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Synthesis of 2′-*O*-Benzoyl-3-keto-6-*O*-propargyl-11,12-carbamoyl Erythromycin A

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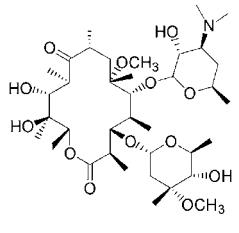
Abstract:

An efficient and practical synthesis of 2′-*O*-benzoyl-3-keto-6-*O*-propargyl-11,12-carbamoyl erythromycin A (4) is described. The semisynthetic macrolide was prepared on large scale in seven steps in 38% overall isolated yield from the readily available bis(trimethylsilyloxy) ether of erythromycin A oxime ketal (5). The chemistry, which required no chromatography, involved selective hydrolysis, alkylation, and hydroxyl protection transformations.

Introduction

There is considerable interest in the scientific community regarding semisynthetic macrolides such as clarithromycin (**1**) and azithromycin (**2**).[1] These erythromycin derivatives are well-known antibacterial agents used clinically to treat upper and lower respiratory tract infections. As with other antibacterial agents, however, their widespread use has resulted in the emergence of resistant bacterial strains. For this reason there is a continuing need to identify new derivatives having improved antibacterial activity. ABT-773 (**3**),[2] for instance, has recently been shown to be a potential antibiotic agent possessing desirable biological properties. With this in mind, we have developed a practical and efficient synthesis of 2′-*O*-benzoyl-3-keto-6-*O*-propargyl-11,12-carbamoyl erythromycin A (**4**), a key intermediate for preparing such new semisynthetic macrolide molecules that may show promise as novel antibiotics.[3]

Our synthetic strategy for the preparation of ketolide **4** from the readily available 2′,4″-di-*O*-trimethylsilyl oxime of erythromycin A (**5**) focused on several synthetic objectives: (1) selective propargylation of the C-6 hydroxy group, (2) deoximation of the C-9 carbonyl, (3) construction of the C-11,C-12 carbamate, (4) selective hydrolysis of the cladinose sugar to provide a free carbonyl at C-3, and (5) appropriate manipulation of the many hydroxyl sites in the molecule. Benzoate derivatives were considered the ideal hydroxyl protected intermediates due to their ease of preparation and removal, stability to both basic and acidic conditions, and tendency to be crystalline solids. The oxime



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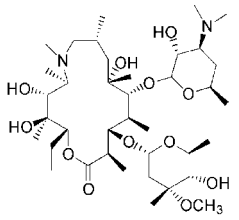

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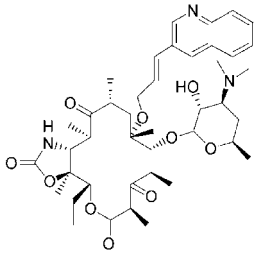

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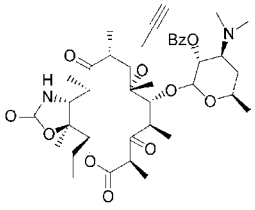

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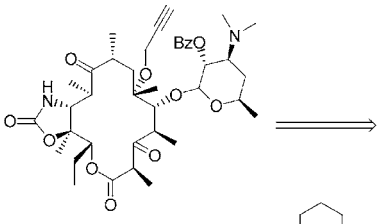
ketal **5** was also chosen as the starting material because the electrophilic C-9 ketone is deactivated, preventing hemiketal formation that complicates later transformations.[4]



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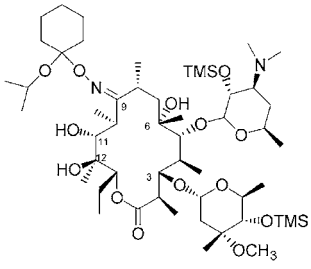

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Results and Discussion

The macrolide was synthesized as outlined in Scheme 1. Desilylation of the readily accessible 2′,4″-bis (trimethylsilyloxy) erythromycin A oxime ketal **5** by treatment with 2.1 equiv of 1 M solution of tetrabutylammonium fluoride in THF for 5 h at room temperature gave the deprotected oxime

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(2) (a) Or, Y.-S.; Ma, Z.; Clark, R. F.; Chu, D. T.; Plattner, J. J.; Griesgraber, G. U.S. Patent 5,866,549, 1999. (b) Rubenstein, E., Ed. *Science* **1994**, *264*, 360. (c) Cohen, M. L. *Trends Microbiol.* **1994**, *2*, 422.

(3) Phan, L. T.; Clark, R. F.; Rupp, M.; Or, Y. S.; Chu, D. T. W.; Ma, Z. *Org. Lett.* **2000**, *2*, 2951.

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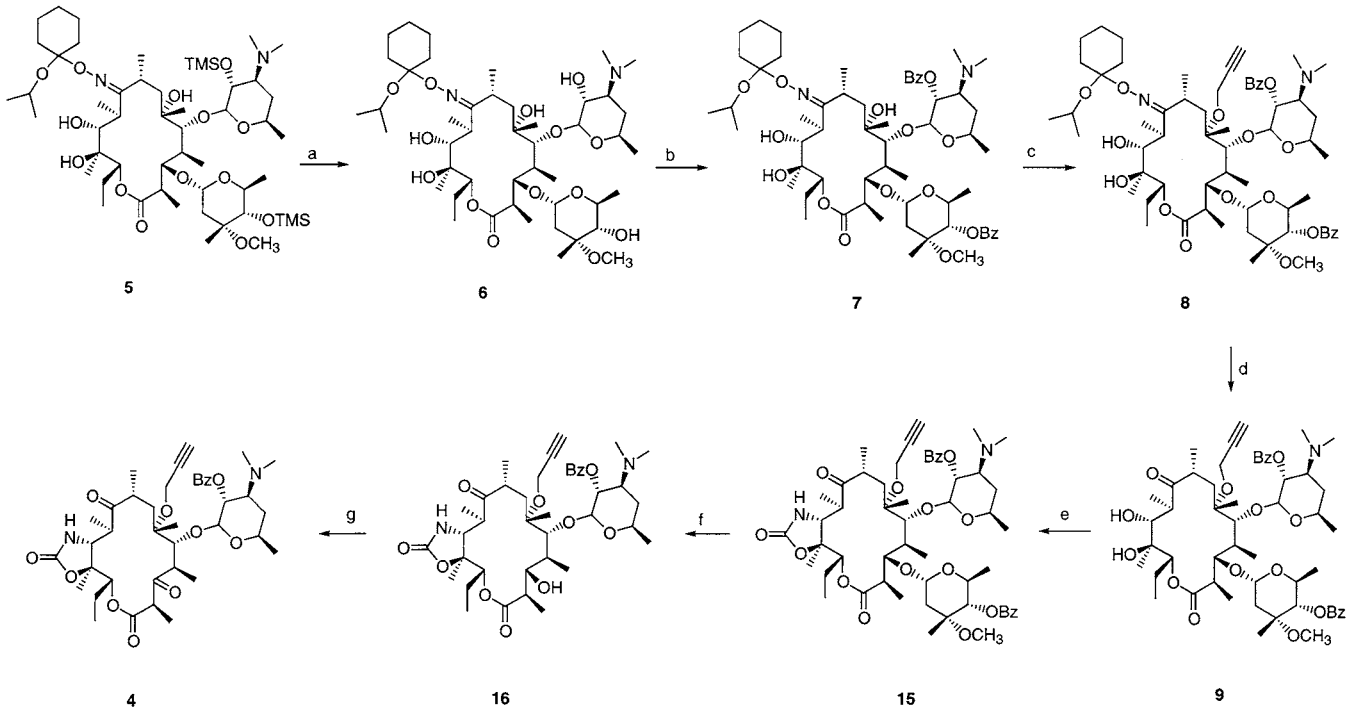
Scheme 1[a]



[a] Key (a) TBAF, 100%; (b) Bz_2O, DMAP, TEA, 87%; (c) Propargyl Br, KOtBu, 78%; (d) $NaNO_2$, HCl, 71%; (e) DBU, CDI, NH_3, KOtBu, 85%; (f) HCl, EtOH, 99%; (g) DMS, NCS, 94%.

ketal **6** in quantitative yield. The product was isolated as a solution in ethyl acetate and azeotropically dried with ethyl acetate to a KF of <0.05% and used directly in the next step. If necessary, the macrolide **6** could be crystallized from acetonitrile.

Oxime ketal **6** was treated with 3 equiv of benzoic anhydride, 1 equiv of 4-(dimethylamino)pyridine and 2 equiv of triethylamine at room temperature in ethyl acetate for 20 h to provide, after crystallization from 95/5: heptane/ethyl acetate, the dibenzoate **7** in 87% yield. Initial attempts to diprotect the oxime ketal without DMAP being present gave inferior results. The 2′-hydroxyl was completely benzoylated, but only after adding DMAP was the 4′-hydroxyl efficiently benzoylated. Although excess benzoic anhydride can be removed by treatment of the reaction mixture with *N*,*N*-dimethylethylenediamine, we found the use of 5% aqueous sodium bicarbonate to be equally effective. Only a trace amount of tribenzoate was detected that was readily rejected in the purification. These first two reactions have been successfully accomplished on 50 kg scale.

With the more reactive C-2′ hydroxyl and C-4″ hydroxyl blocked, alkylation of the less reactive C-6 hydroxyl group[5] was attempted. Propargylation of dibenzoate **7** by simultaneous slow addition of 3.7 equiv of propargyl bromide and 2.3 equiv of KOtBu in THF/DMSO at 0 °C over 3 h provided the acetylene **8** in 78% yield after crystallization from acetonitrile. The major controlling parameters of this reaction were the amounts of each reagent, the rate of their addition, and the temperature. We found that 3 h is the optimal reagent addition time. Significantly longer or shorter times resulted in inferior yields of product. Furthermore, the addition of reagents must be simultaneous; otherwise, yields of less than 15% of desired product are produced. In the presence of only KOtBu, the starting material decomposes over time due to the opening of the lactone ring. The bromide without the base reacts with the substrate amine to form a quarternary salt. Both reagents react together in the absence of the substrate to form polymeric byproducts. To avoid large excesses of either reagent, which are detrimental to the formation of desired product, the reagents are added together to the macrolide in a controlled manner. With regard to the temperature, lower temperatures than 0 °C are impractical since DMSO freezes at this temperature, while higher temperatures (≥20 °C) result in the formation of polymeric byproducts and yields of less than 35%. The corresponding diacetate and ditrimethylsilyl analogues were also propargylated, but the yields (61% and 52%, respectively) were dramatically lower due to their relative instability to the conditions of the reaction and a more difficult purification. This reaction has been reproduced successfully on 20 kg scale.

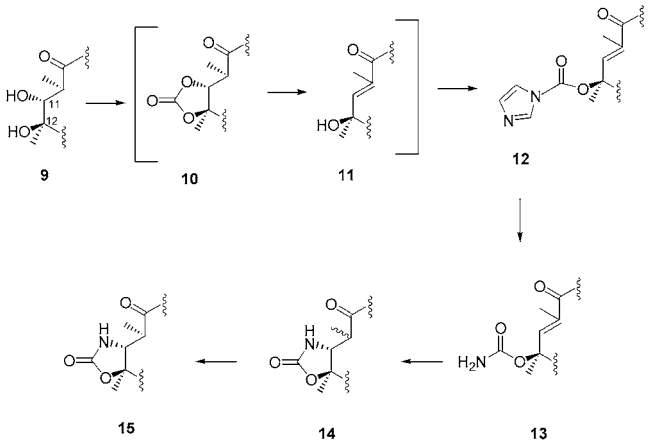
The acetylene **8** was then deoximated via a two-step one-pot procedure. Treatment of acetylene **8** with 8.0 equiv of 2 M HCl in 50% aqueous ethanol at room temperature for 15 min resulted in hydrolysis of the ketal. Subsequent sealing of the reaction vessel, addition of a solution of 7.5 equiv of sodium nitrite in H_2O, and stirring for 15 h provided the ketone **9** in 75% yield as the nitrate salt. The salt was triturated with 1:1 MTBE/cyclohexane and neutralized with 2 equiv of potassium carbonate in methanol to give ketone **9** in 71% isolated yield. For maximum yield, the HCl must be in excess by not more than 0.5 equiv relative to the nitrite

(5) Morimoto, S.; Takanishi, Y.; Adachi, T.; Nagate, T.; Wantanabe, Y.; Omura, S. *J. Antibiot.* **1990**, *43*, 286.

and more than 6 equiv of nitrite are needed. During the sealed reaction pressure builds up (15−40 psi depending on headspace) which may be released after 1 h and resealed. The reaction progresses for another 12 h (accompanied by an increase in pressure). The product precipitates throughout the reaction and is isolated by filtration. The filtrate contains predominantly the *N*-nitroso impurity. If the system is open, inferior yields of product are obtained. Presumably the initial pressure buildup is due to the immediate formation of the key reagent (HONO) that then reacts with the oxime to give two intermediates whose mass is consistent with addition of NO. Nitric oxide (NO) is observed in the headspace from the beginning of the reaction. There is some evidence in the literature that nitrosonium ion is the actual reagent.[6] It is postulated that this reacts with the oxime to give an oxaziridine intermediate with subsequent loss of nitrous oxide to provide the ketone. Headspace gas analysis confirmed that nitrous oxide (N_2O) is produced during this reaction. The effect of releasing the pressure and then resealing was also studied. There was found to be no significant difference in the final yield of product whether the pressure was released after 1 h and resealed or after 18 h. This reaction was performed on 8 kg scale.

Initially, the deoximation was done without the pressure or sealed system; however, under these conditions (6 equiv of sodium nitrite, 5 equiv of HCl at 45 °C for 2 h), the ketone was obtained in 36% yield. Dinitrogen tetroxide (N_2O_4) was also used in this conversion. Reaction of the oxime in a sealed system with 3 equiv of N_2O_4, 6 equiv of sodium nitrite, and 5 equiv of HCl in 50% aqueous ethanol, provided the nitrate salt in 76% yield after trituration with 2-propanol.

The installation of the carbamate functionality[7] was a three-step, one-pot sequence proceeding in 85% yield (equation 1). Treatment of the starting ketone **9** with excess carbonyl diimidazole and 0.2 equiv of DBU in THF/DMF at 40 °C afforded initially the carbonate **10**. The carbonate slowly decomposed over 10 h to the acylimidazolide **12** in quantitative yield via the hydroxyenone **11**. The reaction mixture was cooled to −10 °C and excess liquid ammonia added to form the acyclic carbamate **13** in 95% yield. The reaction mixture was then warmed to 15 °C and treated with 1.1 equiv of KOtBu that mediated the cyclization to a mixture of cyclic carbamates **14**, epimeric at C-10. This mixture equilibrated over 10 h to the desired stereoisomer **15** (98:2 ratio of desired to undesired). After recrystallization from IPA, carbamate **15** was obtained in 85% overall yield from ketone **9**. Among the several bases (LDA, lithium imidazolide, sodium imidazolide) tried in the first step of this sequence, DBU was found to be the most effective and convenient to handle. The use of 0.2 equiv of DBU gives maximum results, while higher amounts of DBU increase the epimers at the cyclization stage and decrease the recovery of desired carbamate. In the next step liquid ammonia is preferred over ammonia gas since the speed of the reaction is dependent on the concentration of ammonia. The carbamate formation has been successfully accomplished on 5 kg scale.



Cleavage of the cladinose sugar proceeded with excellent selectivity. Treatment of **15** in ethanol with 2 N HCl at 45 °C for 12 h afforded carbinol **16** in 99% yield. Higher reaction temperatures resulted in inferior yields. The cladinose related byproducts were entirely removed with one or two MTBE extractions in the aqueous workup. The nitroso impurity originating from the deoximation step was also readily removed in the workup. Although the alcohol can be recrystallized from acetonitrile, the crude product was of sufficient purity to be used in the next step.

The carbinol **16** was oxidized by a modification of the Corey−Kim[8] procedure. Addition of a solution of 1.9 equiv of *N*-chlorosuccinimide in THF to a mixture of the 3-hydroxy macrolide **16** in THF containing 2.1 equiv of dimethyl sulfide and 1.3 equiv of diisopropylethylamine in THF at −10 to −15 °C gave, after 3 h, the 3-ketolide **4** in 94% yield. The major impurity in this reaction was the 3-methylthiomethyl ether (1−2%) whose formation could be minimized by manipulation of the reaction parameters. Initial screening on a small scale identified the reaction conditions necessary to obtain maximum yields of desired product and minimal amounts of impurities. In general, the more polar solvent, more hindered base, lower reaction temperature and order of addition of the reagents, the less thioether produced. THF afforded higher yields and less impurity compared to those with methylene chloride. Among the amines studied (Et_3N, *i*-Pr_2EtN, 1-ethylpiperidine and 2,6-lutidine), *i*-Pr_2EtN provided the best results. Above 0 °C, the thioether level increased. The hydrolysis and oxidation steps have been accomplished on 200 g scale.

If needed, the 2′-benzoyl group is readily removed from the desosamine hydroxyl by extension of the literature methods[7,9] (Scheme 2). Refluxing 3-keto **4** in methanol for 8−12 h afforded the deprotected ketolide **17** in 91% yield. The methyl benzoate byproduct was removed by acidification of the reaction mixture with dilute acid and extraction with MTBE.

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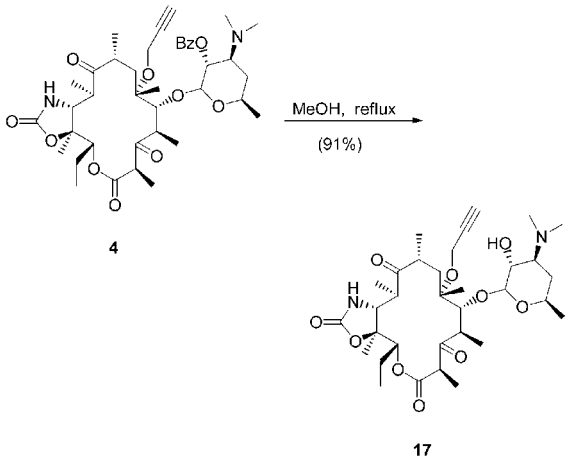
Scheme 2



Conclusions

In summary, a practical process for the preparation of ketolide **4** has been developed. It proceeded in seven steps and 38% overall isolated yield from the readily available bis(trimethylsilyloxy)erythromycin A oxime ketal **5**. The synthesis described utilized the appropriate choice of protecting scheme for this series of molecules, resulting in an efficient and selective propargylation of the C-6 hydroxyl, deoximation of the propargylated macrolide, formation of the carbamate, and hydrolysis of the cladinose sugar. This new flexible process has been done on large scale (most steps demonstrated on multikilogram scale) and requires no chromatography. It is also amenable to the synthesis of a wide variety of analogues and derivatives.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. The bis(trimethylsilyloxy) erythromycin A oxime ketal was supplied by Abbott Laboratories. All reactions with the exception of the deoximation were carried out under an atmosphere of nitrogen. ^{1}H NMR spectra were obtained on a General Electric QE-300 NMR instrument at 300 MHz. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. ^{1}H NMR data are tabulated in the following order: chemical shift, mutiplicity (s, singlet: d, doublet: t, triplet; q, quartet; m, multiplet), number of protons, coupling constant (s) in hertz. Mass spectra were recorded with a Finnagan LCQ mass spectrometer. All new compounds were characterized by full spectroscopic and analytical data, and yields refer to spectroscopically homogeneous materials.

9-Ketaloxime Erythromycin A (6). Into a three-neck flask is charged the bis(trimethylsilyl) ether **5** (250 g). The agitation is started, and then 250 mL of THF is added. To this solution is added all at once 1 M tetrabutylammonium fluoride solution in THF (508 mL). The solution is stirred at room temperature until HPLC or TLC indicates completion. Typical reaction time is 5 h. Ethyl acetate (2250 mL) is added, and the mixture is washed with 750 mL of water and 750 mL of 5% NaCl at 45 °C. The product solution is concentrated to ~400 mL, 1250 mL of EtOAc is added and reconcentrated under vacuum to ~400 mL. This process is repeated once. EtOAc (1250 mL) is again added, and a KF sample is taken. If KF is no more than 0.05%, the solution is used for the benzoylation. The solution is diluted with more EtOAc to a total final volume of 1500 mL. The yield of macrolide **6** by potency assay is quantitative. This solution is used directly for the benzoylation. HPLC conditions: Zorbax SB-C8 4.6 mm × 25 cm, 96:4:0.4 MeOH:H_2O: ethanolamine, column temperature 50 °C, flow rate of 1 mL/min, UV detection at 220 nm. Retention times: starting material 35.1 min, mono-TMS 18.4 min, product 10.9 min. A pure sample was isolated for analysis. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.12 (m, 1H), 4.92 (m, 1H), 4.50 (s, 1H), 4.45 (d, J = 7 Hz, 1H), 4.0−4.15 (m, 2H), 3.70−3.80 (m, 1H), 3.70 (s, 1H), 3.59 (d, J = 8 Hz, 1H), 3.45−3.55 (m, 1H), 3.41 (br s, 1H), 3.32 (s, 3H), 3.15−3.26 (m, 2H), 3.0−3.1 (m, 1H), 2.85−2.94 (m, 1H), 2.75 (q, J = 7 Hz, 1H), 2.35−2.50 (m, 2H), 2.29 (s, 6H), 1.76−2.05 (m, 5H), 1.35−1.70 (m, 16H), 1.42 (s, 3H), 1.01−1.35 (m, 30H), 0.85 (t, J = 7 Hz, 3H); MS *m*/*e* 889 $(M + H)^+$. Anal. Calcd for $C_{46}H_{84}N_2O_{14}$: C, 62.14; H, 9.52; N,3.15. Found: C, 61.96; H, 9.78;N, 3.02.

2′,4″-Di-*O*-benzoyl-9-ketaloxime Erythromycin A (7). Into a 5-L three-neck flask are charged benzoic anhydride (164.2 g) and 4-(dimethylamino)pyridine (29.6 g). The solid mixture is stirred, and the oxime ketal **6**/EtOAc solution (prepared from 250 g of the bis(trimethylsilyl)ether **5**) is added followed by triethylamine (49.0 g). The clear solution is stirred at room temperature under a blanket of nitrogen and monitored by HPLC until the monobenzoate is less than 2.0 area % (usually 20−24 h; however, reaction run as long as 3 days gives no adverse effect). 5% $NaHCO_3$ (1500 mL) is charged to the reactor, and the mixture is vigorouly stirred for 1 h. HPLC shows no benzoic anhydride remains. The organic layer is further washed with of 5% $NaHCO_3$ (1500 mL), 5% KH_2PO_4 (2 × 1500 mL), and water (1500 mL). The product **7**/EtOAc solution is filtered and concentrated to about 400 mL. Heptane (1250 mL) is added and reconcentrated to about 400 mL. Heptane (1250 mL) is added, and the mixture is reconcentrated to ~1000 mL.The final slurry is heated to reflux for about 30 min to dissolve the solids. The solution is cooled to 0−5 °C and stirred for another 2 h. The mother liquor is sampled to make sure the potency is no more than 15 mg/mL. The crystals are filtered and washed with 500 mL of heptane. The wet cake is dried under vacuum at 50 °C to give the dibenzoate **7** (225.6 g, 87%). HPLC conditions: Zorbax RxC8, 4.6 mm × 25 cm; eluent: 50/30/20 CH_3CN/0.1% H_3PO_4/0.03 M KH_2PO_4 to 95/5/5 CH_3CN/0.1% H_3PO_4/0.03 M KH_2PO_4 in 15 min and kept at 95/5/5 for another 5 min; flow rate: 1.5 mL/min; UV detection at 230 nm. Retention time: starting material 4.5 min (very weak), monobenzoate 9.3 min, bis-benzoate 12.5 min, benzoic anhydride 7.4 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (dd, J = 7 Hz, 4H), 7.59 (m, 2H), 7.46 (m, 4H), 5.12 (m, 3H), 4.92 (m, 2H), 4.50 (s, 1H), 4.45 (d, J = 7 Hz, 1H), 3.82−4.10 (m, 3H), 3.65−3.78 (m, 1H), 3.60 (s, 1H), 3.48−3.55 (m, 3H), 3.15 (br s, 1H), 2.91−3.02 (m, 1H), 2.75−2.85 (m, 1H), 2.65 (q, J = 7 Hz, 1H), 2.45−2.52 (m,

1H), 2.35 (s, 6H), 1.65−1.95 (m, 8H), 1.31−1.65 (m, 14H), 1.01−1.30 (m, 25H), 1.04 (d, J = 7 Hz, 3H), 0.72−0.82 (m, 6H); MS m/e 1097 $(M + H)^+$. Anal. Calcd for $C_{60}H_{92}N_2O_{16}$: C,65.67; H, 8.45;N,2.55. Found: C, 65.43; H, 8.66;N, 2.33.

2′,4″-Di-*O*-benzoyl-6-*O*-propargyl-9-ketaloxime Erythromycin A (8). To a 200-gal reactor evacuated and purged with nitrogen is charged with dibenzoate **7** (21.9 kg), THF (77 kg) and DMSO (95 kg). The mixture is agitated and cooled to an internal temp of 0−2 °C. The reagents, propargyl bromide (11.0 kg) and KOtBu (26.0 kg), are diluted with THF (128.0 kg) in pressure canisters and placed on top of a weigh balance. The reagents are then charged to the vessel at a predetermined rate over a period of 3 h, controlled by nitrogen pressure on the canisters. The reaction is complete when the amount of dibenzoate **7** is less than 5 area %. The reaction mixture is diluted with MTBE (280 kg) while maintaining the internal temperature below 10 °C, followed by triethylamine (18.0 kg). Chilled (10 °C) water (280 kg) is then added to the reaction mixture, and the agitation is kept slow to avoid emulsion formation. Aqueous layer is removed, and then 3% sodium chloride is charged; the layers are separated, and the upper organic layer is weighed and assayed before being concentrated under vacuum at a bath temperature of not more than 45 °C. The organic layer is concentrated to approximately 70 L, and then it is solvent-exchanged with acetonitrile (300 kg), causing the product to crystallize. This procedure removes most of the THF, and its presence is limited to <1% by NMR analysis. The suspension is concentrated to approximately 130 L and cooled to 10 °C. The product is then filtered, and the reactor is rinsed with fresh acetonitrile (15 kg); the rinse is then transferred as a wash on the wetcake. The wetcake is dried on the filter for no longer than 6 h and then dried in a vaccum oven with nitrogen purge at 45 °C to yield 18.2 kg (potency adjusted yield 78%) of acetylene **8**. HPLC conditions: Zorbax SB-C8 4.6 mm × 25 cm column; mobile phase is 55% isopropyl alcohol: 45% 44 mM ammonium acetate solution, run time is 25 min; flow rate is 1.0 mL/min; UV detection at 235 nm. Retention times: product 20.9 min, starting material 17.7 min, isomeric monohydroxy 18.3 min, dialkylated 19.3 min, isomeric dialkylated 23.8 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (br d, J = 10.7 Hz, 3.7 Hz, 4H), 7.64 (br dd, J = 16.2 Hz, 13.6 Hz, 2H), 7.45 (q, J = 14.3 Hz, 6.6 Hz, 4H), 5.10 (dd, J = 11.8 Hz, 10.3 Hz, 2H), 4.96 (m, 2H), 4.50 (m, 1H), 4.35 (br s, 1H), 3.82−4.15 (m, 6H), 3.63−3.75 (m, 3H), 3.54 (s, 3H), 3.23 (br s, 1H), 2.91−3.02 (m, 1H), 2.75−2.85 (m, 1H), 2.58 (br q, J = 13.6 Hz, 1H), 2.50 (d, J = 15.0 Hz, 1H), 2.39 (t, J = 2.2 Hz, 1H), 2.33 (br s, 6H), 1.25−2.05 (m, 22H), 0.91−1.25 (m, 26H), 0.7−0.84 (m, 6H); MS m/e 1135 $(M + H)^+$. Anal. Calcd for $C_{63}H_{94}N_2O_{16}$: C,66.67; H, 8.29; N, 2.47. Found C, 66.48; H, 8.37; N, 2.39.

2′,4″-Di-*O*-benzoyl-6-*O*-propargyl Erythromycin A (9). The pressure reactor is first pressure checked at 40 psi. Acetylene **8** (8.0 kg) is charged to the pressure reactor, followed by ethanol (32.0 kg). This slurry is stirred at room temperature; 2 M HCl (28.2 L) is then charged to the reactor and the resulting solution stirred for 15 min. The reactor is sealed. A solution of sodium nitrite (3.65 kg) in water (23 kg) is made up in a second reactor, and this is added to the oxime solution using positive nitrogen pressure. The reaction is allowed to proceed for 3 h. The pressure starts out at approximately 30 psi and increases to 50 psi over 3 h. No cooling is used; the temperature increases to 35−40 °C. The reaction is sampled using the internal pressure, and then the pressure is released. The reactor is sealed again and the reaction allowed to continue. The reactor will build up pressure again (approximately 10−15 psi). The reaction sample is initially gummy, but upon cooling to room temperature it starts to solidify. This is analyzed by HPLC. HPLC conditions: Zorbax SB-C8; flow 1.5 mL/min 30:70 to 90:10 acetonitrile:water (0.1% H_3PO_4) over 15 min; UV detection at 235 nm. Retention times: intermediate (+NO) 9.4 min, oxime 9.8 min, 9-keto 10.2 min, intermediate (+NO) 11.2 min, *N*-nitroso 17.8 min. The pressure is released and the internal temperature adjusted to 25 °C. The mixture is stirred for at least 1 h, before filtering. The wetcake is washed with water (10 kg) and then dried in the vacuum oven at 65 °C to provide 6.0 kg of tan powder. The crude product (nitrate salt of ketone **9**) is 86% potent, obtained in 75% potency adjusted yield. The nitrate salt of ketone **9** (16.9 kg) is charged to the reactor, followed by MTBE (124 kg) and cyclohexane (132 kg). The slurry is heated to 65 °C and held at that temperature for 30 min. The mixture is then cooled to 0 °C and stirred for 2 h. The resulting slurry is filtered and the wetcake washed with 55 kg of cold MTBE:cyclohexane (1:1). The wetcake is blown dry in the filter pot overnight. The wetcake is charged to the reactor along with distilled water (39 kg) and then methanol (154 kg). A 50% (w/w) potassium carbonate solution (4.6 kg) is then immediately added followed by distilled water (95 kg). The resulting slurry is stirred for at least 1 h and then filtered. The wetcake is washed with distilled water (102 kg) and dried in the vacuum oven at 65 °C to provide 15.15 kg of ketone **9** as a tan-colored powder with 93% potency and 71% overall yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03−8.06 (m, 4H), 7.55−7.64 (m, 2H), 7.44−7.50 (m, 4H), 5.05−5.12 (m, 3H), 4.93−5.01(m, 2H), 4.45−4.50 (m, 1H), 3.98−4.09 (dq, J = 8 Hz, 2.2 Hz, 2H), 3.87−3.94 (m, 2H), 3.78 (d, J = 10 Hz, 1H), 3.67−3.70 (m, 2H), 3.56 (s, 3H), 3.16 (br s, 1H), 2.93−3.02 (m, 2H), 2.80−2.86 (m, 1H), 2.60−2.66 (m, 1H), 2.50 (d, J = 15 Hz, 1H), 2.44 (t, J = 2.2 Hz, 1H), 2.33 (s, 6H), 1.62−1.93 (m, 6H), 1.54 (s, 3H), 1.32−1.49 (m, 2H), 1.23 (s, 6H), 1.21 (s, 2H), 1.10−1.16 (m, 7H), 1.04 (s, 3H), 0.95 (d, J = 6 Hz, 3H), 0.75−0.82 (m, 6H); MS m/e 980 $(M + H)^+$. Anal. Calcd for $C_{54}H_{77}NO_{15}$: C, 66.17; H, 7.92; N, 1.43. Found: C,65.99; H 8.03; N, 1.32.

2′,4″-Di-*O*-benzoyl-6-*O*-propargyl-11,12-carbamoyl Erythromycin A (15). To a 30-gal reactor evacuated and purged with nitrogen is charged ketone **9** (4.8 kg), carbonyl diimidazole (2.5 kg), THF (18.0 kg), and DMF (6.4 kg). The mixture is agitated, and DBU (160 g) is then added; the mixture stirred at ambient temperature for 1 h and then heated to 40 °C and for not less than 10 h (typically overnight). It initially forms the carbonate which is then converted to the

acyl imidazolide. The reaction is monitored by HPLC. HPLC conditions: Zorbax SB-C8 4.6 mm × 25 cm column; mobile phase is 30% acetonitrile:70% water (0.1% H_3PO_4) to 90:10 over 15 min; stop time is 20 min; flow rate is 1.5 mL/min; UV detection at 235 nm. Retention times: product, acyl imidazole 11.0 min, carbonate 10.2 min, starting material 10.0 min. The limit here is less than 1% of the carbonate before proceeding to the next step. The reaction mixture is then cooled to −10 °C, and liquid ammonia (∼10 kg) is charged into the reactor; the conversion to acyclic carbamate is complete in about 1 h. No acyl imidazole is detected after 1 h. The excess ammonia is removed by warming the reactor to 15 °C and then bubbling nitrogen into the reaction. HPLC conditions: Zorbax SB-C8 4.6 mm × 25 cm column; mobile phase is 30% acetonitrile:70% water (0.1% H_3PO_4) to 90:10 over 15 min; stop time is 20 min; flow rate is 1.5 mL/min; UV detection at 235 nm. Retention times: product, acyclic carbamate 9.9 min, hydroxyenone 10.6 min (impurity); starting material (acyl imodazole) 11.0 min. Potassium *tert*-butoxide (5.2 kg, 1 M solution in THF) is then added and the reaction mixture stirred at ambient temperature for not less than 10 h. The reaction is complete when the amount of the epimer is less than 1 area %. Typically the amount of the epimer after overnight is 0.7%. HPLC conditions: Zorbax SB-C8 4.6 mm × 25 cm column; mobile phase is 30% acetonitrile:70% water (0.1% H_3PO_4) to 90:10 over 15 min; stop time is 20 min; flow rate is 1.5 mL/min; UV detection at 235 nm. Retention times: product, cyclic carbamate 9.5 min, hydroxyenone 10.6 min (impurity); epimer (impurity) 10.2 min, starting material (acyclic carbamate) 9.9 min. Isopropyl acetate (45 kg) and potassium phosphate solution (25 kg) are then added to the reaction mixture, and the agitation is kept slow to avoid emulsion formation. The aqueous layer is removed (clear but dark colored), and then 5% sodium chloride (25 kg) is charged; the layers are separated, and the upper organic layer is weighed and assayed before being concentrated under vacuum at an internal temperature of not more than 45 °C. The organic layer is concentrated to approximately 30 L, and then additional isopropyl acetate (30 kg) is added; the distillation is continued until the KF of the organics is less than 1 mg/g, and the volume is kept at approximately 30 L. It is then solvent-exchanged with isopropyl alcohol (50 kg) causing the product to crystallize. This procedure removes most of the THF and IPAc. The amount of IPAc remaining is limited to <1% by NMR analysis. The suspension is concentrated to approximately 40 L and cooled to 10 °C. Samples are checked periodically to determine the mother liquor concentration before filtration of the product. Typically the concentration of the mother liquors after 3 h at 10 °C is 14−16 mg/mL. The product is then filtered, and the reactor is rinsed with cold IPA (15 kg); the rinse is then transferred as a wash on the wetcake. The wetcake is dried on the filter for not less than 2 h and then dried in a vaccum oven with nitrogen purge at 60 °C to yield 4.44 kg of carbamate **15** (yield = 89%, potency-adjusted yield = 85%). The mother liquors are assayed and found to contain another 334 g of compound **15**. Carbamate **15** is typically an off-white powder with >96.5 area % by HPLC analysis. Typically it contains about 2% of *N*-nitroso (from deoximation step) and 1.5% hydroxyenone. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (br m, 4H), 7.6 (br dd, J = 7.3 Hz, 7.7 Hz, 2H), 7.45 (dt, J = 7.7 Hz, 1.9 Hz, 4H), 5.78 (br s, 1H), 5.04 (m, 3H), 4.9 (d, 7.3 Hz, 2H), 4.45 (m, 1H), 4.0 (m, 2H), 3.85 (m, 3H), 3.75 (br s, 1H), 3.67 (br d, J = 6.3 Hz, 1H), 3.52 (s, 3H), 3.0−2.72 (m, 3H), 2.43−2.6 (m, 4H), 2.33 (s, 6H), 1.9−1.6 (m, 10H), 1.48 (br s, 3 H), 1.35 (br s, 3H), 1.2−1.3 (m, 8H), 1.15 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 5.9 Hz, 3H), 0.85 (t, J = 7.7 Hz, 3H), 0.76 (d, J = 7.4 Hz, 3H); MS *m*/*e* 1005 $(M + H)^+$. Anal. Calcd for $C_{55}H_{76}N_2O_{15}$: C, 65.72; H, 7.62; N, 2.79. Found: C, 65.59; H, 7.81; N, 2.48.

2′-*O*-Benzoyl-3-hydroxy-6-*O*-propargyl-11,12-carbamoyl Erythromycin A (16). Crystalline carbamate **15** (268 g, 0.266 mol) is charged to a 5-L three-necked round-bottomed flask, equipped with a thermocouple, nitrogen inlet tube, and mechanical stirring apparatus. EtOH (1.3 L) and 2 N HCl (1.3 L) are added, and the mixture is heated to 45 °C until starting material is not detected by HPLC (typically 12 h). HPLC conditions: Zorbax SB-C8 4.6 mm × 25 cm column; mobile phase is 70% water (0.1% H_3PO_4):30% acetonitrile to 10:90 over 15 min; flow rate is 1.5 mL/min; UV detection at 235 nm. Retention times: desired product 5.2 min, starting material 8.9 min. After cooling to room temperature, water (1.3 L) is added and the ethanol removed. MTBE (500 mL) is added, and the layers are agitated and separated. This is done twice more. The aqueous product-containing layer (bottom) is diluted with isopropyl acetate (2.2 L) and treated with 30% K_2CO_3 solution (500 mL) with good mixing. The layers are separated. The organic layer is removed in vacuo to provide carbinol **16** as a foam which can be further dried under high vacuum to an off-white powder (200 g, 99%). Carbinol **16** is of sufficient purity (96%) to be used directly in the next step. An analytical sample was prepared by crystallization from acetonitrile. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (m, 2H), 7.58 (br dd, J = 7.3 Hz, 7.7 Hz, 1H), 7.46 (dt, J = 7.7 Hz, 1.9 Hz, 2H), 5.77 (br s, 1H), 5.04 (m, 3H), 4.78 (d, 7.3 Hz, 1H), 3.80 (m, 4H), 3.57 (br d, J = 6.3 Hz, 2H), 2.93−2.99 (m, 1H), 2.82 (m, 1H), 2.45−2.64 (m, 3H), 2.32 (br s, 6H), 1.42−2.00 (m, 6 H), 1.40 (s, 2H), 1.34 (s, 2H), 1.22−1.29 (m, 9H), 1.12 (d, J = 6.6 Hz, 3H), 1.07 (d, J = 6.6 Hz, 3H), 0.85 (t, J = 7.7 Hz, 3H), 0.72 (d, J = 7.4 Hz, 3H); MS *m*/*e* 743 $(M + H)^+$. Anal. Calcd for $C_{40}H_{58}N_2O_{11}$: C, 64.67; H, 7.87; N, 3.77. Found: C, 64.31; H, 7.97; N, 3.45.

2′-*O*-Benzoyl-3-Keto-6-*O*-propargyl-11,12-carbamoyl Erthromycin A (4). 1.5 L of carbinol **16** (208 g, 280 mmol) in THF is charged to a jacketed flask, followed by dimethyl sulfide (37 g, 590 mmol) and diisopropylethylamine (47 g, 364 mmol). The solution is cooled to ∼−13 °C. *N*-Chlorosuccinimide (71 g, 532 mmol) is dissolved in THF (240 mL) and added to the flask at a rate so as to maintain the internal temperature at −11 to −13 °C. The mixture is then stirred at −15 ± 5 °C for 3 h. HPLC conditions: Zorbax RxC8, 4.6 mm × 25 cm; 30/70 acetonitrile/0.1% H_3PO_4 to 90/10 acetonitrile/0.1% H_3PO_4 in 15 min; flow rate: 1.5 mL/min; UV detection at 235 nm. Retention time: starting

material 5.2 min, product 6 min, 3-methylthiomethyl ether 7.1 min. Isopropyl acetate (3 L) is added followed by 0.5 N NaOH (1.2 L). The mixture is warmed to room temperature and stirred for 1 h. The organic layer is washed with 5% NaCl (2 × 600 mL) and brine (2 × 600 mL). The product layer is concentrated under vacuum to obtain at first a yellow amorphous solid which when dried under high vacuum turns into a white foam. Slurrying the solid in warm water followed by filtration and drying afforded the 3-ketomacrolide **4** as a white solid in 94% yield (196 g) after trituration with 10% EtOAc/heptane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (m, 2H), 7.58 (br dd, J = 7.3 Hz, 7.7 Hz, 1H), 7.46 (dt, J = 7.7 Hz, 1.9 Hz, 2H), 5.72 (br s, 1H), 4.94−5.12 (m, 3H), 4.59 (d, 7.3 Hz, 1H), 4.35 (d, J = 7 Hz, 1H), 3.87 (s, 1H), 3.55−3.77 (m, 4H), 2.93−3.07 (m, 1H), 2.86 (m, 1H), δ 2.52−2.64 (m, 2H), 2.36 (t, J = 3 Hz, 1H), 2.31 (br s, 6H), 1.86−2.00 (m, 2 H), 1.40−1.62 (m, 8H), 1.28−1.35 (m, 6H), 1.24 (d, J = 6.6 Hz, 3H), 1.08−1.17 (m, 4H), 0.96 (d, J = 7 Hz, 3H), 0.85 (t, J = 7 Hz, 3H); MS m/e 741 $(M + H)^+$. Anal. Calcd for $C_{40}H_{56}N_2O_{11}$: C, 64.85; H, 7.62; N, 3.78. Found C, 64.68; H, 7.74; N, 3.51.

3-Keto-6-*O*-propargyl-11,12-carbamoyl Erythromycin A (17). The 3-ketolide **4** (10 g, 13.5 mmol) in methanol (100 mL) is refluxed for 15 h. The methanol is removed, and MTBE (100 mL) is added followed by 0.5 N HCl (100 mL). After 20 min of mixing, the aqueous layer is washed with MTBE (2 × 100 mL) to remove methyl benzoate byproduct. HPLC conditions: Zorbax RxC8, 4.6 mm × 25 cm; 30/70 acetonitrile/0.1% H_3PO_4 to 90/10 acetonitrile/0.1% H_3PO_4 in 15 min; flow rate: 1.5 mL/min; UV detection at 220 nm. Retention time: starting material 6 min, product 5.2 min, methyl benzoate 7.8 min. The product/HCl layer is diluted with EtOAc (200 mL) and neutralized with 30% K_2CO_3 to reach pH 11. After 30 min mixing the organic layer is washed with 5% NaCl (2 × 100 mL), filtered to remove some insolubles and the solvent removed. The crude solid is triturated with hot heptane/EtOAc for 1 h and then allowed to cool to room temperature overnight. The ppt is collected and dried under vacuum to afford the deprotected macrolide **17** (7.8 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.72 (br s, 1H), 4.94−5.12 (m, 3H), 4.59 (d, 7.3 Hz, 1H), 4.35 (d, J = 7 Hz, 1H), 3.87 (s, 1H), 3.55−3.77 (m, 4H), 2.93−3.07 (m, 1H), 2.86 (m, 1H), 2.52−2.64 (m, 2H), 2.36 (t, J = 3 Hz, 1H), 2.31 (br s, 6H), 1.86−2.00 (m, 2 H), 1.40−1.62 (m, 8H), 1.28−1.35 (m, 6H), 1.24 (d, J = 6.6 Hz, 3H), 1.08−1.17 (m, 5H), 0.96 (d, J = 7 Hz, 3H), 0.85 (t, J = 7 Hz, 3H); MS m/e 637 $(M + H)^+$. Anal. Calcd for $C_{33}H_{52}N_2O_{10}$: C, 62.24; H, 8.23; N, 4.40. Found C, 62.38; H, 8.22; N, 4.12.

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