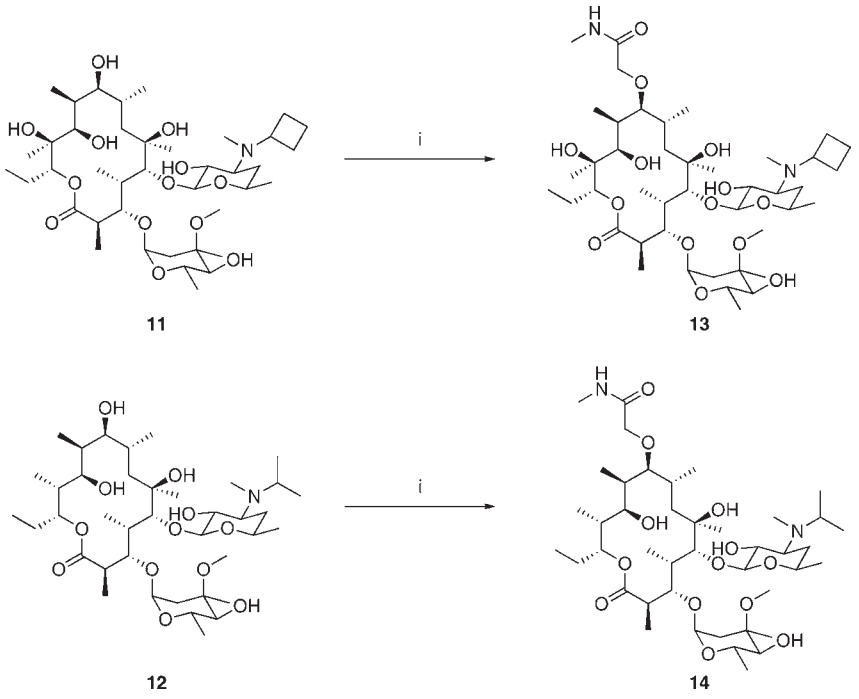


Figure 2. Methylamides with increased linkers, **8a**−**8c**, **9**, **10**.

Scheme 5. Synthesis *N*-Cyclobutyl and EryB Methylacetamides **13**, **14**[a]



[a] (i) ^{t}BuOK, **6a**, DME, rt, 2 h.

the amide to be positioned more favorably. Further investigation would be necessary to determine the significance of this effect. Both **9** and **10** are significantly less potent than their corresponding flexible compounds **8b** and **8c**, respectively. In addition, increasing the number of carbons in the side chain increases the amount of hERG inhibition observed, however, the increase is not as pronounced as when the carbons are introduced onto the nitrogen (e.g., **8a** vs **7f**).

In the in vitro assays, the *N*-methyl amide **7a** gave the most desirable combination of properties. Thus, it was decided to investigate transferring this group to other scaffolds. We selected the 9-dihydro-*N*-desmethyl-*N*-cyclobutylerythromycin A **11** and 9-dihydroerythromycin B **12** scaffolds. Again, alkylation proceeds under the standard conditions to obtain the *N*-cyclobutyl and erythromycin B derivatives **13** and **14**, respectively (Scheme 5).

The compounds were tested in the in vitro screens (Table 3). The *N*-cyclobutyl derivative **12** shows a 10-fold loss in potency relative to **7a** and increased inhibition of the hERG channel. By contrast, the erythromycin B derivative **14** is very similar to **7a** in terms of potency, however, the hERG inhibition is increased (Table 3).

Table 3. In Vitro Assay Results for Compounds **13** and **14**

compd	potency EC_{50} (nM)	tachyphylaxis %@ 4th dose	*Streptococcus pneumoniae* ATCC 6301 MIC (μg/mL)	hERG % inhibition @30 μM	hERG % inhibition @300 μM
7a	58	89 ± 8	128	7	37
13	510	nd[a]	> 128	12	53
14	90	86 ± 4	> 128	21	67

[a] nd = not determined

Conclusion

The 9-*O*-acetamides are potent motilin agonists. Furthermore, introduction of the acetamide eliminates antibiotic activity. The nature of the substituent on the acetamide impacts both the potency and the inhibition of the hERG channel. In general, as the lipophilicity of the amide is increased, the potency is increased as well as the hERG inhibition. Introducing polar groups moderates the hERG inhibition but also lowers the potency of the molecules as motilin agonists. Introducing carbons between the macrolide core and the amide group does not have as profound an effect on the hERG inhibition as does adding them to the amide. However, the potency of the compounds is lowered, suggesting that the amide may have a specific interaction with the motilin receptor. It appears that this interaction may require the amide N−H as the dimethylamide is 10-fold lower in potency relative to the corresponding monomethylamide (**4b** vs **7a**).

The methylacetamide **7a** has an optimal in vitro profile that predicts both prokinetic efficacy and safety. It is 20-fold more potent than **1** as a motilin agonist and shows no measurable tachyphylaxis. Furthermore, it has no detectable antimicrobial activity when tested against over 200 strains of clinically relevant bacteria (data not shown). Importantly, **7a** does not interact with the hERG channel at concentrations necessary for complete agonism of the motilin receptor. The motilin agonist EC_{50}:hERG blockade IC_{50} ratio of **7a**, used as an in vitro predictor of cardiovascular safety, is more than 200-fold improved relative to erythromycin A **1** (Table 4).

Table 4. Predicted Cardiovascular Safety Window of Benchmark Compounds

compd	motilin agonist potency EC_{50} (nM)	hERG current inhibition IC_{50} (uM)	hERG IC_{50}/ agonist EC_{50}	fold improvement vs **1**
EryA **1**	1200	39	32.5	
3	260	10	38	1.6
7a	58	411	7086	218

Table 5. ^{1}H NMR Resonances for Macrolide Portion of Compounds **3**, **4a**, **4b**, **7a**−**7i**[a]

	3	4a	4b	7a	7b	7c	7d	7e	7f	7g	7h	7i
H-2	2.80	2.64	2.67	2.65	2.67	2.72	2.70	2.71	2.72	2.64	2.68	2.69
H-3	4.22	4.00	4.02	4.00	4.06	4.05	4.03	4.02	4.25	4.04	3.98	4.08
H-4	1.89	1.82	1.94	1.81	1.82	1.84	1.84	1.81	1.85	1.85	1.80	1.85
H-5	3.73	3.52	3.56	3.56	3.56	3.58	3.57	3.57	3.88	3.59	3.55	3.57
H-7a	1.61	1.93	1.86	1.86	1.61	1.62	1.63	1.77	1.81	1.97	1.62	1.64
H-7b	1.34	1.24	1.30	1.24	1.35	1.25	1.23	1.25	1.26	1.25	1.25	1.26
H-8	2.14	2.54	2.47	2.45	2.46	2.41	2.46	2.39	2.43	2.54	2.44	2.59
H-9	2.81	3.22	3.00	3.17	3.16	3.12	3.18	3.10	3.13	3.23	3.16	3.17
H-10	1.98	2.15	2.07	2.15	2.12	2.14	2.16	2.11	2.15	2.16	2.12	2.17
H-11	3.73	3.88	3.70	3.84	3.86	3.84	3.85	3.80	3.58	3.88	3.76	3.86
H-13	4.88	4.85	5.02	4.83	4.82	4.83	4.81	4.83	4.84	4.83	4.85	4.86
H-14a	1.92	1.87	1.98	1.84	1.88	1.90	1.88	1.90	1.88	1.90	1.86	1.93
H-14b	1.49	1.48	1.44	1.47	1.48	1.51	1.52	1.49	1.50	1.53	1.46	1.51
H-15	0.88	0.88	0.92	0.87	0.88	0.88	0.90	0.88	0.90	0.92	0.86	0.91
H-1′	4.59	4.58	4.63	4.58	4.56	4.56	4.56	4.57	4.55	4.59	4.58	4.58
H-2′	3.21	3.20	3.26	3.20	3.21	3.21	3.22	3.21	3.21	3.27	3.20	3.23
H-3′	2.62	2.61	2.65	2.60	2.61	2.59	2.60	2.60	2.59	2.66	2.61	2.63
H-4′a	1.61	1.63	1.67	1.61	1.86	1.77	1.88	1.62	1.62	1.66	1.89	1.89
H-4′b	1.39	1.34	1.42	1.43	1.25	1.38	1.39	1.37	1.39	1.42	1.35	1.40
H-5′	3.58	3.58	3.61	3.57	3.54	3.57	3.55	3.55	3.54	3.60	3.57	3.57
NCH_3	2.21	2.19	2.24	2.18	2.19	2.19	2.21	2.20	2.20	2.34	2.19	2.22
NCH	2.91	2.89	2.92	2.87	2.88	2.89	2.90	2.89	2.90	2.93	2.88	2.91
H-1″	5.02	5.03	5.01	5.05	5.02	5.02	5.03	5.04	5.02	5.06	5.07	5.03
H-2″a	2.39	2.38	2.42	2.39	2.38	2.39	2.39	2.40	2.39	2.42	2.40	2.41
H-2″b	1.60	1.59	1.61	1.56	1.55	1.57	1.57	1.57	1.56	1.58	1.58	1.58
H-4″	3.04	3.07	3.05	3.01	3.01	3.02	3.03	3.03	3.02	3.08	3.03	2.85
H-5″	4.03	4.00	4.03	3.97	4.03	4.00	4.00	3.99	4.00	4.01	4.01	4.02
NH		7.38		7.60	7.44	7.17	8.24	7.56	7.48	10.76	7.85	7.54
OCH_3	3.34	3.33	3.37	3.33	3.33	3.33	3.34	3.34	3.34	3.36	3.33	3.36
H-9′a		4.18	4.49	4.12	4.18	4.13	4.29	4.20	4.22	4.24	4.19	4.28
H-9′b		4.02	3.99	4.12	3.98	3.89	4.07	3.89	3.95	4.20	4.04	3.96

[a] H-16, H-17, H-18, H-19, H-20, H-21, H-6′, $NCH(C\underline{H}_3)_2$, H-6″, and H-7″ are between 1.25 and 1.00 ppm.

This compound was taken forward into pharmacokinetic and gastric emptying studies, which will be reported in due course.

Experimental Section

Unless otherwise noted, ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 K using a Bruker DRX 400 MHz spectrometer, where possible spectra were assigned using COSY, HSQC, and HMBC experiments. Infrared spectra were obtained using a Perkin-Elmer Spectrum One FTIR with an attenuated total reflectance accessory containing a zinc selenide plate. High resolution mass spectra were obtained by flow injection with manual peak-matching using an Applied Biosystems Mariner TOF spectrometer with a turbo-ion spray source. All final compounds were >95% pure by LC-MS when detecting by evaporative light scattering detection (ELSD) using a linear gradient of 15% (5 mM NH_4OAc in CH_3CN-MeOH [4:1]) in 5 mM NH_4OAc in water to 100% (5 mM NH_4OAc in CH_3CN-MeOH [4:1]) over 10 min using a 3 um, 4.6 mm × 150 mm Varian Metasil Basic or Phenomenex Luna C-18(2) column. All numbering is consistent with that shown in Figure 1.

Synthesis of (9*S*)-9-Dihydro-*N*-desmethyl-*N*-isopropylerythromycin A 3. Erythromycin A **1** (20.0 g, 27.3 mmol, 1.0 equiv) was dissolved in 2-propanol−ether (1:1 v/v, 400 mL) and cooled to 0 °C. Sodium borohydride (2.1 g, 54.5 mmol, 2.0 equiv) was added in two aliquots. The mixture was warmed to room temperature and stirred for 3 h. The excess borohydride was destroyed by the addition of phosphate buffer (1.0M, pH 6.0, approximately 50 mL) before adding triethanolamine (80 mL) and stirring at room temperature for 2 h. The organics were extracted with EtOAc (4 × 300 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Column chromatography (33% acetone−hexane, 1% triethylamine) yielded 9-dihydroerythromycin (17.2 g, 86%). A mixture of 9-dihydroerythromycin A (17.20 g, 23.4 mmol, 1.0 equiv) and sodium acetate (9.75 g, 119.0 mmol, 5.0 equiv) in methanol−water (4:1 v/v, 400 mL) was heated to 50 °C. Iodine (7.25 g, 28.6 mmol, 1.2 equiv) was added in two aliquots over 30 min. During the reaction, NaOH (3M, 7.9 mL) was added in small portions to maintain the pH > 8. Completion of the reaction was determined by TLC (typically 4 h). The majority of the solvent was removed under vacuum, and the residue extracted with EtOAc (3 × 100 mL). The combined organics were dried (Na_2SO_4) and concentrated under reduced pressure to obtain the demethylated product (15.6 g) as a yellow solid, which was used for next step without further purification. The crude 9-dihydro-*N*-desmethylerythromycin (2.50 g, 3.41 mmol), diisopropylethylamine (6.1 mL, 10 equiv), and 2-iodopropane (10.2 mL, 30 equiv) were dissolved in CH_3CN (50 mL) and heated to 70 °C for 24 h. Water (100 mL) and $NaHCO_3$ (150 mL) were added and the organics extracted with EtOAc (3 × 150 mL). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (silica, 25% acetone−hexane, 1% triethylamine) yielded **3** (1.80 g, 75% yield for 2 steps) as a white solid; ^{1}H NMR δ (Table 5). ^{13}C NMR δ 177.6, 103.0, 96.2, 83.7, 83.2, 79.3, 79.3, 78.3, 75.6, 75.1, 73.1, 70.8, 70.7, 69.7, 66.4, 62.7, 60.8, 52.9, 49.8, 45.2, 42.3, 37.1, 35.2, 34.8, 33.5, 32.4, 31.4, 25.9, 22.1, 22.0, 21.7, 21.5, 20.8, 20.2, 18.7, 16.8, 15.3, 14.8, 11.6, 9.6. *m*/*z*: 765 $[M + H]^+$.

General Procedure for the Synthesis of the Bromoacetamides 6a−6m. To a mixture of bromoacetyl bromide (1.0 equiv) and sodium bicarbonate (2.0 equiv) in tetrahydrofuran at −78 °C was added the amine (1.5 equiv). The solution was allowed to warm to room temperature over 14 h. The mixture was partitioned between EtOAc and $NaHCO_3$. The organics were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to yield the bromoacetamide, which was used without further purification.

Table 6. ^{13}C NMR Resonances for Macrolide Portion of Compounds **7a**−**7h**[a]

	7a	7b	7c	7d	7e	7f	7g	7h
C-1	177.4	177.5	177.6	177.7	177.6	177.6	177.5	177.8
C-2	44.0	44.1	44.2	44.2	44.2	44.2	44.1	44.2
C-3	77.3	77.5	77.7	77.5	77.8	78.0	77.3	77.3
C-4	43.6	43.2	42.7	43.2	44.2	44.1	43.7	43.5
C-5	84.4	84.4	84.1	84.4	84.1	84.2	85.2	84.2
C-6	75.8	75.8	75.7	75.9	75.7	75.8	76.0	75.7
C-7	38.3	37.9	37.5	37.8	37.7	37.5	38.5	37.8
C-8	32.2	31.9	31.7	32.0	32.0	31.6	32.3	32.3
C-9	94.3	94.0	94.0	94.2	94.1	93.9	94.9	93.6
C-10	32.8	32.5	32.2	32.5	32.3	32.1	33.0	32.4
C-11	70.0	70.0	69.8	69.9	69.4	69.4	70.0	69.8
C-12	74.4	74.3	74.1	74.5	74.1	74.1	74.9	74.4
C-13	77.7	77.5	77.5	77.6	77.3	77.3	77.6	77.3
C-14	22.2	22.1	22.0	22.6	22.0	22.0	22.4	22.4
C-15	11.4	11.3	11.3	11.3	11.3	11.3	11.4	11.2
C-16	12.9	13.2	13.5	13.3	13.4	13.6	12.9	13.2
C-17	9.3	9.3	9.2	9.2	9.1	9.2	9.5	9.1
C-19	16.8	16.8	16.8	16.9	16.7	16.8	16.9	16.9
C-20	14.5	14.7	15.4	14.9	15.1	15.5	14.5	15.1
C-1′	102.1	102.3	102.3	102.4	102.3	102.4	102.5	102.1
C-2′	70.0	70.0	70.1	70.0	70.4	70.1	70.2	70.0
C-3′	62.1	62.2	62.3	62.2	62.2	62.4	62.3	62.1
C-4′	32.8	32.9	32.9	32.9	32.9	32.9	33.0	32.8
C-5′	69.5	69.4	69.4	69.4	68.8	70.0	69.7	69.5
NCH_3	31.0	31.0	31.0	31.0	31.0	31.0	31.2	31.1
NCH	52.7	52.6	53.0	52.7	52.6	52.6	53.0	52.7
C-1″	94.6	94.8	95.0	94.9	94.9	95.1	94.9	94.7
C-2″	34.5	34.6	34.6	34.6	34.6	34.7	34.6	34.5
C-3″	72.8	72.1	72.8	71.9	72.8	72.8	72.7	72.8
C-4″	77.3	77.8	77.8	77.7	77.3	77.5	77.7	77.7
C-5″	65.6	65.6	65.6	65.7	65.7	65.6	65.8	65.8
C-6″	17.5	17.7	17.9	17.6	17.8	17.9	17.7	17.6
OCH_3	49.3	49.3	49.3	49.3	49.3	49.3	49.4	49.3
C-9′	72.8	72.8	71.5	72.8	71.9	71.3	72.9	72.1
C-9″	170.4	169.5	168.7	170.7	171.2	168.7	166.7	170.7

[a] C-18, C-21, C-6′, $NCH(\underline{C}H_3)_2$ and C-7″ are between 22.0 and 19.0 ppm.

General Procedure of the Synthesis of the 9-*O*-Acetamides. To a solution of 9-dihydro-*N*-desmethyl-*N*-isopropylerythromycin A **3** (0.100 g, 0.313 mmol, 1.0 equiv) in dimethoxyethane (1.0 mL) was added potassium *tert*-butoxide (0.20 mL of a 1.0 M solution in tetrahydrofuran, 0.196 mmol, 1.5 equiv). The solution was stirred at room temperature for 10 min before adding a solution of the haloacetamide (0.157 mmol in 0.5 mL of dimethoxyethane, 1.2 equiv). The solution was stirred at room temperature for 2 h before adding $NaHCO_3$ (20 mL). The organics were extracted with EtOAc (3 × 20 mL), combined, dried ($MgSO_4$), and concentrated under reduced pressure. Column chromatography (silica, 30 → 60% acetone−hexane, 0.1% triethylamine) yielded the 9-*O*-acetamide as a white solid.

4a: Using the general procedure on a 0.362 mmol scale yielded **4a** (0.220 g, 73%) as a white solid; ^{1}H NMR δ (Table 5). ^{13}C NMR δ 177.7, 173.2, 102.6, 95.1, 94.7, 85.4, 78.0, 77.7, 76.3, 74.6, 73.3, 72.3, 70.7, 70.4, 69.9, 66.1, 62.5, 53.1, 49.7, 44.4, 44.0, 38.8, 35.0, 33.3, 33.1, 31.5, 22.7, 22.3, 21.9, 21.6, 21.5, 20.8, 19.9, 18.2, 17.4, 15.2, 13.4, 11.8, 9.7. *m*/*z*: 822 $[M + H]^+$, 663. *m*/*z*: 822.0 $[M + H]^+$ (found $[M + H]^+$, 821.5385; $C_{41}H_{77}N_2O_{14}$ requires $[M + H]^+$ 821.5369).

4b: Using the general procedure on a 0.362 mmol scale yielded **4b** (0.212 g, 69%) as a white solid; ^{1}H NMR δ (Table 5) 2.97 (3H, s, 1 × NCH_3), 2.87 (3H, s, 1 × NCH_3). ^{13}C NMR δ 176.9, 170.1, 102.4, 95.3, 93.3, 85.5, 78.2, 78.1, 77.9, 75.2, 74.0, 73.4, 70.5, 69.8, 69.6, 68.2, 65.8, 62.6, 53.0, 49.7, 44.4, 43.8, 36.9, 35.8, 35.6, 35.0, 33.3, 32.7, 32.1, 31.5, 31.4, 23.3, 22.8, 21.9, 21.7, 21.5, 20.8, 19.0, 18.1, 17.4, 16.5, 13.7, 12.0, 9.5. *m*/*z*: 850 $[M + H]^+$ (found $[M + H]^+$, 849.5673; $C_{43}H_{80}N_2O_{14}$ requires $[M + H]^+$ 849.5682).

7a: Using the general procedure on a 0.131 mmol scale yielded **7a** (0.067 g, 61%) as a white solid. IR (film) 3394, 2973, 2937, 1713, 1674, 1547, 1362, 1179, 1087, 1056, 1029, 997 cm^{-1}. ^{1}H NMR δ (Table 5) 2.75 (3H, d, *J* 5.0 Hz, NCH_3). ^{13}C NMR δ (Table 6) 25.6. *m*/*z*: 836 $[M + H]^+$, 678 (found $[M + H]^+$, 835.5498; $C_{42}H_{78}N_2O_{14}$ requires $[M + H]^+$ 835.5526).

7b: Using the general procedure on a 0.131 mmol scale yielded **7b** (0.072 g, 65%) as a white solid. IR (film) 3421, 2973, 2936, 1732, 1661, 1539, 1451, 1378, 1179, 1084, 1054, 1029, 998 cm^{-1}. ^{1}H NMR δ (Table 5) 3.26 (2H, m, NCH_2CH_3), 1.22 (3H, s, NCH_2CH_3). ^{13}C NMR δ (Table 6) 33.8, 15.0. *m*/*z*: 850 $[M+H]^+$, 692 (found $[M+H]^+$, 849.5698; $C_{43}H_{80}N_2O_{14}$ requires $[M+H]^+$ 849.5682).

7c:Using the general procedure on a 0.131 mmol scale yielded **7c** (0.051 g, 45%) as a white solid. IR (film) 3458, 2971, 1737, 1660, 1538, 1455, 1366, 1230, 1176, 1085, 1054, 998 cm^{-1}. ^{1}H NMR δ (Table 5) 4.07 (1H, m, $NHCH(CH_3)_2$), 1.17 (6H, m, $NHCH(CH_3)_2$). ^{13}C NMR δ (Table 6) 40.9, 21.3. *m*/*z*: 864 $[M + H]^+$, 706 (found $[M + H]^+$, 863.5818; $C_{44}H_{82}N_2O_{14}$ requires $[M + H]^+$ 863.5839).

7d: Using the general procedure on a 0.038 mmol scale yielded **7d** (0.019 g, 45%) as a white solid. IR (film) 3438, 2972, 2936, 1731, 1691, 1535, 1452, 1380, 1163, 1085, 1055, 997, 900, 864 cm^{-1}. ^{1}H NMR δ (Table 5) 3.88 (2H, m, $NHCH_2CF_3$). *m*/*z*: 904 $[M + H]^+$, 746 (found $[M + H]^+$, 903.5385; $C_{43}H_{77}N_2O_{14}F_3$ requires $[M + H]^+$ 903.5400).

7e: Using the general procedure on a 0.131 mmol scale yielded **7e** (0.063 g, 56%) as a white solid. ^{1}H NMR δ (Table 5) 2.71 (1H, m, NHC<u>H</u>), 0.73 (2H,m, 2H of CH_2CH_2), 0.57 (2H, m, 2H of CH_2CH_2). ^{13}C NMR δ (Table 6) 22.1, 6.0 (2C). *m*/*z*: 862 $[M + H]^+$, 703 (found $[M + H]^+$, 861.5695; $C_{44}H_{80}N_2O_{14}$ requires $[M + H]^+$ 861.5682).

7f: Using the general procedure on a 0.131 mmol scale yielded **7f** (0.058 g, 51%) as a white solid. IR (film) 3422, 2972, 2937, 1732, 1660, 1537, 1451, 1379, 1179, 1085, 1054, 1028, 998, 900, 863 cm^{-1}. ^{1}H NMR δ (Table 5) 4.38 (1H, app sextet, *J* 8.0 Hz, NCH), 2.28 (2H, m, 1 × H-2cyb and 1 × H-4cyb), 1.94 (2H, m, 1 × H-2cyb and 1 × H-4cyb), 1.69 (2H, m, 2 × H-3cyb). ^{13}C NMR δ (Table 6) 44.1, 30.8, 30.7, 15.2. *m*/*z*: 876 $[M + H]^+$, 718 (found $[M + H]^+$, 874.5833; $C_{45}H_{82}N_2O_{14}$ requires $[M + H]^+$ 874.5839).

7g: Using the general procedure on a 0.085 mmol scale with bromoacetic acid 2-(trimethylsilyl)ethyl ether yielded the 9-*O*-acetic acid-2-(trimethylsilyl)ether ester (0.045 g, 57%), which was dissolved in dimethylformamide (1.0 mL) and cooled to 0 °C before adding TBAF (0.015 g, 0.059 mmol, 1.2 equiv). The solution was stirred at 0 °C for 5 h before EDCI (0.014 g, 0.074 mmol, 1.5 equiv), hydroxybenzotriazole (0.013 g, 0.098 mmol, 2.0 equiv), and methoxylamine hydrochloride (0.008 g, 0.098 mmol, 2.0 equiv). The solution was stirred at room temperature for 18 h before diluting with EtOAc (15 mL) and washing with $NaHCO_3$ (15 mL) and brine (15 mL). The organics were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica, 30 → 50% acetone−hexane, 1% triethylamine) yielded **7g** (0.009 g, 22%) as a white solid. IR (film) 3457, 2971, 1733, 1684, 1456, 1380, 1179, 1083, 999, 899 cm^{-1}. ^{1}H NMR δ (Table 5) 3.76 (3H, s, CH_3). ^{13}C NMR δ (Table 6) 64.1. *m*/*z*: 852 $[M + H]^+$, 754 (found $[M + H]^+$, 851.5490; $C_{42}H_{78}N_2O_{15}$ requires $[M + H]^+$ 851.5475).

7h: Using the general procedure on a 0.131 mmol scale yielded 9-*O*-(2-*O*-*tert*-butyldimethylsilyl)ethanolylacetamide (0.101 g, 79%) as a white solid, which was dissolved in tetrahydrofuran (1.0 mL) and cooled to 0 °C. TBAF (0.020 g, 0.114 mmol, 1.1 equiv) was added and the solution stirred at 0 °C for 2.5 h before adding $NaHCO_3$ (15 mL). The organics were extracted with EtOAc (3 × 15 mL), combined, washed with brine (25 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography (silica, 55% acetone−hexane, 1%

Table 7. ^{1}H NMR Resonances for Macrolide Portion of Compounds **7j**−**7m**, **8a**−**8c**, **9**, **10**, **13**, and **14**[a]

	7j	7k	7l	7m	8a	8b	8c	9	10	13	14
H-2	2.72	2.66	2.72	2.71	2.67	2.68	2.66	2.69	2.62	2.69	2.57
H-3	4.05	4.02	4.00	4.00	3.92	3.94	3.94	3.99	4.00	3.99	4.05
H-4	1.77	1.82	1.82	1.80	1.80	1.79	1.79	1.80	1.82	1.70	1.87
H-5	3.56	3.56	3.56	3.55	3.54	3.60	3.61	3.52	3.58	3.53	3.54
H-7a	1.80	1.61	1.85	1.84	1.78	1.72	1.66	1.91	1.91	1.95	1.96
H-7b	1.27	1.37	1.27	1.23	1.20	1.13	1.18	1.21	1.22	1.26	1.36
H-8	2.39	2.55	2.52	2.51	2.40	2.40	2.40	2.66	2.61	2.50	2.48
H-9	3.10	3.16	3.21	3.19	3.08	3.05	3.02	3.25	3.23	3.18	3.26
H-10	2.12	2.14	2.20	2.25	2.05	2.02	2.02	2.09	2.12	1.96	2.02
H-11	3.84	3.83	3.89	3.87	3.77	3.72	3.73	3.79	3.86	3.86	3.62
H-13	4.77	4.79	4.90	4.91	4.92	4.94	4.93	5.01	4.91	4.82	5.00
H-14a	1.90	1.86	1.93	1.96	1.94	1.92	1.90	1.91	1.91	1.86	1.80
H-14b	1.49	1.43	1.40	1.52	1.44	1.46	1.44	1.44	1.46	1.56	1.39
H-15	0.87	0.87	0.92	0.93	0.90	0.89	0.89	0.88	0.91	0.87	0.80
H-1′	4.52	4.55	4.59	4.59	4.64	4.66	4.66	4.62	4.65	4.56	4.52
H-2′	3.19	3.21	3.25	3.24	3.23	3.22	3.22	3.23	3.25	3.22	3.17
H-3′	2.57	2.56	2.70	2.60	2.63	2.64	2.64	2.57	2.63	2.51	2.57
H-4′a	1.61	1.86	1.64	1.62	1.63	1.65	1.65	1.60	1.63	1.72	1.59
H-4′b	1.37	1.22	1.38	1.40	1.38	1.40	1.35	1.38	1.42	1.46	1.39
H-5′	3.52	3.53	3.54	3.54	3.62	3.62	3.61	3.55	3.60	3.53	3.54
NCH_3	2.18	2.19	2.23	2.21	2.22	2.21	2.21	2.20	2.22	2.02	2.15
NCH	2.88	2.88	2.93	2.89	2.96	2.90	2.89	2.88	2.91	3.07	2.84
H-1″	4.99	4.96	5.04	5.05	5.12	5.09	5.08	5.15	5.08	5.04	4.92
H-2″a	2.37	2.35	2.39	2.39	2.42	2.41	2.40	2.39	2.38	2.38	2.34
H-2″b	1.55	1.48	1.54	1.55	1.58	1.58	1.60	1.54	1.57	1.63	1.51
H-4″	3.01	2.96	2.95	2.94	3.04	3.04	3.04	2.91	3.00	3.00	2.96
H-5″	4.03	3.97	3.99	4.00	4.02	4.00	4.00	3.92	3.95	3.96	3.93
NH	7.55	7.88	9.73	9.86	6.47	5.88	5.75	6.86	6.18	7.56	7.85
OCH_3	3.32	3.32	3.34	3.34	3.36	3.36	3.35	3.34	3.35	3.32	3.29
H-9′a	4.24	4.27	4.53	4.51	3.66	3.47	3.74	4.92	4.90	4.13	4.11
H-9′b	3.89	4.06	4.01	4.00	3.52	3.40	3.33	4.41	4.45	4.08	3.90

[a] H-16, H-17, H-18, H-19, H-20, H-21, H-6′, $NCH(C\underline{H}_3)_2$, H-6″, and H-7″ are between 1.25 and 1.00 ppm.

triethylamine) yielded **7h** (0.063 mg, 70%) as a white solid. IR (film) 3423, 2972, 2936, 1732, 1661, 1542, 1451, 1379, 1179, 1082, 1053, 997, 899, 863 cm^{-1}. ^{1}H NMR δ (Table 5) 3.68 (2H, br t, J 5.0 Hz, $CH_2C\underline{H}_2OH$), 3.50−3.43 (1H, m, 1H of $C\underline{H}_2CH_2OH$), 3.37−3.31 (1H, m, 1H of $C\underline{H}_2CH_2OH$). ^{13}C NMR δ (Table 6) 62.0, 42.0. m/z: 866 $[M + H]^+$, 708 (found $[M+H]^+$, 865.5655; $C_{43}H_{80}N_2O_{15}$ requires $[M+H]^+$ 865.5632).

7i: Using the general procedure on a 0.131 mmol scale yielded **7i** as a white solid. ^{1}H NMR δ (Table 5) 3.47 (2H, m, 2H of OCH_2CH_2O), 3.41 (2H, m, 2H of OCH_2CH_2O), 3.36 (3H, s, OCH_3). m/z: 880 $[M+H]^+$, 722.

7j: Using the general procedure on a 0.131 mmol scale yielded **7j** (0.086 g, 73%) as a white solid. ^{1}H NMR δ (Table 7) 3.97 (1H, m, NHCH), 3.93 (1H, 2H of $CH(CH_2C\underline{H}_2)_2O$), 3.43 (1H, t, J 11.0 Hz, 2H of $CH(CH_2C\underline{H}_2)_2O$), 1.82 (2H, m, 2H of $CH(C\underline{H}_2CH_2)_2O$), 1.59 (2H, m, 2H of $CH(C\underline{H}_2CH_2)_2O$). ^{13}C NMR δ (Table 8) 45.2, 66.9, 32.8. m/z: 906 $[M + H]^+$, 748 (found $[M + H]^+$, 905.5957; $C_{46}H_{84}N_2O_{15}$ requires $[M + H]^+$ 905.5946).

7k: Using the general procedure on a 0.131 mmol scale yielded **7k** (0.083 g, 70%) as a white solid. IR (film) 3431, 2972, 2936, 1731, 1663, 1536, 1454, 1379, 1266, 1179, 1084, 1052, 1028, 997, 957, 899 cm^{-1}. ^{1}H NMR δ (Table 7) 7.33−7.23 (5H, m, ArH), 4.43 (2H, t, J 6.5 Hz, $PhCH_2$), 8.26 (1H, m, ArH-5). ^{13}C NMR δ (Table 8) 138.2, 128.5, 127.6, 127.6, 42.9. m/z: 912 $[M+H]^+$, 754 (found $[M + H]^+$, 911.5813; $C_{48}H_{82}N_2O_{14}$ requires $[M + H]^+$ 911.5839).

7l: Using the general procedure on a 0.079 mmol scale yielded **7ll** (0.025 g, 35%) as a white solid. IR (film) 3446, 2972, 2934, 1730, 1538, 1455, 1413, 1379, 1279, 1178, 1083, 1056, 998, 900 cm^{-1}. ^{1}H NMR δ (Table 7) 9.53 (1H, d, J 1.0 Hz, ArH-3), 8.34 (1H, d, J 2.5 Hz, ArH-6), 8.26 (1H, m, ArH-5). ^{13}C NMR δ (Table 8) 147.8, 142.0, 140.3, 137.5. m/z: 900 $[M + H]^+$, 742 (found $[M + H]^+$, 899.5563; $C_{45}H_{78}N_4O_{14}$ requires $[M + H]^+$ 899.5587).

7m: Using the general procedure on a 0.089 mmol scale yielded **7m** (0.014 g, 18%) as a white solid. IR (film) 3452, 2972, 2935, 1726, 1570, 1507, 1454, 1389, 1309, 1179, 1084, 1054, 1028, 997, 900 cm^{-1}. ^{1}H NMR δ (Table 7) 8.90 (1H, d, J 1.0 Hz, ArH-2), 8.62 (1H, d, J 5.5 Hz, ArH-5), 8.19 (1H, dd, J 5.5, 1.5 Hz, ArH-4). ^{13}C NMR δ (Table 8) 158.4, 158.3, 156.9, 110.6. m/z: 922 $[M + Na]^+$, 900 $[M + H]^+$, 742 (found $[M + H]^+$, 899.5552; $C_{45}H_{78}N_4O_{14}$ requires $[M+H]^+$ 899.5587).

8a: Using the general procedure on a 0.107 mmol scale yielded **8a** (0.014 g, 15%) as a white solid. IR (film) 3445, 2971, 2934, 1732, 1656, 1552, 1451, 1378, 1178, 1081, 1053, 1035, 998, 900 cm^{-1}. ^{1}H NMR δ (Table 7) 2.77 (3H, d, J 5.0 Hz, $NHCH_3$), 2.31 (1H, m, 1H of $OCH_2CH_2C\underline{H}_2CON$), 2.21 (1H, m, 1H of $OCH_2CH_2C\underline{H}_2CON$), 1.91 (2H, m, $OCH_2C\underline{H}_2CH_2CON$). ^{13}C NMR δ (Table 8) 33.1, 26.2 (2C). m/z: 864 $[M+H]^+$, 706 (found $[M+H]^+$, 863.5814; $C_{44}H_{82}N_2O_{14}$ requires $[M+H]^+$ 863.5839).

8b: Using the general procedure on a 0.224 mmol scale yielded **8b** (0.051 g, 26%) as a white solid. IR (film) 3434, 2971, 2936, 1733, 1650, 1552, 1451, 1379, 1178, 1082, 1029, 999, 900 cm^{-1}. ^{1}H NMR δ (Table 7) 2.79 (3H, d, J 5.0 Hz, $NHCH_3$), 2.18 (2H, m, $CH_2C\underline{H}_2CON$), 1.68−1.59 (4H, m, $OCH_2C\underline{H}_2CH_2CH_2$-CON). ^{13}C NMR δ (Table 8) 36.3, 29.3, 26.2, 23.1. m/z: 878 $[M + H]^+$, 720 (found $[M + H]^+$, 877.5978; $C_{45}H_{84}N_2O_{14}$ requires $[M+H]^+$ 877.5995).

8c: Using the general procedure on a 0.128 mmol scale yielded **8c** (0.067 g, 59%) as a white solid. IR (film) 3417, 2935, 1732, 1650, 1553, 1452, 1378, 1178, 1082, 1055, 1035, 998, 900 cm^{-1}. ^{1}H NMR δ (Table 7) 2.78 (3H, d, J 5.0 Hz, $NHCH_3$), 2.14 (2H, m, $CH_2C\underline{H}_2CON$), 1.63−1.52 (5H, m, 5H of $OCH_2CH_2CH_2$-$C\underline{H}_2CH_2CON$), 1.34 (1H, m, 1H of $OCH_2CH_2CH_2C\underline{H}_2$-$C\underline{H}_2CON$). ^{13}C NMR δ (Table 8) 36.4, 29.6, 26.2, 25.8, 25.5.

Table 8. ^{13}C NMR Resonances for Macrolide Portion of Compounds **7j**−**7m**, **8a**−**8c**, **9**, and **10**[a]

	7j	7k	7l	7m	8a	8b	8c	9	10
C-1	177.6	177.4	177.5	177.4	177.1	177.1	177.0	177.7	177.1
C-2	44.3	44.0	44.2	44.2	44.5	44.4	44.3	44.5	44.2
C-3	77.6	77.3	77.8	77.8	77.9	77.9	77.6	77.5	77.1
C-4	42.5	43.2	43.4	43.5	43.9	43.8	43.9	44.2	44.0
C-5	84.1	84.5	84.5	84.3	84.6	84.0	83.7	85.0	84.8
C-6	75.9	75.7	76.1	76.1	75.3	75.1	75.1	75.2	75.4
C-7	37.3	37.9	38.0	37.9	38.0	37.8	38.0	37.6	38.4
C-8	31.9	31.8	31.2	31.2	31.4	31.6	31.3	30.6	30.3
C-9	94.1	94.0	93.9	93.7	92.6	92.8	92.9	91.8	91.9
C-10	32.0	32.4	32.4	32.3	32.3	32.3	32.3	32.3	32.5
C-11	65.9	70.0	69.3	69.1	70.2	70.0	70.0	70.2	70.1
C-12	74.2	74.2	74.1	74.1	74.5	74.1	74.0	73.9	74.0
C-13	77.3	77.3	77.6	77.5	77.3	76.7	77.2	77.0	77.1
C-14	21.9	21.5	22.3	22.3	22.5	22.6	22.2	22.2	22.5
C-15	11.2	11.4	11.5	11.5	11.5	11.6	11.5	11.3	11.7
C-16	13.6	13.2	13.2	13.2	13.3	13.4	13.2	13.3	12.8
C-17	9.2	9.3	9.2	9.1	9.1	9.1	9.1	9.0	9.3
C-19	16.8	16.8	17.1	17.2	16.5	16.4	16.4	16.6	16.6
C-20	15.6	15.0	15.5	15.5	15.1	14.9	14.8	15.3	14.7
C-1′	102.4	102.4	102.3	102.2	102.1	101.9	101.9	101.9	102.0
C-2′	70.0	70.0	70.1	70.1	70.2	70.2	70.2	70.1	70.1
C-3′	62.4	62.2	62.3	62.2	62.0	62.0	62.0	62.0	62.0
C-4′	32.7	32.9	32.9	32.9	32.9	32.9	33.0	32.9	32.9
C-5′	69.3	69.4	69.4	67.5	69.5	69.4	69.4	69.5	69.4
NCH_3	30.9	31.0	31.2	31.1	31.2	31.2	31.2	31.2	31.2
NCH	52.6	52.6	52.9	52.7	52.7	52.7	52.7	52.7	52.8
C-1″	95.1	95.0	95.0	94.9	94.9	94.8	94.8	94.6	94.5
C-2″	34.6	34.5	34.5	34.5	34.7	34.7	34.7	34.5	34.6
C-3″	72.7	72.8	72.8	72.8	72.9	72.9	72.9	72.9	73.0
C-4″	77.8	77.7	77.7	77.6	77.9	77.9	77.9	77.7	77.7
C-5″	65.6	65.6	65.8	65.8	65.8	65.8	65.8	65.7	65.7
C-6″	17.9	17.7	17.7	17.6	18.0	18.0	17.9	17.2	17.7
OCH_3	49.3	49.3	49.3	49.3	49.3	49.3	49.3	49.3	49.3
C-9′	71.5	72.0	70.9	70.9	71.2	71.5	71.7	71.8	71.9
C-9″	169.1	169.8	168.8	170.0	173.3	173.3	173.6	167.9	167.9

[a] C-18, C-21, C-6′, $NCH(\underline{C}H_3)_2$, and C-7″ are between 22.0 and 19.0 ppm.

m/*z*: 892 $[M + H]^+$, 734 (found $[M + H]^+$, 891.6127; $C_{46}H_{86}N_2O_{14}$ requires $[M + H]^+$ 891.6152).

9: Using the general procedure on a 0.082 mmol scale yielded **9** (0.052 g, 69%) as a white solid. ^{1}H NMR δ (Table 7) 7.81 (1H, d, *J* 7.5 Hz, ArH-4 or ArH-6), 7.69 (1H, s, ArH-2), 7.38−7.30 (2H, m, ArH-4 or ArH-6 and ArH-5), 2.99 (3H, d, *J* 5.0 Hz, NCH_3). ^{13}C NMR δ (Table 8) 138.2, 135.0, 129.6, 128.5, 127.1, 124.5, 26.8. *m*/*z*: 912 $[M + H]^+$, 754 (found $[M + H]^+$, 911.5838; $C_{48}H_{82}N_2O_{14}$ requires $[M + H]^+$ 911.5839).

10: Using the general procedure on a 0.063 mmol scale yielded **10** (0.027 g, 47%) as a white solid. IR (film) 3461, 2972, 1732, 1648, 1549, 1452, 1378, 1178, 1081, 998, 900 cm^{-1}. ^{1}H NMR δ (Table 7) 7.70 (2H, d, *J* 8.5 Hz, ArH-3, ArH-5), 7.36 (2H, d, *J* 8.5 Hz, ArH-2, ArH-6), 3.01 (3H, d, *J* 5.0 Hz, NCH_3). ^{13}C NMR δ (Table 8) 141.4, 134.0, 127.6, 127.0, 26.8. *m*/*z*: 912 $[M + H]^+$, 754 (found $[M + H]^+$, 911.5833; $C_{48}H_{82}N_2O_{14}$ requires $[M + H]^+$ 911.5839).

13: Using the standard coupling procedure with **11** yielded **13** as a white solid. ^{1}H NMR δ (Table 7) 2.74 (3H, d, *J* 4.5 Hz, NCH_3), 2.14 (2H, m, 2H of Ncyb), 1.80 (2H, m, 2H of Ncyb), 1.51 (2H, m, 2H of Ncyb). ^{13}C NMR δ 177.8, 170.8, 102.7, 95.0, 94.7, 85.2, 78.1, 77.4, 76.2, 74.8, 73.2, 73.1, 70.6, 70.1, 70.0, 66.0, 60.0, 57.2, 49.7, 46.6, 44.3, 44.0, 38.8, 35.0, 33.2, 32.5, 31.3, 29.9, 28.9, 28.6, 26.0, 22.7, 22.2, 22.0, 21.6, 19.9, 17.9, 17.3, 15.0, 14.4, 11.9, 11.8, 9.7. *m*/*z* 848 $[M + H]^+$ (found $[M + H]^+$, 847.5529; $C_{43}H_{78}N_2O_{14}$ requires $[M + H]^+$ 847.5526).

14: Using the standard coupling procedure with **12** on a 0.66 mmol scale yielded **14** as a white solid. ^{1}H NMR δ (Table 7) 2.70 (3H, d, *J* 4.5 Hz, NCH_3), 1.68 (1H, m, H-12). ^{13}C NMR δ 178.0, 171.1, 102.7, 95.2, 93.8, 85.4, 78.1, 77.8, 76.1, 75.0, 73.2, 71.1, 70.4, 69.7, 65.9, 62.6, 53.0, 49.7, 44.0, 39.2, 37.6, 35.1, 33.2, 31.4, 25.9, 24.7, 21.9, 21.7, 21.5, 20.8, 20.4, 18.0, 13.1, 12.0, 10.2, 10.1, 9.6. *m*/*z*: 842 $[M + Na]^+$, 820 $[M + H]^+$, 662 (found $[M + H]^+$, 819.5572; $C_{42}H_{78}N_2O_{13}$ requires $[M + H]^+$ 819.5577).

In Vitro Assays. Motilin agonist potency and tachyphylaxis were measured in a rabbit smooth muscle contractility assays as previously reported.[29,30] EC_{50} values are the concentration that caused 50% of the maximal possible contraction. Tachyphylaxis is reported as the % contractility response obtained from an EC_{90} drug concentration following three cycles of administration and washout. hERG inhibition was measured at 37 °C using a stably transfected HEK cell line at expressing the hERG mRNA.[32] For routine screening, compounds were tested in replicate at 30 and 300 μM. For measurement of the IC_{50} of **1**, **3**, and **7a**, duplicate measurements were made at 1, 3, 10, 50, 100, and 300 μM.

Antibacterial activity was assessed by determining the minimal growth inhibitory concentration (MIC) of each compound.[42] For routine screening, the highly erythromycin-sensitive strain *Streptococcus pneumoniae* ATCC6301 was used. For **7a**, a panel of ∼200 strains were used and were representative of those commonly found in the gut flora, and as well as those which are commonly know to develop resistance to antibiotics. The strains were isolated from a variety of clinical specimens and are part of a collection maintained at the Clinical Microbiology Laboratories at the University of Rochester Medical Center and included approximately 10 isolates each of the following strains: *Enterococcus faecalis*, *Enterococcus faecium*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus coagulase*, *Streptococcus bovis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Corynebacterium jeikeium*, *Corynebacterium species* (not *jeikeium*), *Lactobacillus species*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Hemophilus influenzae*, *Moraxella catarrhalis*, *Bacteroides fragilis*, *Clostridium difficile*, *Clostridium perfringens*, *and Propionbacterium acnes*.

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Supporting Information Available: A full NMR data set is included for compound **7a** along with LC-MS and IR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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